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FORMULATION AND TEST OF ANTIBACTERIAL ACTIVITY OF PURIFICATION EXTRACT OF MUTMETE SEED (*Myristica fragrans*) AGAINST STREPTOCOCCUS MUTANS

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Abstract

Streptococcus mutans naturally occurs in the human oral cavity, is a microorganism that plays an important role in the initiation of dental caries. One of the efforts to prevent dental caries is the use of natural ingredients that contain antibacterial substances that can interfere with the growth or kill bacteria. Nutmeg is a natural ingredient that has antibacterial properties. Bioactive compounds in nutmeg seeds have antibacterial properties. This research aimed to determine the formulation and concentration of nutmeg seed extract which has antibacterial activity against Streptococcus mutans. The type of research used is a laboratory experiment with a post test only group design. The treatment group used purified extract of nutmeg seeds with concentrations of 25%, 20%, 15%, 10%, 7.5%, 5.0%, 2.5%, 1.25%, the positive control group used mouthwash I, T and L. The test was carried out three repetitions at each concentration, then the inhibition zone was measured using the well diffusion method. Antibacterial activity testing was carried out using the well method, the data were analyzed using the Shapiro-Wilk test, the Levene test, the one way annova test and the PST hock test. Obtained the average inhibition of nutmeg seeds against Streptococcus mutans at a concentration of 25% (18.33 mm). 20% concentration (17.16 mm), 15% concentration (16.16 mm), 10% concentration (14.66 mm), 7.5% concentration (13.33 mm), 5% concentration (6.66 mm), the concentration of 2.5% (2.6 mm) and the smallest concentration of 1.25% had no inhibition (0 mm), while in the control group I (4 mm), T (3 mm) and L (2 mm). The higher the concentration of purified extract of nutmeg seeds, the higher the antibacterial activity against Streptococcus mutans. further research on the concentration of nutmeg seed extract preparations was carried out in vivo tests on experimental animals in order to obtain purified extract preparations of nutmeg seeds that are safe and can be an alternative choice in maintaining oral health.

Keywords: Antibacterial, Nutmeg, Streptococcus mutans

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Introduction

Streptococcus mutans is naturally present in the human oral cavity, is one of the microorganisms that causes dental caries and will get worse if not treated immediately (Warganegara and Restina, 2016). Caries is a demineralization process caused by interactions between microorganisms, teeth, saliva and substrate. Streptococcus mutans, one of the many etiological factors of dental caries, which is a microorganism capable of acquiring new properties for expression as a determinant of pathogenicity determines virulence under certain environmental conditions. Streptococcus mutans has the ability to survive in an acidic environment and special interactions with other microorganisms (Soesilowati, 2020). These microorganisms are cariogenic bacteria because they are able to convert glucose and carbohydrates in food into acids through a fermentation process and these microorganisms produce lactic, formic, acetic and propionic acids, which are products of carbohydrate metabolism (Struzycka, 2014).

Under normal circumstances the teeth are always moistened with saliva. Saliva has a role to maintain the preservation of teeth (bechal.S.J, 2002). As soon as the tooth surface is cleaned the pellicle which is a thin layer of salivary protein adheres to the tooth surface. The pellicle consists of modified glycoproteins, within a few hours microorganisms are found in the pellicle of the teeth to form plaque. The substrate, in this case carbohydrates, is a food source for plaque microorganisms. Carbohydrates will be fermented by Streptococcus mutans and form acids. While at the same time the ammonia produced by the enzyme urease in saliva can help host defense in neutralizing the acids formed in dental plaque. In addition, saliva also functions as a natural cleanser in the mouth against cariogenic substrates, but if the acidic atmosphere in the mouth is left continuously without cleaning for a long time, coupled with a small flow of saliva, it will cause demineralization (Schuurs, 1988). Streptococcus mutans is a gram-negative bacterium. positive bacteria which are more sensitive to antibacterial compounds than gram-negative bacteria (Nugroho, 2016).

Given the high number of dental and oral health problems, especially dental caries, which is experienced by almost half of the world's population. The results of the 2018 Basic Health Research (Riskesdas) state that the prevalence of caries in Indonesia is 88.8%. In order to realize Caries Free Indonesia 2030, the Ministry of Health is preparing strategic plans and action plans for dental and oral health efforts, one of which is the implementation of dental and oral health efforts (Ministry of Health, 2019)

Efforts to support dental health services, especially to prevent and overcome the problem of dental caries, have been carried out a lot, one of which is research on natural ingredients to be used as medicines. The use of natural ingredients as medicine rarely causes adverse side effects compared to drugs made from synthetic materials (Purnamasari, 2010).

The use of extracts from plants that have antibacterial properties has been widely studied to inhibit the growth of Streptococcus bacteria, one of which is the use of nutmeg (Danica, 2022). Nutmeg consists of skin, meat, mace and nutmeg seeds. Every part of nutmeg has an inhibitory power against the growth of the

Streptococcus mutans bacteria, and the greatest inhibition is in nutmeg (Dewi, 2022). Nutmeg has several parts where each part of the nutmeg has an active substance as an antimicrobial (Syarifah et al, 2018). For more specific purposes, nutmeg seeds can be made as natural antibacterial agents because they contain unique compounds that act as antibacterials (Zuzuana and Weny, 2016). The results of the phytochemical tests conducted by Thomas and Krishnakumari (2015), that nutmeg seed extract (Myristica fragrans) confirmed the presence of secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, steroids/tripenoids and tannins.

Based on the description above, researchers are interested in conducting research on the formulation and antibacterial test of purified extract of nutmeg (Myristica fragrans) against Streptococcus mutans.

Research Method

The research was conducted using laboratory experimental methods. The research phase included collecting and processing samples, making nutmeg extract, diluting the nutmeg extract, testing the antibacterial activity of nutmeg extract against Streptococcus mutans. The observed parameter is the minimum inhibitory concentration of Streptococcus mutans in mm.

The tools used in this study include: Petri dish, syringe, vortex, ose, bunsen, test tube, rack, autoclave, inoculum tube, sterile cotton swab, micropipette, tip, incubator, hot plate, erlenmeyer, boor prop and calipers shove. The materials used in this study included: extract samples, blood agar media, physiological NaCl, sterile distilled water and Mc. Farlands 0.5.

Media preparation begins with weighing the powder for each type of media to be used, such as blood agar media, then the powder is put into an Erlenmeyer, dissolved with distilled water and heated using a hot plate stirrer. Then sterilized using an autoclave at 121oC for 15 minutes at a pressure of 1 atmosphere. Then the blood agar media is added with 5-8% sheep blood and homogenized and then poured into a petri dish that already has a borer prop and allowed to freeze at room temperature.

Tools and materials

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Media Creation

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allowed to freeze at room temperature. Especially for blood media, 5-8% sheep blood is added and homogenized.

Streptococcus mutans working procedure

I. Bacterial suspension
The preparation of the bacterial suspension begins by inserting physiological NaCl into the inoculum tube and inserting the Streptococcus mutans bacteria colony into the tube. After that it was homogenized using a vortex and the bacterial suspension was equalized to 0.5 Mc-Farland turbidity.

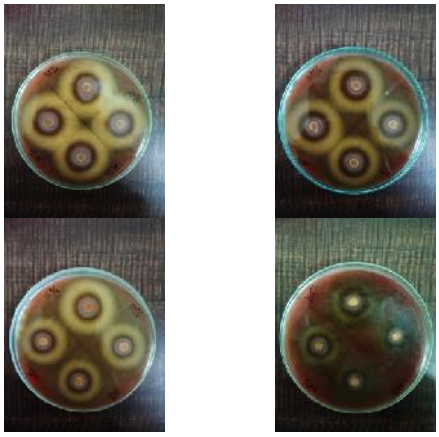
II. Extract dilution
The concentration of the extract that was diluted was 25%, 20%, 15%, 10%, 7.5%, 5.0%, 2.5%, 1.25%. Pipette the sample (35%) then add sterile distilled water, pipet and add distilled water according to the dilution calculation, then vortex until homogeneous.

III. Antibacterial test by pitting method
The test was carried out by dipping a sterile cotton swab in the bacterial suspension and then rubbing it on the surface of the Blood agar medium in a petri dish until smooth, letting it dry for 3-5 minutes. Drop the sample in the wells that have been formed by the drill prop, in 1 petri dish there are 4 wells and the sample tested consists of 8 concentrations namely: 25%, 20%, 15%, 10%, 7.5%, 5.0%, 2.5% and 1.25%. Then incubated at 37°C for 24 hours.

IV. Measurement of Inhibition Zone Diameter
Measurement of the inhibition zone using the well diffusion method, namely measuring the inhibition zone around the well vertically and horizontally using calipers in millimeters (mm).

Results and Discussion

The results of the inhibition zone diameter test can be seen in Figure 1 below



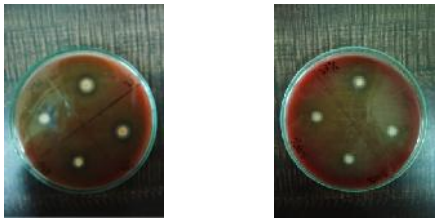


Figure 2. Results of Observation of Preparations

Based on Figure 1 above, the inhibitory power of the purified extract of nutmeg (Myristica fragrams) can be seen in table 1 below.

Table 1
Test Results of Repeated Extract of Purified Nutmeg Seeds (Myristica fragrams) on the Growth of Streptococcus mutans

Test	**1,25%**	**2,5%**	**5%**	**7,5%**	**10%**	**15%**	**20%**	**25%**	K T	K L	K I
P 1	0	3	8	13	14	16	17	18	3	2	4
P II	0	3	6	13	15	16	17,5	18	3	2	4
P III	0	2	6	14	15	16,5	17	19	3	2	4

Notes
PI = Repetition 1
PII = Repetition 2
PIII = Repetition 3
KT = Tantum Verde Control
KL = Listerin control
KI = 2% Iodide Control

The inhibition data that has been measured are interpreted based on the criteria set by Davis and Stout (1971) which state that the strength of the antibacterial activity is 20 mm or more which is very strong, 10-20 mm means strong, 5-10 mm means medium and 5 mm or less means weak. Based on table 1, the average inhibition for three repetitions at each solution concentration can be seen in table 2 below:

Table 2
Average Diameter of Inhibition Zone of Purified Nutmeg Seed Extract (Myristica fragrams) on the Growth of Streptococcus mutans

Extract Concentration	Average	Resistance Category
1,25%	0 mm	Low
2,5%	2,67 mm	Low
5%	6,67 mm	Medium
7,5%	13,33 mm	Strong
10%	14,67 mm	Strong
15%	16,17 mm	Strong
20%	17,17 mm	Strong
25%	18,33 mm	Strong
Kontrol T	3,00 mm	Low
Kontrol L	2,00 mm	Low
Kontrol I	4,00 mm	Low

From the data in table 2 it can be seen that the increase in the inhibition zone on the preparation is in line with the increase in the concentration of the extract it contains. The higher the concentration of the extract in the preparation, the higher the content of antibacterial substances in it so that it inhibits bacterial growth more optimally.

The results of the antibacterial activity test of purified extract of nutmeg seeds (Myristica Fragrams) on the growth of Streptococcus mutans were carried out three times at each concentration of 25%, 20%, 15%, 10%, 7.5%, 5%, 2.5% and 1.25% indicates that the purified extract of nutmeg seeds (Myristica Fragrams) can inhibit the growth of Streptococcus mutans with strong inhibition ranging from 7.5% to 25%, and the greatest inhibition is at the highest concentration of 25% with an average inhibition of 18 .33mm. Moderate inhibition at a concentration of 5% with an average of 6.67mm. The inhibition was weak at concentrations of 2.5% and 1.25% and the control group had the smallest inhibition at a concentration of 2.5% with an average of 2.67 mm, and no inhibition was found for nutmeg seeds at a concentration of 1.25% (0 mm). The study was also conducted on three control groups using commercial mouthwash with 2% Iodine an average of 4 mm, Listerine with an average of 2 mm and Tantum verde with an average of 3 mm.

Antibacterial activity as seen from the formation of inhibition zones or clear zones inhibiting the growth of Streptococcus mutans due to secondary metabolites contained in the ethanol extract of nutmeg seeds (Myristica fragrans Houtt). Streptococcus mutans is a Gram positive bacteria that is more sensitive to antibacterial compounds than Gram negative bacteria. Antibacterial compounds can prevent peptidoglycan synthesis in bacterial cells (Nugroho, 2016).

The inhibition zone formed due to the presence of active substances in nutmeg seeds, namely flavonoids, tannins, saponins. Flavonoids are one of the chemical compounds that are bacteriostatic which can damage the cytoplasmic membrane which can cause leakage of important metabolites and inactivate bacterial enzyme systems. This damage allows nucleotides and amino acids to seep out, this

situation can cause bacterial death (Isromarlina, 2020). Tannins are also phenolic compounds that work by inhibiting bacterial growth by reducing surface tension, so that bacterial permeability increases, damage and increased permeability of bacterial cells causes inhibited cell growth and ultimately causes cell death (Dewi, 2022). The mechanism of action of saponins as an antibacterial by denaturing proteins, because the surface active substances of saponins are similar to detergents, saponins can be used as antibacterials where the surface tension of the bacterial cell wall is reduced and the permeability of the bacterial membrane is damaged. The survival of bacteria will be disrupted due to damage to the cell membrane. Then the saponins will diffuse through the cytoplasmic membrane so that the stability of the membrane will be disrupted causing the cytoplasm to leak and leave the cell resulting in cell death (Suranto, 2002). Referring to Jawetzs, et al. (1992) in Suranto, et al. (2002) the combined activity of several antibacterial compounds can be more effective than the effectiveness of each compound. But it is also possible, antibacterial compounds which have the greatest percentage can affect the effectiveness of their work. On the other hand, the combined activity of several antibacterial compounds is also less effective than the effectiveness of each compound. -each compound.

Research on the inhibition test of nutmeg seed extract (Myristicae fragrans) on the growth of Staphylococcus aureus and Streptococcus pyogenes found that nutmeg extract has the potential to have an inhibitory effect on the growth of Streptoccoccus aureus and Streptoccoccus pyogenes (Paisia, 2016). According to Wei Kevin Zhang (2016) in the Journal of Food and Nutrition Research and Valtccho D Jeliazkov in Chemistry of Spice from Russia, nutmeg contains excellent antibacterial and anti-inflammatory substances in the oral cavity.

Other research states that the ethanol extract of pre-extracted nutmeg seeds can be formulated into mouthwash preparations, which meet the requirements for stability (color, smell, and taste, turbidity and sediment), pH, and cycling test. Pre-extracted nutmeg ethanol extract mouthwash preparations have antibacterial activity. against Streptococcus mutans where the effective concentration is 50 mg/ml (5%). The ethanol extract mouthwash of nutmeg seeds has antibacterial activity against Streptococcus mutans

The results of statistical analysis tests were used to compare the concentrations of nutmeg seed extract with various concentrations using the ANOVA test. F count is 343.76, while F table with df (0.05) is 2.49 with a p-value of 1.033 or 0.000103. Because F count > F table (3,43,76) > (2,49) with a value or p value (0,0000103) < alpha (0,05), it can be concluded that there is a difference in the average inhibition of nutmeg against bacteria Streptococcus mutans.

Conclusion

Based on the research that has been done, it can be concluded that the purified extract of nutmeg seeds (Myristica fragrams) has an inhibitory power against Streptococcus Mutans bacteria, the higher the concentration of nutmeg seed extract, the stronger the inhibition of the extract against Streptococcus Mutans bacteria.

Recomendation

It is recommended that further researchers conduct other research on the inhibition of nutmeg against other bacteria in the mouth

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