THE EFFECT OF ETHANOLIC EXTRACT OF *Syzygium cumini* LEAVES ON THE GROWTH OF *Streptococcus mutans*

PENGARUH EKSTRAK ETANOLIK DAUN JAMBLANG (Syzygium cumini) TERHADAP PERTUMBUHAN Streptococcus mutans

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Abstract

Streptococcus mutans plays an important role in the pathogenesis of caries. This bacteria has virulence properties involve in the formation of biofilm on tooth surface. Due to its antibacterial effect, Jamblang leaf/*Syzygium cumini* L may be used as an agent to prevent caries. This study aimed to elucidate the effects of ethanolic extract of *Syzygium cumini* L on the growth of *S. mutans.* Analysis of active compounds was carried out using *thin layer chromatography* (TLC) and *Liquid Chromatograph-Mass Spectrography* (LC-MS). Samples of *S. mutans* were isolated from children's carious deciduous molar teeth. Growth test was done by dilusion technique. Aquadest was used as a negative control. TLC and LC-MS showed the presence of the flavonoid, tannin, and terpenoid *Kruskall-Wallis* test showed significant differences ($p<0.05$) among the groups, indicating that Jamblang leaves ethanolic extract decreased the growth of *S.mutans.* The higher concentrations of the extract, the less number of *S.mutans colonies* grown. No colony of *S. mutans* at 22.5% of extract's concentration. *Syzygium cumini* L ethanolic extract reduces the growth of *S. mutans.* Concentration of 22.5% *Syzygium cumini* L ethanolic extract has bacteriocid effect.

Keywords: Ethanolic extract of *Syzygium cumini* L, growth, *Streptococcus mutans*

Abstract

Streptococcus mutans mempunyai peranan penting sebagai penyebab karies. Bakteri ini memiliki sifat virulensi yang terlibat dalam pembentukan biofilm kariogenik pada permukaan gigi. Daun Jamblang/*Syzygium cumini* L mempunyai efek antibakteri sehingga dapat digunakan sebagai bahan untuk mencegah karies. Penelitian ini bertujuan untuk mengetahui efek ekstrak etanolik *Syzygium cumini* L terhadap pertumbuhan *S. mutans.* Analisa senyawa aktif dilakukan dengan kromatografi lapis tipis (KLT) dan *Liquid Chromatograph-Mass Spectrography* (LC-MS). *Streptococcus mutans* diisolasi dari gigi molar sulung anak yang karies. Uji pertumbuhan dilakukan dengan teknik dilusi. Akuades digunakan sebagai kontrol negatif. Hasil analisis KLT dan LC-MS menunjukkan adanya kandungan flavonoid tannin, dan terpenoid. Uji *Kruskall-Wallis* menunjukkan adanya perbedaan yang signifikan ($p<0,05$) antara semua kelompok yang diuji, mengindikasikan ekstrak etanolik *Syzygium cumini* L menurunkan pertumbuhan *S.mutans.* Semakin tinggi konsentrasi ekstrak, semakin sedikit jumlah koloni *S. mutans* yang tumbuh. Pada konsentrasi 22,5% tidak ditemukan lagi pertumbuhan *S. mutans.* Ekstrak etanolik *Syzygium cumini* L menurunkan pertumbuhan *S. mutans.* Efek bakteriosid didapatkan pada ekstrak konsentrasi 22,5%.

Kata kunci: Ekstrak etanol *Syzygium cumini* L, pertumbuhan, *Streptococcus mutans*

INTRODUCTION

Caries is a bacterial infection disease that causes destruction of the hard tissue of teeth. This disease occurs as the result of interactions between teeth, saliva, cariogenic bacteria, food substrate, and time.[1] Caries is still a major dental and oral problem in children.[2] Children aged 4-6 years (preschool age) are an age group that is in desperate need of attention in dental care. This is because children of that age often

consume sweet food, and their ability to maintain oral hygiene is still lacking.[3] One of the pathogenic bacteria that plays an important role in the pathogenesis of caries is Streptococcus mutans (S. Mutans).[1,2] *Streptococcus mutans* has virulence factors that cause these bacteria to play a role in the occurence of caries. One of the factors is the ability to synthesize and secrete *glucosyltransferase* (gtf) enzymes.[4,5] *Glucosyltransferase* consists of gtfB, gtfC, and gtfD. *Glucosyltransferase* B and gtf C produce glucan bond of α (1-3) from sucrose, which are very sticky and insoluble in water. Glucans play an important role for attachment and biofilm accumulation.[4,6] *Streptococcus mutans* has a *surface protein peptide antigen* (SpaP), namely B antigen (AgB) and I/II antigen (AgI/II) which are the surface proteins of *S. mutans*. This protein functions to mediate *S. Mutans* attachment to the tooth surface.[4.7]

Syzygium cumini L is a wild plant that is widely used for health.[8,9] *Syzygium cumini L* has antimicrobial effects on several bacteria and fungi, including *Staphylococcus aureus, Bacillus subtilis, Salmonella typhi, Pseudomonas aeruginosa, Escherichia colli, Candida albicans dan Candida crusei.*[9,10] *Syzygium cumini L* contains a mixture of polyphenols, especially flavonoid glycosides and tannins. In addition, it also contains terpenoid compounds.[10,11] Plants that contain flavonoids are known to have antibacterial effects.[12] This study aims to determine the effect of *Syzygium cumini L* ethanolic extract on the growth of *S. mutans* that is isolated from dental caries of preschool children (4-6 years), a follow-up study from previous studies using *S. mutans* OMZ 175.[13] *Streptococcus mutans* that is used in this study was isolated from children's dental caries because the OMZ 175 *Streptococcus mutans* that was used in the preliminary study came from humans, but no information was found whether it came from caries or normal lesions, and from children or adults. Therefore, to confirm the test, the *S. mutans* which was isolated from child caries was used.

MATERIAL AND METHOD

The research method used is experimental laboratory. The research subjects were kindergarten students in Prujakan, Ngaglik, Sleman. The subjects selected met the criteria of 4-6 years old, suffering from caries that reached dentine in primary molars. Sampling was done by taking soft dentine on carious lesions, using excavators and growing it on *Trypticase-soy yeast extract 20% sucrose with bacitracin* (TYS20B).[14] Research has obtained permission from the Ethics and Advocacy Unit of the Faculty of Dentistry, Gadjah Mada University (FKG UGM).

Syzygium cumini L was taken from Nggembongan village, Sentolo sub-district, Kulon Progo regency, Yogyakarta. *Syzygium cumini L* which has been cleaned with water, is finely pollinated using a grinder. *Syzygium cumini L* extract was obtained by maceration using 70% ethanol and diluted with 20% DMSO. The active compound content of *Syzygium cumini L* extract was analyzed using TLC at UGM Integrated Research and Testing Laboratory (LPPT). The analysis procedure using LC-MS was conducted at the Chemical Research Center Laboratory, Indonesian Institute of Sciences (LIPI), Serpong, West Java.

Streptococcus mutans that was grown on TYS20B media, was put in a wax lid, and incubated for 2x24 hours at 370C.[14] The morphology of *S. mutans* colonies on TYS20B media gives a round-shape image, 0.5-2μm diameter, and bright white color. In Gram coloring gives Gram positive results with morphology of round cells and cell arrangements forming chains. *S. mutans* colonies were then cultured on blood agar and in liquid BHI with concentration of 10^6CFU/ml. The identification of *S. mutans* was done by looking at the shape of the colony and the description of hemolysis in blood agar, catalase test, *Optochin* test, tolerance to 6.5% NaCl, mannitol, sorbitol, inulin and amylum.[15] Test of the effect of *Syzygium cumini L* ethanolic extract on *S. mutans* growth was carried out by dilution method. As much as one ml of *Syzygium cumini L* ethanolic extract was made in three concentrations, i.e 30%, 40%, and 45%. The extract was diluted using 20% DMSO. Then 1 ml of bacterial suspension was added in BHI (10^6 CFU/ml), so the final concentration of the extract was 15%, 20%, 22.5%. Aquades was used as negative controls. All tubes were incubated at 37^0C for 24 hours. After being diluted in stages using NaCl, then cultured on Muller Hinton Agar (MHA) media. After being incubated for 24 hours at 37^0C, the number of colonies of bacteria that grow were counted.

RESULT

The results of TLC showed the presence of flavornoid active compounds, mainly quercetin (16.6%), tannin (0.19%), and terpenoids. The results of the analysis and identification of compounds in *Syzygium cumini L* ethanolic extract using LC-MS showed the presence of flavonoid compounds, namely *myericetin, 3-0-a-L-rhamnocyl myricetin,* and *taxifolin,* also found terpenoid substance i.e *eugenyl acetate,* and *tricosanoyl lupeol.*

There was a decrease in the number of *S. mutans* colonies after being exposed to *Syzygium cumini L.* etha-nolic extract. The effect of this decrease increased in accordance with the increase in extract concentration. The number of *S. mutans* was found mostly in negative controls (Table 1).

Table 1. Number of *S. mutans* colonies (CFU/ml) in various concentrations of *Syzygium cumini* L ethanolic extract

Group	$\bar{X} \pm SD$
Concentration of extract 22.5%	0.00 ± 0.00
Concentration of extract 20 %	$4866.67 \pm 1.13 \times 10^3$
Concentration of extract 15%	$5.16 \times 10^4 \pm 2.44 \times 10^3$
Negative control	$1.82 \times 10^{12} \pm 9.99 \times 10^{10}$

The results of the normality test using *Kosmogorof-Smirnov* showed that the data tested were normally distributed. The homogeneity test shows the data tested is not homogeneous so that the parametric test of variance analysis (ANOVA) cannot be done. The test used was the *Kruskal-Wallis* non-parametric test.

The results of the *Kruskall-Wallis* test showed a significant difference ($p < 0.05$) in the number of *S. mutans* colonies between groups. These results indicate that exposure to *Syzygium cumini L* ethanolic extract affects the number of *S. mutans* colonies. *Mann-Whitney* test results show a significant difference in the number of *S. mutans* colonies ($p < 0.05$) among all extract concentrations tested (Table 2).

Table 2. Summary of Mann-Whitney test results of *S. mutans* colonies between groups of *Syzygium cumini* L ethanolic extract concentrations

No.	Between groups		Z	Significance
1.	Extract 22.5	Extract 20%	-4.992	0.000*
		Extract 15%	-4.992	0.000*
		Negative control	-4.989	0.000*
2.	20%	Extract 15%	-4.674	0.000*
		Negative control	-4.671	0.000*
3.	15%	Negative control	-4.671	0.000*

Note: * = significant different ($p<0.05$)

DISCUSSION

The results of this study indicate that the ethanolic extract of *Syzygium cumini L* was able to reduce the number of *S. mutans* colonies. The higher the extract concentration, the greater the growth barriers that occur. This is due to the components of antibacterial substances in the extract, namely flavonoids, tannins and terpenoids. Increased extract concentration results in a greater decrease in growth. This is related to the increase in the amount of active compounds from *Syzygium cumini L.*[16,17] Flavonoids in *Syzygium cumini L* extract can interfere with the activity of transpeptidase peptidoglycan which disrupts the formation of cell walls, resulting in cell lysis. Flavonoids also form complex compounds with extracellular proteins, thus disrupting *S. mutans* cell membrane integrity. The disruption of cell membrane integrity, will inhibit protein synthesis or nucleic acid metabolism, inhibit enzyme activity, and inhibit energy metabolism.[18] Inhibition of energy metabolism by flavonoid compounds by inhibiting the accumulation of intracellular polysaccharides (IPS) from S. mutans, so that the function of IPS as a source of endogenous carbohydrates is disrupted. This condition causes inhibition of *S. mutans* growth.[19]

Flavonoids compound of *Syzygium cumini L* namely mirisetin and quercetin inhibit nucleic acid synthesis. The inhibitory effect of nucleic acid synthesis is related to the presence of ring B from flavornoids. Ring B of flavonoids plays a role in the intercalation or hydrogen bonding with nucleic acids.[12] *Syzygium cumini L* ethanolic extract in this study contained quercetin. *Syzygium cumini L* ethanolic extract in this study contained quercetin. Quercetin compounds can inhibit the activity of the enzyme *deoxyribonucleic acid girase* (DNA girase) and *Adenosine triphosphatase* (ATPase). The AT Pase enzyme acts to pump protons (H^+) from the cytoplasm out of the cell, so that the cytoplasmic pH remains in physiological state. If the activity of AT Pase enzyme is inhibited, the cytoplasmic pH becomes acidic, and affects the aciduric nature of *S.*

mutans, as well as *S. mutans* metabolism as a whole. DNA gyrase enzymes function to open the twists of the DNA chain, so as to reduce the temporary tension of double-stranded DNA. This DNA gyrase enzyme is often the target of antibiotics.[4,7] The antibacterial mechanism of tannin compounds is similar to flavonoids, which interfere with membrane lipid and protein interfaces.[20] Tannin compounds can damage bacterial cell membranes, induce the formation of bonding complexes to microbial enzymes or substrates. Tannin compounds can also be toxic to bacteria.[21]

Terpenoids contained in the ethanolic extract of *Syzygium cumini L* can provide antibacterial effects against *S. mutans* through the interaction of lipophilic terpenoid compounds with lipid elements from *S. mutans* cell membrane. This interaction causes disruption of the *S. mutans* structure and makes it more permeable.[22] The terpenoids have membranotropic properties which interfere with the stability of the membrane structure which results in a decrease in membrane functional integrity.[23] The terpenoid compounds in 22.5% *Syzygium cumini L* ethanolic extract in this study are likely to be able to lyse membranes or coagulate cytoplasm from *S. mutans* cells, thus stopping *S. mutans* growth.[22] This condition is in accordance with the research of Akiyama et al (2001) which proved that, antimicrobial materials that are bacteriostatic can be bacteriocid if used in high dosage.[24] Carvone terpenoid compounds in *Syzygium cumini L* extract can interfere with the pH gradient and cell membrane potential, thus disrupting the general metabolism of cells. Terpenoid compounds can be bacteriostatic and bacteriocide, depending on their concentration.[19] In this study, terpenoid compounds in *Syzygium cumini L* extract were bacteriostatic at a concentration of $\leq$ 20%, and were bacteriocid at a concentration of 22.5%. The conclusion of this study is that the *Syzygium cumini* L ethanolic extract has the ability to decrease the growth of *S. mutans*. Bacteriosid effect is found in the extract concentration of 22.5%. Further study is required to isolate the active substance of *Syzygium cumini L* so as to understand more in details the active compound that roles in the mechanism of growth inhibition.

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