

# The Antibacterial Effects of *Syzygium Cumini* Fruit Mouthwash Against *Streptococcus mutans* as an Agent of Dental Caries

Santi Chismirina<sup>1</sup>, Ridha Andayani<sup>1</sup>, Suzanna Sungkar<sup>2</sup>

<sup>1</sup>Department of Biology Oral, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

<sup>2</sup>Departement of Pedodontic, Faculty of Dentistry, Syiah Kuala University, Banda Aceh, Indonesia

\*Corresponding Author: Santi Chismirina, [santi\\_chismirina@unsyiah.ac.id](mailto:santi_chismirina@unsyiah.ac.id)

## Abstract

Caries is a disease of the oral cavity characterized by tooth decay due to the dissolution of minerals on the teeth by *Streptococcus mutans* (*S. mutans*) attached to the teeth surface. One way to prevent the bacteria from decaying the teeth is by gargling with a mouthwash containing antibacterial ingredients from herbs. The results of our previous research on the *Syzygium cumini* fruit showed that it was one of the natural ingredients with antibacterial compounds against *S. mutans*. This study aimed to determine the antibacterial effects of the *Syzygium cumini* fruit which was formulated in the form of mouthwash against *S. mutans*. *Syzygium cumini* fruit was made into mouthwash preparations which were then tested for stability, including for the organoleptic and antibacterial tests. The results of the organoleptic test showed that the *Syzygium cumini* fruit mouthwash with formula 4 was the best choice for the mouthwash formula. Antibacterial test results showed that the *Syzygium cumini* fruit could inhibit the growth of *S. mutans*, characterized by the formation of a halo zone with an average diameter of 8 mm. This diameter indicated that the *Syzygium cumini* fruit mouthwash had moderate inhibition against *S. mutans*.

**Keywords:** *Syzygium cumini*, mouthwash, *Streptococcus mutans*, dental caries

## 1. Introduction

One of the oral dental diseases that most Indonesians suffered from was dental caries. Based on data from Basic Health Research called *Riskesdas* in 2018, the prevalence of this disease was 45.3%. Out of the proportion, 54% of it was experienced by the 5- to 9-year age group. According to the United States Surgeon General's 2000 report, dental caries is the most common chronic disease among children (Dean, 2016). Caries is a microbiological infectious disease and progressive due to the dissolution of the mineral structure of teeth by acids produced by *Streptococcus mutans* (*S. mutans*) as the main caries agent (Banas, 2004; Forssten et al, 2010; Hemagaran & Neelakantan, 2014). Dental caries occurs due to the interaction of several factors, namely agent, substrate, host, and time (Krzyściak et al, 2014; Hegazy & Salama, 2016). Dental caries has a very dangerous impact on the health of the body because the antigen from the bacteria which causes caries, namely *S. mutans*, plays a role in triggering systemic diseases such as endocarditis and nephritis (Nemoto et al, 2008; Matsuri et al, 2010; Oktrianda, 2011).

The process of dental caries can be prevented or slowed down by conducting an intervention on one of the contributing factors. One way is to clean plaque using mouthwash (Featherstone, 2000; Hegazy & Salama, 2016). Mouthwash is a solution that contains antibacterial properties that are useful for maintaining the cleanliness and health of the oral cavity (Raja and Kumari, 2013). Mouthwash is used as a mouth rinse so that the mouth is fresh and the breath does not smell bad (Benjamin et al, 2016; Nofita et al, 2018). Mouthwash is highly recommended for

use after brushing teeth because it can reach the surface area in the oral cavity that is difficult to reach by a toothbrush so that food debris that sticks between the teeth can be removed (Bodiba, 2017; Farias et al, 2019).

Generally, mouthwash contains synthetic antibacterial ingredients such as alcohol, but in its use, there are various unwanted side effects such as dry mouth and even triggering oral cancer. Therefore, currently, there are many studies on the exploration of natural ingredients such as herbal plants that have antibacterial effects as the main bioactive ingredients in mouthwash as a substitute for synthetic materials (McCullough & Farah, 2008; Prasad et al, 2016). The industries which engaged in dentistry products are aggressively formulating mouthwashes from herbal plants, along with the increasing consumer interest in products made from natural ingredients. *Syzygium cumini* plant commonly known as “Jamblang” is one of the herbal plants containing main active compounds of flavonoids with antibacterial effects and rich minerals (Faria et al, 2011; Swami et al, 2012; Jahan & Reddy, 2018).

The results of our previous research indicated that *Syzygium cumini* fruit extract was able to inhibit the growth of *S. mutans* in vitro (Chismirina, et al, 2011). Likewise, the results of some other studies showed that the ethanolic extract of “Jamblang” leaves (*Syzygium cumini*) could reduce the growth of *S. mutans* (Sungkar, et al, 2018). The antibacterial ability and mineral content in the *Syzygium cumini* fruit was the basis for this research, namely to explore the antibacterial potential of “Jamblang” fruit as the main bioactive ingredient of mouthwash against the growth of *S. mutans*, which is the main bacteria causing dental caries.

## 2. Method

The research on the antibacterial effects of *Syzygium cumini* fruit in the form of a mouthwash formulation was carried out by the Dentistry Laboratory of FKG Unsyiah and the Pharmacology Laboratory of FMIPA Unsyiah. The first stage of this research was collecting *Syzygium cumini* fruit from Krueng Raya, Aceh Besar. In this stage, fresh and healthy *Syzygium cumini* fruit was selected. The pulp was then separated from the seeds manually. The weight was 10g, blended with 50 mL of distilled water, and made into juice. After that, its phytochemicals were examined.

The second stage of this research was preparing a mouthwash formula consisting of the *Syzygium cumini* fruit juice, glycin, propylenglycol, sodium saccharin, menthol, and aquadest. There were 4 formulas in total with different levels of concentration for mouthwash preparations. The composition of the *Syzygium cumini* fruit mouthwash formula is shown in Table 1.

**Table 1. Composition of Four *Syzygium cumini* Fruit Mouthwash Formulas**

Ingredient (mL)	Formula			
	F1	F2	F3	F4
<i>Syzygium cumini</i> Fruit Juice	0	2	4	6
Glycerin	10	10	10	10
Propylene-glycol	10	10	10	10
Sodium Saccharin	0.1	0.1	0.1	0.1
Menthol	0.25	0.25	0.25	0.25
Aquadest	100	100	100	100

The next stage was to evaluate the stability of the mouthwash preparations by doing a physical evaluation, namely organoleptic observations on smell, color, taste, turbidity, and the presence of sediment. Organoleptic examinations were carried out by a limited panelist of 5 people.

Panelists assessed the taste, color, and aroma of mouthwash. The final stage was to test the antibacterial activity of mouthwash preparations against *S. mutans* by using the paper disc diffusion method. *Streptococcus mutans* which had been cultured on *S. mutans* specific medium, namely TYS20B, on the spread plate was reacted with a paper disc which had been immersed in *Syzygium cumini* fruit mouthwash and incubated at 37°C for 18-24 hours. The results were then observed. The data obtained from the results of the antibacterial test from mouthwash were analyzed through a multivariate test.

### 3. Results and Discussions

The results of the phytochemical test of the Jamblang fruit juice (*Syzygium cumini*) showed that “Jamblang” fruit juice) contained saponins, flavonoids, phenols, tannins, alkaloids, and steroids as shown in Table 2 below.

**Table 2. Phytochemical Test Results of Jamblang Fruit Juice (*Syzygium cumini*)**

Chemical Content	Test Result	
Saponins	+	
Flavonoids	+	
Phenols	+	
Tannins	+	
Alkaloids	Mayer	+
	Wagner	+
	Dragendorff	-
Steroids	+	

Description: (+) Content  
(-) No Content

The results of the stability evaluation of the *Syzygium cumini* fruit juice mouthwash using the organoleptic test are shown in Table 3. The results of the phytochemical test showed that the juice contained antibacterial compounds, such as saponins, flavonoids, phenols, tannins, alkaloids, and steroids. These compounds are active compounds against bacteria. Organoleptic tests were carried out to assess the visual and characteristics of the *Syzygium cumini* fruit juice mouthwash preparation.

**Table 3. Organoleptic Test Results of the Jamblang Fruit Juice Mouthwash (*Syzygium cumini*)**

Jamblang Fruit Juice Mouthwash preparations ( <i>Syzygium cumini</i> )	Taste	Color	Aroma
<b>Formula 1</b>	Sweet and slightly spicy	Brownish	Very Strong Menthol
<b>Formula 2</b>	Sweet, and slightly “Jamblang” fruit chelating taste	Brownish Purple	Menthol, but not as strong as Formula 1
<b>Formula 3</b>	Sweet, and strong chelating taste of “Jamblang” fruit	Purple with a little dark brownish	Menthol, but not as strong as Formula 1 and 2
<b>Formula 4</b>	Sweet, and very strong chelating taste of “Jamblang”	Faded Purple	Menthol, but not as strong as Formula 1,2 and 3

fruit

From Table 3, it can be seen that the increasing amount of Jamblang fruit juice (*Syzygium cumini*) will affect the taste, color, and aroma of the mouthwash preparation. The strongest taste of *Syzygium cumini* fruit is found in the formula with highest fruit juice content, namely Formula 4 with a concentration of 6 mL of Jamblang fruit juice (*Syzygium cumini*). Formula 4 mouthwash formula also has a clear faded purple color, without sediment, and with menthol aroma that is not as strong as Formula 1, 2, and 3. Formula 4 thus has an attractive color and aroma preferred by panelists.

The results of the antibacterial test of “Jamblang” fruit mouthwash (*Syzygium cumini*) against the growth of *S. mutans* using the disc diffusion method are shown in Table 4.

**Table 4. The Antibacterial Test Results of Jamblang Fruit Juice Mouthwash (*Syzygium cumini*) against *S. mutans***

Mouthwash Formula	Inhibition Zone Diameter (mm)			Average	Davis and Stout Inhibition Ability
	I	II	III		
<b>F1 (Negative Control)</b>	4.7	4.5	4.3	4.5	Weak
<b>F2</b>	7.6	7.5	7.2	7.4	Moderate
<b>F3</b>	8	7.9	7.5	7.8	Moderate
<b>F4</b>	8.1	7.8	8.2	8	Moderate
<b>F5 (Positive Control)</b>	7.6	7.8	7.4	7.6	Moderate

The results of the antibacterial test of *Syzygium cumini* fruit mouthwash showed that the mouthwash could inhibit the growth of *S. mutans* characterized by the formation of a halo zone around the paper disc with an average diameter of 7.7 mm. This diameter indicated that the *Syzygium cumini* fruit mouthwash had a moderate antibacterial ability against *S. mutans* based on the classification of bacterial growth inhibition, according to Davis and Stout (Davis and Stout, 1971). The diameter of the inhibition zone became wider along with the increased concentration of *Syzygium cumini* fruit in the mouthwash formula.

The ability of mouthwash from *Syzygium cumini* fruit juice to inhibit the growth of *S. mutans* is apparently due to its main bioactive content, namely flavonoids, and is supported by other active compounds such as alkaloids, tannins, triterpenoids, monoterpenes, and essential oils. *Syzygium cumini* also contained quercetin, myricetin, and essential oils such as myrtenol, kuarsetin, and camphorol which also have antibacterial effects (Ramya et al, 2013; Haroon et al, 2015; Ghosh et al, 2017; Bijauliya et al, 2017). Flavonoids can damage bacterial cell membranes and cause the release of intracellular compounds by forming complex compounds with extracellular proteins and dissolved proteins. In addition, flavonoids also play a role in inhibiting the synthesis of DNA (Deoxyribonucleic Acid) and RNA (Ribonucleic acid) bacteria and inhibiting energy metabolism by inhibiting the use of oxygen by bacteria (Chismirina et al, 2011; Silva et al, 2017).

Phenolic compounds are also known as active compounds fighting against bacteria. Phenol works by increasing the permeability of the cytoplasmic membrane causing the leakage of intracellular components and coagulation of the cytoplasm, resulting in bacterial cell lysis. Phenolic compounds are antibacterial which are bactericidal. This compound has broad-spectrum

antimicrobial activity against Gram-positive and Gram-negative bacteria so that phenolic compounds can be used as disinfectants. The mechanism of action of tannins as an antibacterial is to inhibit the reverse transcriptase enzyme and DNA topoisomerase so that bacterial cells cannot be formed. Tannins have an antibacterial activity because of their ability to activate microbial cell adhesion, inactivate enzymes, and disrupt protein transport to the inner layer of cells. Tannins also target the cell wall polypeptides so that the cell wall formation becomes imperfect. This results in bacterial cell lysis due to osmotic and physical pressure, causing the bacterial cells to die (Sasidharan et al, 2011; Alizadeh and Imani, 2018).

Alkaloids as antibacterial works by interfering with the peptidoglycan components that make up the bacterial cell wall so that the cell wall layer is not formed properly. As a result, the bacterial cell wall layer breaks and makes the cells die. In addition, alkaloids are also known as DNA intercalators and inhibit bacterial cell topoisomerase enzymes. Steroids work as anti-bacteria by interacting with cell phospholipid membranes which are permeable to lipophilic compounds. This causes the integrity of the membrane to decrease and changes the morphology of the cell membrane, making the cells brittle and lysis (Haroon et al, 2015; Ghosh et al, 2017; Bijauliya et al, 2017).

Several factors influence the antibacterial activity of a substance. The main factors are the properties of the tested bacteria, the concentration of the extract used and the length of time the bacteria contact with the antibacterial agents. *Streptococcus mutans* is a Gram-positive bacterium with a cell wall structure that is simpler than that of Gram-negative bacteria, making it vulnerable and easy to be damaged by antibacterial compounds (Banas, 2004). In addition, the concentration of the extract used also affects the antibacterial activity of the extract. Pelczar and Chan (1988) stated that the higher the extract concentration, the larger the diameter of the inhibition zone formed (Bodiba et al, 2017). This is evidenced by an increase in the diameter of the inhibition zone along with the increasing concentration of the extract in the mouthwash preparation (Table 4).

#### 4. Conclusions

From the results of this study, it can be concluded that the mouthwash of *Syzygium cumini* fruit has the ability to inhibit the growth of *S. mutans*, indicated by the formation of a clear zone around the paper disc. The inhibitory ability of *Syzygium cumini* fruit juice increases along with the increasing amount of *Syzygium cumini* fruit juice used in the mouthwash formula. This indicates that the higher the concentration of *Syzygium cumini* fruit, the stronger the ability of *Syzygium cumini* fruit juice to inhibit the growth of *S. mutans*, as evidenced by the wider formation of the inhibition zone diameter.

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