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RESEARCH ARTICLE

Effect of Nutmeg (*Myristica Fragrans*) Methanolic Extract to the Growth of Dental Plaque Bacteria

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ABSTRACT

Background: *One of the causes of dental caries is Streptococcus mutant bacteria which has the ability to change the sugar content of food waste into lactic acid as the initial process of caries. One of the efforts to overcome dental caries is to use natural ingredients such as nutmeg. Every part of nutmeg has active substances that are efficacious as antimicrobial, antibacterial, antioxidant, antifungal and anti-inflammatory. Pulpam, seeds and fuli of nutmeg showed as potential extracts in inhibiting the growth of Streptococcus mutans.* ***Objective:*** *This study aimed to analyze methanol extracts from pericarpium, pulpam, fuli and nutmeg seeds against the growth of Streptococcus mutans bacteria that causes dental plaque.* ***Methods:*** *The research was conducted by diffusion method using BHI agar media and incubated anaerobically at 37°C for 24 hours.* ***Results:*** *The results of the calculation of the average diameter of the inhibition zones from the extract of fuli, seeds, pulpam and pericarpium of the nutmeg plant were 19.00 mm, 25.33 mm, 15.66 mm, 22.66 mm, and 21.83 mm, respectively. Data analysis using ANOVA (one way) showed that there were significant differences in all groups at p<0.05. The results of the LSD test showed that there were significant differences in all treatment groups.* ***Conclusion:*** *Methanol extract from fruit and fruit parts of nutmeg showed the ability to inhibit the growth of bacteria that cause dental plaque.*

Keywords: *Dental Plaque, Myristica Fragrans, Bacteria, Streptococcus mutans, Dental Caries*

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INTRODUCTION

Dental and oral health problems, especially dental caries, are a disease that affects almost half of the world's population. The results of the Basic Health Research (Riskesdas) in 2018 stated that the prevalence of caries in Indonesia was 88.8%.[1] In realizing a caries-free Indonesia by 2030, Ministry of Health in preparing strategic plans and action plans for dental and oral health efforts, one of them is the implementation of dental and oral health efforts.[2]

Many efforts have been made to support dental health services, especially to prevent and overcome dental caries problems, one of them is research on natural ingredients to be used as medicines. The use of natural ingredients as medicine causes very few adverse side effects compared to synthetic drugs.[3,4] One of the plants that has the potential to overcome disease and health problems is spices.[5] Several studies have shown that spices have antimicrobial properties. The main component that gives spices antimicrobial properties is the essential oil. Essential oils are also known as volatile oils or etheric oils and are sometimes referred to as spice oils.[6]

Nutmeg (*Myristica fragrans* Houtt) is one of Indonesia's original spices that has economic value and multipurpose because every part of this plant can be used in various industries such as the food industry, medicine and cosmetics.[7] Nutmeg is one of the important export commodities for Indonesia, because Indonesia is the largest exporter of nutmeg seeds and *fuli* (mace), which is around 60% of the world's nutmeg needs.[8] Fuli is a seed coat or a thin, bright red membrane that covers the skin of the nutmeg which is often referred to as mace or many also call it mace. Nutmeg production in West Sumatra from 2008 to 2013 has a tendency to increase. Nutmeg production in West Sumatra in 2013 was around 1,332 tons and the land used was 3,683 Ha.[9]

Nutmeg consists of four parts namely *pericarpium*, *pulpam, fuli*, and seeds. All parts of the nutmeg can be used for various purposes, among which the best known in the market are fuli and seeds as spices, while nutmeg oil is usually used for medicine.[10] Nutmeg contains common compounds such as carbohydrates, proteins, structural fats, and minerals (potassium, magnesium and phosphorus), especially essential oils which have high economic value.[11] In addition, every part of the nutmeg has an active substance as an antimicrobial substance[12] antibacterial, antioxidant, antifungal, and anti-inflammatory.[13,14,15]

Nutmeg contains anti-inflammatory properties, is rich in monoterpenes such as terpineol, pinene and sabinene. All of these substances are anti-inflammatory compounds that can prevent inflammation, Besides having antioxidant properties, Nutmeg shows some anti-bacterial effects, especially against some harmful bacteria such as *Streptococcus mutans* which is one of the bacteria that causes dental caries.[16] Nutmeg seeds and fruit contain saponins as antibacterial, essential oils are oils produced by one of the metabolic processes that contain trimyristin and myristicin as antioxidants, anti-inflammatory and antibacterial.[17] Nutmeg seeds produce a special etheric oil and fat.

Other supporting data is a study conducted by Shafiei Z et al (2012) on the antibacterial activity of nutmeg against oral cavity pathogens. The results showed that the active compound trimyristin contained in nutmeg functions as an antibacterial against gram-positive bacteria such as Streptococcus mutans, Streptococcus sobrinus and lactobacillus. These bacteria are thought to be bacteria that can cause dental caries. Meanwhile, gram-negative bacteria such as *aggregatibacteractino-mycetemcomitans*, *Porphyromonas gingivalis*, are closely associated with periodontitis.[18] Other research on invitro antimicrobial activity of phytochemical extract of *Myristica fragrans* on *Streptococcus mutans* stated that the ethanol extract of the pulp, seeds and fuli of nutmeg showed a strong potential against the pathogen *Streptococcus mutans.*[19]

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Preliminary test results from the author show that gargling with nutmeg powder causes a fresh taste in the mouth. Based on the description of the background above, the author is interested to analyze extracts from *pericarpium*, *pulpam*, fuli, and nutmeg seeds against the growth of *Streptococcus mutans* bacteria that causes dental plaque.

MATERIALS AND METHODS

Materials and apparatus

The materials used in this study are Fruit and fruit parts of nutmeg (*pericarpium, pulpam, fuli*, and seeds) obtained from the Bukittinggi area. West Sumatra, *Streptococcus mutans* culture *(*collection from the Bacteriology Laboratory of the Faculty of Medicine of Andalas University*), Brain Heart Infusion Broth* (BHI) media, *70%* ethanol, methanol, distilled water, McFarland 0.5. The tools used include Petri dishes, test tubes, centrifuges, shakers, autoclaves and incubators

Preparation of nutmeg extract

Fruit and fruit parts of nutmeg (pericarpium, pulpam, fuli, and seeds) were washed and dried in the oven at 40oC for 48 hours, then powdered by using electrical blender and ready to extract by maceration methods. 25 grams of Each of powder the fruit and parts of the nutmeg (pericarpium, pulp, mace, and seeds) dissolved with 150 ml methanol solvent in a erlenmeyer then macerated for 72 hours and shaking periodically. The precipitated residue was separated from the solvent by filtration and concentrated by using vacuum evaporator at 50°C to produce a crude extract. The crude extract extracted again twice with 150 ml solvent to obtain a clear colour residue..

Evaluation of antimicrobial activity

S. mutans strains were grown in brain heart infusion (BHI) broth (Difco, Detroit, MI, USA) or on BHI agar plates overnight at 37 ◦C in 95% air and 5% *CO2 (*v/v*)*. Disk diffusion method was used to measure S. mutans sensitivity to the experimental group consisting of Fruit and fruit parts of nutmeg (pericarpium, pulpam, fuli, and seeds). Thirty mL of freshly prepared and autoclaved brain heart infusion (BHI) agar was poured into sterile glass petri dishes. The media were cooled to room temperature, and stored in a refrigerator until use. Plates were examined for sterility before use by incubating at 35 °C for 48 h. Three to five well isolated colonies of the same morphological type were selected from a blood agar plate culture and transferred with a sterile loop into a tube containing 5 mL of BHI broth that was then incubated at 37 °C for 24 h. The turbidity of the broth culture was adjusted to 0.5 McFarland standards. Transferred immediately 50 µL of the broth to the middle of a dry BHI agar and spread uniformly over the entire agar surface using a sterile L spreader. Filter paper discs of 6 mm diameter were prepared from Whatman filter paper No. 1, placed in a petri dish and sterilized in a hot air oven at 160 °C for 2 h. Thereafter, discs were impregnated with 20 µL of each of the experimental groups (pericarpium, pulpam, fuli, and seeds), 3 disks for each group, and placed immediately over the plates. Sterile distilled water were used as negative control. The plates were incubated for 24 h at 37 °C. After incubation, the plates were observed for uniform culture growth (granular, frosted glass appearance) and formation of inhibition zones around the discs that were measured in millimeters. The mean of 3 measurements of the diameter of each inhibition zone for each disk was calculated. The test was repeated twice for accuracy

DATA ANALYSIS

Data was analyzed using SPSS 15.0 for windows (SPSS Inc, Chicago, IL, USA, 2001). One-sample Shapiro Wilk test was used to assess the normality of data distribution. One-Way ANOVA was used to compare the antibacterial effect of Fruit and fruit parts of nutmeg (pericarpium, pulpam, fuli, and seeds). Least Significant Difference (LSD) test was

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performed to determine the difference in significance between each group.

RESULT

The results of the study were the calculation of the mean diameter and standard deviation of the inhibition zone of the extract from each part of the sample on the growth of *Streptococcus mutans.*

Table 1. Average results of inhibition zone of extract

Extract group	N	Average inhibition zone ± SD
Fuli	3	19 ±2
Seed	3	25,33 ±2,08
Pulpam	3	15,66 ±1,52
Pericarpium	3	22,66 ± 1,52
Fructus	3	21,83 ± 0.28
Negative control (DMSO)	3	0
Total	**18**	**104.5 ± 7.42**

Based on the research data (Table 1), it was obtained data that there was a clear zone (inhibition) of the nutmeg extract and the part of the nutmeg on the growth of *Streptococcus mutans* bacteria. while the negative control (Sterile distilled water) did not give a clear area. The smallest inhibitory power was produced by the pulp extract of 15.66 mm and the largest inhibition was produced by the nutmeg seed extract of 25.33 mm. From Figure 1 below, it can be seen that the seeds have a higher inhibitory diameter than the fruit and other parts.

The ANOVA test results show that there are significant differences in each test group at $p<0.05$, Based on the results in table 1, it can be concluded that the extract of the fruit and parts of the fruit can inhibit the growth of *Streptococcus mutans* bacteria and needs to be continued with the LSD test.

Table 2. LSD test results

Extract group	**Mean**
Fuli	19[a]
Seed	25,33 [b]
Pulpam	15,66 [c]
Pericarpium	22,66 [d]
Fructus	21,83 [d]

The LSD test results in table 2 above show that there is a significant difference between Fuli on seeds, pulp, pericarpium, and fruit. Likewise, Seeds have a significant difference with other parts. Meanwhile, there is no significant difference between the pericarpium and the fructus.

DISCUSSION

Nutmeg and parts of the nutmeg which include pericarpium, pulpam, fuli, and seeds according to preliminary test, contain active compounds of essential oils and other common chemical compounds found in fruit such as carbohydrates, proteins, structural fats, and minerals. fuli and seeds of nutmeg have the ability to inhibit the growth of Gram Negative and Positive bacteria. Meanwhile, according to previous research, nutmeg seeds contain phenols, terpenoids, flavonoids and alkaloids that have antibacterial activity.[20] In addition, the essential oils and saponins found in the pulpam of nutmeg are also antibacterial substances. Antibacterial substances in fuli of nutmeg include flavonoids, phenols, saponins and tannins which are also able to inhibit bacterial growth. The results of other studies also provide information that the pulpam of nutmeg also contains phenolic compounds and antioxidants that have potential as antimicrobials.[21] Each active substance contained in nutmeg and other fruit parts has a specific mechanism of action in carrying out its function as an antibacterial. Flavonoids will form complex compounds with extracellular proteins to damage bacterial cell membranesFlavonoids also play a role in inhibiting energy metabolism. Flavonoid compounds will interfere with energy metabolism, where sufficient energy is needed for the absorption of various metabolites and for macromolecular biosynthesis.[22] Phenolic compounds and antioxidants are able to inactivate the development of oxidation reactions by preventing the formation of free radicals that attack macromolecular components such as proteins, lipid membranes and DNA that

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cause diseases such as cancer, diabetes mellitus and inflammation.[23]

The results of the study as shown in table 1 showed the inhibitory ability of extracts of Fruit and fruit parts of nutmeg (*pericarpium, pulpam, mace,* and *seeds*) against Streptococcus mutans bacteria. This is evidenced by the formation of a clear zone around the filter paper disc Fruit and fruit parts of nutmeg extract (*pericarpium, pulpam, mace,* and *seeds*). The inhibition zones formed were generated from each Petri dishes that have different diameter sizes and non-uniform shapes. Therefore, measurements were made by measuring the vertical diameter and horizontal diameter of the zone inhibition formed around the filter paperdisc. Table 1 shows that the petri dish containing the seed extract has the largest inhibition zone diameter of 25.33 mm and the petri dish containing the pulp extract has the smallest inhibition zone of 15.66 mm. The description of the calculation results is inserted into the diagram to see the size of the comparison between the intervention group and the control group.

Nutmeg seed extract produced the greatest inhibition compared to other parts of the fruit, even compared to the whole fruit. This is due to the influence of the content of the extracted active compound and the concentration of the test material. The concentration of the test substance can affect the molecular weight to be greater so that the viscosity is thicker. Research by Katalinic *et al.* 2006 showed that the essential oil contained in nutmeg seeds has strong antioxidant properties. The high antioxidant activity occurs due to the synergism between the components of the essential oil.[24] Nutmeg seeds contain active compounds such as 5-Octadecanoic acid, myristicin, phenol, terpineol, and 9-octadecenoic in large enough quantities.[25] Extracts from nutmeg seeds also have antioxidant activity from the alkaloid group and vitamin C.[26]

Strptococcus mutans is a bacterium that has the ability to resist almost all anti-septic drugs. These bacteria have the ability of intrinsic resistance and acquired resistance. Intrinsic resistance is a characteristic present in almost or all strains of the species in which the gene for intrinsic resistance is carried on the chromosome. Meanwhile, acquired resistance is resistance that is obtained due to DNA mutations or the formation of new DNA through plasmid transfer and transposons. The resistant genes in these bacteria are stored in plasmids so they can be transferred at any time. With this resistance *Streptococcus mutans* bacteria can be resistant to many substances, it is assumed that *Streptococcus mutans* bacteria also have the possibility of being resistant to nutmeg extract because of the same mechanism of action based on phenol content. This study showed that extracts of Fruit and fruit parts of nutmeg (*pericarpium, pulpam, mace,* and *seeds*) had inhibitory power against *Streptococcus mutans* bacteria, because there were compounds that functioned as antimicrobials against *Streptococcus mutans* bacteria so that breadfruit leaves. In the future research, this plant extract can be developed and processed into medicinal preparations or mixed ingredients in toothpaste and mouthwash to treat dental caries and other oral infections.[26,27,28]

CONCLUSION

In conclusion, our results indicated that fruit extract and fruit parts of nutmeg has an inhibitory effect on the growth of Streptococcus mutans as bacteria that cause dental plaque compared to negative control

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