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Antibacterial Effect Of Ethanolic Extract Of Duku Seed (*Lansium Domesticum*) Against *Streptococcus Mutans*

M. Reza Eka Chandra[1], Shanty Chairani[1*], Listia Eka Merdekawati[2]

[1]Dentistry Program, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia
[2]South Sumatera Provincial Dental Hospital, Palembang, Indonesia
*Correspondence author email: shanty.c@fk.unsri.ac.id

Abstract

Introduction: Caries is a major oral health disease in Indonesia. *Streptococcus mutans* (*S. mutans*) is the main microorganism causes caries. Caries prevention can be done by mechanical or chemical methods. Plants can be used as an alternative for antibacterial chemical agent, one of which is duku seed. Ethanolic extract of duku seed showed the presence of alkaloids, flavonoids, saponins, and tannins that have antibacterial effect. **Purpose:** The purpose of this study was to evaluate the antibacterial effect of ethanolic extract of duku seed against *S. mutans.* **Method:** Present study was an in vitro laboratory study using post-test only control group design. Duku seed extracted by maceration with ethanol 96%. Antibacterial effect was carried out using microdilution method for MIC and MBC90 by ethanolic extract of duku seed with concentration of 0,19-100%. Inhibition zone was evaluated using disc diffusion method by ethanolic extract of duku seed with concentration of 1,56-12,5%, chlorhexidine gluconate 0,2% (positive control), and aquadest (negative control). Results were analyzed using one way ANOVA and Tukey's LSD post-hoc. **Result:** The results showed that MIC and MBC90 were 0,19% and 3,12%, respectively. Ethanolic extract of duku seed at concentration of 3,12% showed the maximum inhibition zone of 4,50 mm, but still lower than chlorhexidine gluconate 0,2%. **Conclusion:** Ethanolic extract of duku seed has antibacterial effect against *S. mutans*, so it can be used as an alternative plant for dental caries prevention.

Keywords: Antibacterial activity; Duku seed; *Lansium domesticum*; *Streptococcus mutans*

Introduction

Dental caries is a major oral health disease in Indonesia. Caries is multifactorial disease that occurs related to main factors and predisposition factors. The main etiology of caries are host, agent, diet, and time.[1]

Streptococcus mutans (*S. mutans*) is the main microorganism that causes caries. Synthesis of extracellular polysaccharides, adhesion, biofilm formation, and acid formation are the typical virulence of *S. mutans.*[2] Caries prevention can be done by mechanical or chemical methods.[3] Plants can be used as an alternative for antibacterial agent.[4]

Duku (*Lansium domesticum*) is the main commodity plant from South Sumatera region which is widely consumed by the community. Duku seed could not be consumed because its bitter taste and will become waste.[5] All parts of duku fruit such as flesh, peels, and seeds can be used in various purposes, including medical purposes. Alimon et al (2014) reported that

extract of duku seed contains alkaloids, saponins, tannins, and flavonoids that act as an antibacterial agent.[6] These secondary metabolites have antibacterial activity because these compounds change the arrangement of the extracellular matrix, inhibit the production of microbial enzymes, and disrupt bacterial cell walls.[7,8]

Duku has been widely researched as an antibacterial against microorganisms. Korompis et al. (2010) reported that the ethanol extract of duku seed had antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* at a concentration of 100%, and *Vibrio cholerae* at a concentration of 75%.[9] Chaisawadi et al (2011) reported that ethanolic extract of dried duku seed had a higher antibacterial effect against *Bacillus cereus* and *Staphylococcus aureus* than those that were not dried.[10] Therefore, the present study aimed to evaluate the antibacterial effect of ethanolic extract of duku seed against *S. mutans*.

Methods

Preparation of extracts

Duku seeds was harvested from cultivation in Rasuan Village, Madang Suku I, Ogan Komering Ulu Timur, South Sumatera. Duku seeds was separated from the fruit and washed under tap water. After that, the collected material was filtered and dried with oven at 60ºC for 24 hours. Then, duku seeds was mashed with a blender into powder.

The powder was soaked in ethanol 96% in a 1:2 (w/v) ratio for 24 hours, and then filtered using Whatmann filter paper to get the filtrate. Maceration was repeated for three times to get all the filtrates.The filtrate was evaporated using rotary vacuum evaporator. Extract obtained was collected in a tight and stoppered sterile amber bottle to avoid direct sunlight and kept in the refrigerator.

Culture media

The bacteria used in this study was *Streptococcus mutans* (ATCC 25175). Blood agar and nutrient broth was used as the media for bacteria. The bacteria were growth anaerobically and incubated at 37ºC for 24 hours.The cells were suspended into sterile 0,9% NaCl. Bacterial suspension was standardized matching a turbidity equivalent to 0,5 McFarland standard ($1,5x10^8$ CFU/ml).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration 90 (MBC)

The MIC and MBC90 were determined by using microdilution method. 200 µl of nutrient broth, bacterial suspension, ethanolic extract of duku seed with concentrations of 0,19%-100%, and chlorhexidine gluconate 0,2% as positive control were added into wells. Ethanolic extract of duku seed and chlorhexidine gluconate 0,2% were tested in triplicate. The plate were incubated at 37ºC for 24 hours. The MIC was determined by the lowest concentration of the tested antimicrobial agent that inhibits the visible growth of the microorganism and showing a clear well as detected by the unaided eye.

In accordance with the results of MIC determination, the bacterial cultures from the wells with test samples were transferred to blood agar plate and incubated at 37ºC for 24 hours.The lowest concentration that resulted in no visible growth of bacteria on the blood agar plate was recorded as the MBC90 value.

Determination of the inhibition zone

Disc diffusion method was used to determine the inhibition zone in this study. Bacterial suspension were spread on the blood agar with a cotton swab. Chlorhexidine gluconate 0,2% was used as the positive control and aquadest was used as the negative control. All samples were applied to the paper disc and placed on the agar plate with sterile tweezers. The plates were incubated at 37ºC for 24 hours. A vernier caliper was used to measure the diameter of the inhibition zone in milimeter. Each samples was tested with four repetitions.

Inhibition zone data were analyzed using ANOVA followed by Tukey's HSD for multiple comparisons in the statistical software. The value of $p<0,05$ was considered statistically significant.

Results

MIC and MBC90 value

A microdilution method and agar dilution were used to determine MIC & MBC90 with chlorhexidine gluconate 0,2% as positive control. The results of MIC & MBC90 are shown in Table 1.

Table 1. Results of MIC & MBC90 ethanolic extract of duku seed

	Extract Concentrations										CHX
	100%	50%	25%	12,50%	6,25%	3,12%	1,56%	0,79%	0,39%	0,19%	0,2%
MIC	+	+	+	—	—	—	—	—	—	—	—
MBC90	+	+	+	+	—	—	+	+	+	+	—

(+): Detected bacterial growth, (—): No bacterial growth

The MIC value of ethanolic extract of duku seed was 0,19%. Chlorhexidine gluconate 0,2% as positive control was clear, indicating that it had antibacterial effect. The MBC90 value of ethanolic extract of duku seed was 3,12%.

1.1. The inhibition zone

The inhibition zone of ethanolic extract of duku seed 1,56%, 3,12%, 6,25%, and 12,50% were done using agar disc diffusion method with chlorhexidine gluconate 0,2% as positive control and aquadest as negative control. The results inhibition zone of ethanolic extract are shown in Table 2.

Table 2. Results of inhibition zone ethanolic extract of duku seed

Sample	Mean of Inhibition Zone (mm)
Ethanolic extract of duku seed 1,56%	3,10
Ethanolic extract of duku seed 3,12%	4,50
Ethanolic extract of duku seed 6,25%	3,25
Ethanolic extract of duku seed 12,50%	2,60
Chlorhexidine gluconate 0,2% (Control +)	9,20
Aquadest (Control -)	0

The results showed that ethanolic extract of duku seed had antibacterial effect against *S. mutans* at all concentrations. Ethanolic extract of duku seed at concentration of 3,12% showed the maximum zone of inhibition of 4,50 mm, meanwhile chlorhexidine gluconate 0,2% as

positive control had zone of inhibition of 9,20 mm. Aquadest as negative control did not have zone of inhibition.

The results showed that there was significant difference ($p<0,05$) between the antibacterial effect of the various concentrations of ethanolic extract of duku seed. The results of Tukey's HSD showed that there was significant difference variations between the extract and controls. Ethanolic extract of duku seed 1,56%, 6,25%, and 12,5% showed no significant difference ($p>0,05$). Ethanolic extract of duku seed 3,12% showed significant difference compared to other concentrations ($p<0,05$). There were significant differences between all concentrations of ethanolic extract of duku seed compared to negative and positive control ($p<0.05$).

Discussions

The results of the study showed that ethanolic extract of duku seed has antibacterial effect against *S. mutans*. Ethanolic extract of duku seed showed the presence of alkaloids, flavonoids, saponins, and tannins that had antibacterial effect.[11] The result of this study was similar to Alimon et al (2014) that reported duku seed extract had antibacterial effect against gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*.[6]

MIC in the present study showed the clear zone in the range of 0,19-12,5%. These results were not following the concentration-dependent pattern, in which the higher the concentration of an extract, the greater the antibacterial effect.[12] Regarding with the MIC results, this study was following the hormesis. Hormesis pattern is a dose-response relationship where low concentrations of the effector trigger responses opposite to high concentrations. One of the dose-response relationship characterized by increased in positive effect at low doses and decreased in the effect at high doses. Hormesis pattern could be caused by the several secondary metabolites of ethanolic extract of duku seed and bacterial adaptation of *S. mutans* DNA.[13]

Hormesis pattern of ethanolic extract of duku seed could be assured using another MIC testing with the spectrophotometric method. Makolit et al (2017) reported the MIC results of extract of noni fruit (*Morinda citrifolia L.*) against *S. mutans* with turbidity method has showed that clear zone at the concentrations of 0,39-12,5%, which follows the hormesis pattern. MIC of noni fruit extract subsequently was measured by the spectrophotometric method which showed that the concentration of 50% and 100% inhibited the bacterial growth compared to

smaller concentrations, which follows concentration-dependent pattern.[14] These results showed that different method of MIC testing could exhibit different results. The use of the spectrophotometric method could be carried out in future studies to see the hormesis pattern of the antibacterial effect of ethanolic extract of duku seed against *S. mutans*.

MBC90 of ethanolic extract of duku seed of present study was 3,12%. MBC value of an extract is expected bigger or equal to the MIC value.[15] These finding indicated that increasing ethanolic extract of duku seed concentrations from 0,19% to 3,12% will change the bacteriostatic effect into bactericide.[16]

Inhibition zone test showed that ethanolic extract of duku seed 3,12% had the largest inhibition zone. The extract might have high potency even at low concentrations.[17] The results of present study was similar to Subandrate et al (2016) that reported ethanolic extract of duku seed from three concentrations tested (100 mg/kg, 200 mg/kg, and 300 mg/kg) had showed that 100 mg/kg was having the largest effect and decrease at higher concentrations.[18] Mahmudah et al(2017) also reported that extract of finger root with concentration of 50 mg/ml had exhibited the largest inhibition zone compared to other concentrations against *S. mutans*.[19]

All the concentrations of ethanolic extract of duku seed showed inhibition zone, but still lower than chlorhexidine gluconate 0,2%. This could be due to differences in the antibacterial mechanism between ethanolic extract of duku seed and chlorhexidine gluconate 0,2%. Chlorhexidine gluconate 0,2% has an ability to attach to surfaces.[20] Meanwhile, the ability to attach to surfaces of ethanolic extract of duku seed is still questionable. Chlorhexidine gluconate 0,2% is having several side effects in the oral cavity such as oral staining, impaired taste buds, and oral lesions.[21]A few studies reported that ethanolic extract of duku seed had low toxicity.Klungsupya et al (2014) reported that ethanolic extract of duku seed had no toxic effect on lymphoblast cells.[22] Similar study reported by Mayanti et al (2020) that ethanolic extract of duku seed with dose of 5.000 mg/kg possessed no significant toxic effect on wistar rat.[23] The minimal toxicity effect of duku seed extract could become the advantages of these plant so that it could be developed as an alternative plants for caries prevention.

Antibacterial effect of ethanolic extract of duku seed is expected to be equivalent to the effect of chlorhexidine gluconate 0,2% by modifying the formulation, such as combine with another extract plants that has antibacterial effect. Shekar et al (2019) reported that combinations of akasia leaf (*A. nilotica*), salam koja leaf (*M. koenigii*), eucalyptus, and guava

leaf (*P. guajava*) had antibacterial effect against *S. mutans* rather than chlorhexidine gluconate 0,2% and its single extract.[24] Similar study by Fitriani et al (2016) reported that combinations of lime juice and honey had greater antibacterial activity against *S. mutans* compared to chlorhexidine gluconate 0,2% and its single extract.[25] Based on these studies, combining ethanolic extract of duku seed with other extracts might improve the antibacterial effect of ethanolic extract of duku seed thus having antibacterial effect similar to 0.2% chlorhexidine gluconate.

Conclusion

The current study showed that ethanolic extract of duku seed has antibacterial effect against *S. mutans*, so it could be used as an alternative plant for dental caries prevention.

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