

Effect of Nutmeg Flesh (*Myristica fragrans* Houtt) against *Streptococcus mutans* growth

Putri Rahmi NOVIYANDRI¹, NURHADISAH¹, Santi CHISMIRINA¹

¹ Department of Oral Biology, Faculty of Dentistry, of Syiah Kuala, Banda Aceh, Aceh, Indonesia

* Corresponding Author. E-mail: putri@unsyiah.ac.id

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ABSTRACT *Streptococcus mutans* is an oral commensal. Its bacteria are involved in the dental caries pathogenesis. Nutmeg flesh (*Myristica fragrans* Houtt) is one of the native plants of Indonesia. Nutmeg flesh (*Myristica fragrans* Houtt) contains antibacterial properties such as alkaloids, flavonoids, tannins, terpenoids, saponins, and essential oil. The purpose of this study is to determine the effect of nutmeg flesh (*Myristica fragrans* Houtt) extract in the inhibition of *Streptococcus mutans* growth. The extract of nutmeg flesh (*Myristica fragrans* Houtt) was made using the maceration method with ethanol 96% as the solvent. The Diffusion methods to identify the inhibition of *Streptococcus mutans* growth. The concentration of nutmeg flesh (*Myristica fragrans* Houtt) extract used in this study were 25%, 50%, 75%, and 100. The one-way ANOVA analysis showed that there was the effect of nutmeg flesh (*Myristica fragrans* Houtt) extract on *Streptococcus mutans* growth with value $p < 0.05$, and then data was tested by Least Significant Difference (LSD). This study's conclusion showed an effect of nutmeg flesh (*Myristica fragrans* Houtt) extract in inhibiting *Streptococcus mutans* growth. The concentration of nutmeg flesh (*Myristica fragrans* Houtt) extract, which has the greatest inhibition zone on the growth of *Streptococcus mutans*, is at a concentration of 100% is 33,53 mm.

KEYWORDS: Nutmeg flesh (*Myristica fragrans* Houtt), *Streptococcus mutans*

INTRODUCTION

Oral health is one of the main problems that concern everyone to achieve the best therapy and treatment. One of oral health is dental health.¹ Based on the latest data research by the Oral Health Media Center in 2012, it shows that 60-90% of school-age children and almost all adults in the world have dental issues.² One of the most common dental problems is caries.³ According to the World Health Organization (WHO), in 2012, the highest prevalence of caries was in Asia and Latin America. In Indonesia, the prevalence of caries reaches 60-80% of the population and currently ranks the sixth most common disease.

Caries is an infectious disease that results in progressive loss of dental minerals, resulting in enamel, dentin, cementum, and pulp.⁴ There is a strong relationship between caries and dental plaque.⁵ Plaque is a group of bacteria that can cause tooth decay.⁶ Dominant bacteria as The trigger for caries is *Streptococcus mutans* (*S. mutans*). These bacteria ferment sugars and produce acids that can accelerate the maturation of plaque and cause the tooth surface's pH to become acidic (pH below 5.5). Acid pH conditions are known to cause the surface

of the teeth to dissolve and form white spots that tend to become carious.⁷

Streptococcus mutans is a Gram-positive bacteria, non-motile (immobile), and facultative anaerobes.⁷ In the absence of treatment or allowed, *Streptococcus mutans* in dental plaque will continue to increase and make plaque deposits thicker. Caries can be prevented by reducing the number of plaque deposits on the tooth surface by mechanical cleanings, such as brushing teeth using antibacterial agents to suppress the growth of *S. mutans*. The addition of active ingredients containing natural or synthetic ingredients applies to antibacterial ingredients. At present, the back to nature trend is making people more interested in using natural ingredients for health because it has minimal side effects, is cheaper, and easier to obtain.^{5, 8, 9}

Indonesia is widely recognized as the country with the most extensive biodiversity in the world after Brazil. In Indonesia, there are about 30,000 types of plants and 7000, of which are believed to have medicinal properties. Approximately 2500 species identify as medicinal plants.¹⁰ One of the plants known to Indonesia as traditional medicine is

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nutmeg (*Myristica fragrans* Houtt). This plant has many benefits and benefits for health. Nutmeg consists of flesh, seeds (nuts), mace, which can process into nutmeg oil (oleoresin) and extracts (volatile).¹¹

Nutmeg (*Myristica fragrans* Houtt) is a native Indonesian plant originating from the Banda Islands and Maluku, which spread to Aceh, North Sulawesi, and Papua.¹⁰ The flesh of the nutmeg contains aromatic substances consisting of essential oils. It is namely myristicin and monoterpenes, which are natural ingredients used to reduce bacterial infections.¹² The results of phytochemical tests

conducted by Atmaja (2017) showed that the ethanol extract of the nutmeg flesh contained alkaloids, saponins, tannins, flavonoids terpenoids, which are known to have antibacterial effects.¹¹ Research conducted by Nurhasanah (2014) states that the nutmeg flesh extract (*Myristica fragrans* Houtt) can inhibit Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative such as *Escherichia coli*.¹² Based on the above, researchers are interested in researching the effect of nutmeg pulp extract (*Myristica fragrans* Houtt) against the growth of *S. mutans*.

MATERIALS AND METHODS

This research was conducted in October 2018 in the Faculty of Veterinary, Syiah Kuala University, Banda Aceh. This research is an experimental laboratory design with a post-test only control group. Nutmeg Extract (*Myristica fragrans* Houtt) was prepared by using the maceration method.^{11,13} Phytochemical test was conducted to determine chemical content in nutmeg flesh (*Myristica fragrans* Houtt). In this study, a phytochemical test was conducted to determine alkaloids, flavonoids, tannins, terpenoids, and saponins from the nutmeg flesh extract (*Myristica fragrans* Houtt). The pure extract (100%) that has been obtained is diluted with distilled water to get a concentration of 100%, 75%, 50%, and 25%, then homogenized using a vortex. The dilution formula adopted by Tampedje et al.¹⁴

Streptococcus mutans was cultured in Trypticase Soy with Sucrose and Bacitracin (TYS20B) with the T streak technique. The Petri dishes inoculated with the bacteria are then placed in an anaerobic jar and incubated at 37°C for 1 x 24 hours in an incubator.¹⁵ The *S. mutans* confirmation test was carried out by using Gram stain, which demonstrates a purple color for Gram-positive bacteria.¹⁶ Using ose, *Streptococcus mutans* put into a

tube containing 5ml of 0.85% NaCl solution, then homogenized with a vortex. The turbidity of the solution was equalized using Mc densitometer. Farland 0.5 (1.5×10^8 CFU/ml).¹⁵ Analyzing of inhibition of nutmeg flesh (*Myristica fragrans* Houtt) extract against *S. mutans* was carried out using the well diffusion method using Mueller Hinton Agar (MHA) media.¹⁷

Then three Petri dish MHAs were made into six wells with a diameter of 6mm and a thickness of 4 cm. Then *S. mutans* was inoculated using a hole punch device. Then filled the well with 50 µl of 100%, 75%, 50%, and 25% nutmeg pulp extract, then incubated at 37°C for 24 hours in an incubator.¹⁷ Positive control used chlorhexidine 0.2% and for negative control using distilled water. The formation of the zone of inhibition around the well is measured using a caliper in millimeters using the overall diameter minus the well diameter.^{12,18} The results were assessed based on Davis and Stout classification.¹⁹ Data analysis was performed using SPSS software. Data analysis used One Way Analysis of Variance (ANOVA) with the significant difference is $p < 0.05$.

RESULTS

Phytochemical test results showed alkaloids, flavonoids, tannins, terpenoids, and saponins in the nutmeg pulp extract (*Myristica fragrans* Houtt). The culture results of *Streptococcus mutans* on Trypticase Soy with Sucrose and Bacitracin (TYS20B) media with the T streak technique and incubated for 1 x 24 hour at 37°C showed a yellowish-white colony of *S. mutans* with a convex surface. The results of Gram stain show that purple Gram-positive bacteria are cocci and chains form. The average inhibition zone of nutmeg flesh (*Myristica fragrans* Houtt) extract with a concentration of 25% was 18.23 mm, which is

the minimum inhibition zone in this study, indicating a robust inhibitory ability of bacterial growth. Nutmeg flesh extract with a concentration of 50% had an inhibition zone of 25.43 mm and was classified as very strong. The Nutmeg flesh extract with a concentration of 75% and 100% had an inhibition zone of 31.2mm and 33.53mm, which was classified as robust inhibition of bacterial growth. The distilled water showing no clear area, while the positive control using 0.2% CHX had 19.23mm inhibition power, had a robust inhibitory ability of bacterial growth. The one-way ANOVA test showed

a value of 0.000 ($p < 0.05$), which proved the effect of nutmeg flesh of (*Myristica fragrans* Houtt) extract on the growth of *S. mutans*. The Least Significant Difference (LSD) also confirmed a significant

difference ($p < 0.05$) between all treatment groups, except for the nutmeg flesh (*Myristica fragrans* Houtt) extract with a concentration of 25 % and positive control

DISCUSSION

Nutmeg (*Myristica fragrans* Houtt) is an original Indonesian plant and has a high economic value in the market. The economic value of this plant is found in the fruit, especially in the seeds and mace. Utilization of nutmeg flesh (*Myristica fragrans* Houtt) is still limited, even though it has health properties, one of which is an antibacterial agent.^{12, 21}

The nutmeg flesh (*Myristica fragrans* Houtt) is dried before extraction. This method is known to maintain secondary metabolite compounds that require low temperatures, such as flavonoids, which are easily oxidized and sensitive to heat.²² The nutmeg flesh (*Myristica fragrans* Houtt) was extracted by maceration method. This method was chosen because it is cheap, simple, and easy to do, and it can avoid the destruction of thermolabile compounds. In this study, the nutmeg flesh (*Myristica fragrans* Houtt) maceration's process sample was soaked using 96% ethanol as a solvent. Ethanol is used because it is non-toxic and more comfortable to penetrate plant cell membranes so that it dissolves secondary metabolites and produces perfect secondary metabolites.²³⁻²⁵

Streptococcus mutans is a Gram-positive, non-motile, and facultative anaerobic bacteria. *Streptococcus mutans* bacteria can form colonies and adhere to the surface of teeth, synthesize sucrose from carbohydrates, and produce acids that can make the oral cavity acidic (pH below 5.5). The pH condition, which continues to be sour, cause the tooth surface to dissolve and form white spots that become carious.⁶ The *S. mutans* culture results show the presence of yellowish-white colonies with a convex surface. After the culture process, the bacteria colony was confirmed with Gram stain, and the results showed purple Gram-Positive bacteria with the character of a cocci shape and chains. The resulting purple color comes from the crystal violet substance at the time of the Gram stain. This color is retained by *S. mutans* bacteria, which are Gram-positive bacteria that are known to have a thick peptidoglycan layer in which there are teichoic and lipoteichoic compounds that can retain the dye.^{12, 26}

ANOVA analysis showed that the flesh of nutmeg (*Myristica fragrans* Houtt) extract had a significant effect on the growth of *S. mutans*. The LSD test result confirmed a significant difference between all analyzed groups except 25%

concentration and positive control. The impact of nutmeg flesh (*Myristica fragrans* Houtt) extract at various concentrations, namely: 50%, 75%, and 100% had a better ability to inhibit *S. mutans* than CHX 0.2%. This effect is due to the higher content of antibacterial compounds in the nutmeg flesh (*Myristica fragrans* Houtt). Both concentration factor and type of antibacterial agent are determined to inhibit the growth of *S. mutans*. There was no significant difference between the 25% concentration and 0.2% CHX because although the concentrations were different, the 25% concentration of nutmeg flesh extract (*Myristica fragrans* Houtt) had almost the same potential as CHX 0.2% in inhibiting *S. mutans*.²⁷ Although in this study, the compound activity was not tested quantitatively, it was found that the extract of the nutmeg flesh (*Myristica fragrans* Houtt) had a good effect on the inhibitor of *S. mutans* growth.

Based on the result, the classification of inhibitory power according to Davis and Stout shows that the extract of the nutmeg flesh (*Myristica fragrans* Houtt) can inhibit the growth of *S. mutans*. The inhibition zone diameter of the extract concentration was 25%, had an average inhibition zone diameter of 18.23mm, included in the strong inhibition category. The concentrations of 50%, 75%, and 100% have an average inhibition diameter of 25.43mm, 31.2 mm, and 33.53 mm, which are categorized as robust inhibition. The 25% concentration of nutmeg pulp extract is the minimum inhibition zone with an average diameter of 18.23mm, while the 100% concentration is the maximum concentration with an average diameter of 33.53mm. Based on these categories, it can see that the nutmeg flesh extract (*Myristica fragrans* Houtt) already has an excellent ability to inhibit the growth of *S. mutans* in the strong to powerful category.¹⁵

The results showed that an increase in the percentage of extract concentration caused an increase in the bacterial inhibition zone diameter. This was due to the higher the concentration, the more active substances contained therein to affect the formed inhibition zone. This study's results are in line with the research obtained by Atmaja (2017) in responding to *S. aureus* bacteria by using extraction sources from flesh, mace, and nutmeg. The extraction source with a concentration of 25-100% nutmeg flesh (*Myristica fragrans* Houtt) has an

average diameter of 7.5-10.37mm.¹¹ Research by Atmaja (2017) conducted using the disc diffusion technique is different from this study, which uses the well diffusion technique. As is known, disc diffusion testing is by dropping blank disc paper with the extract to be tested. In contrast, the well diffusion method employing the extract being tested is inserted into each well to diffuse into the agar medium resulting in the osmolarity of the overall extract concentration expected with this extract concentration. Complete inhibition of bacterial growth.²⁸

Nurhasanah (2014) reported that giving nutmeg flesh (*Myristica fragrans* Houtt) extract treatment various concentrations inhibits Gram-positive *S. aureus* growth Gram-negative bacteria *E. Coli*. In this study, the extract of nutmeg flesh (*Myristica fragrans* Houtt) contains several antibacterial compounds such as alkaloids, flavonoids, tannins, terpenoids saponins. The results prove antibacterial compounds. These antibacterial compounds are known to have effective methods in inhibiting bacterial growth.^{11, 12}

Alkaloids have antibacterial properties by disrupting peptidoglycan in bacterial cells causing cell death because the walls are not formed intact. Flavonoids can damage cell membranes and denature proteins and nucleic acids, thereby

disrupting bacterial metabolism. Saponins work as antibacterial by disrupting the stability of the bacterial cell membrane resulting in bacterial lysis. Tannins eliminate bacteria by shrinking the cell wall or cell membrane to interfere with the bacteria's permeability. Terpenoids inhibit bacterial growth by reacting with porin on bacterial cell membranes and forming strong polymer bonds, consequently disrupting wall formation and bacterial cell permeability. The flesh nutmeg (*Myristica fragrans* Houtt) also contains aromatic substances consisting of essential oils such as myristicin and monoterpenes, which are known to have potential as antibacterial agents by disrupting the process of stabilizing cell wall or membrane formation.^{29, 30}

The chlorhexidine and distilled water were on the right track as positive and negative controls. Chlorhexidine is one of the antibacterial ingredients, the chemical formula of 1,6 bis-p-chlorophenylhigualidohexane, a type of cation and derived from desquaniid derivatives. The inhibition mechanism is to precipitate cytoplasmic acid proteins to disrupt the ' permeability walls. Permeability uses the cell membrane to leak from various directions.^{9,31} On the other hand, the use of distilled water has no potential to inhibit antibacterial activity.

CONCLUSION

The extract of nutmeg flesh (*Myristica fragrans* Houtt) affected the growth of *S. mutans* bacteria. The concentration of nutmeg flesh extract, which had the

largest inhibition zone against the growth of *Streptococcus mutans* was at a concentration of 100% with a diameter of 33.53 mm.

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