Inhibition Power Test of White Rice Bran Extract (*Oryza Sativa L.*) With the Solution of Ethanol and Aquades on *Streptococcus Mutans* (In Vitro) Bacteria

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Abstract

White rice bran (*Oryza sativa L.*) is a byproduct of the rice milling process and is only used as livestock material. Rice bran contains a number of phenolic compounds, the largest group of phenolic compounds are flavonoids. *Streptococcus mutans* (*S. mutans*) is one of the gram-positive bacteria that cause caries pathogens that cause corrosion in tooth enamel. The growth of Streptococcus mutans must be inhibited so as not to become pathogens and cause caries by giving antibacterial ingredients.

The general purpose of this study was to determine the inhibition of white rice bran extract (Oryza sativa L.) by using Ethanol and Aquades solvents against Streptococcus mutans bacteria.

The type of research used in this research is laboratory experimental research. The design of this study is posttest only control group design using the Kirby Bauer agar / diffusion method. In this study 5 repetitions were carried out with treatment concentrations of 12.5%, 25%, 50%, 75% and positive control (chlorhekaisidin). The measuring instrument in this study uses a caliper with millimeters (mm).

Kruskall Wallis test results showed a significant difference in the administration of white rice bran extract on the diameter of the inhibitory zone of Streptococcus mutans bacteria formed at a concentration of 12.5%, 25%, 50%, 75% and positive control with mean on each ethanol solvent each was 11.03 mm, 12.40 mm, 13.43 mm, 15.15 mm and 16.45 mm, and in the solvents of distilled water were respectively each 13.63 mm, 14.63 mm, 15.23 mm, 15,50 mm and 16, 25 mm. Data test results obtained p = 0,000 and 0,001 (p <0,05). This shows that white rice bran extract is classified as weak medium which can inhibit the growth of Streptococcus mutans bacteria.

White rice bran extract (Oryza sativa L.) has inhibitory effect on Streptococcus mutans bacteria both using ethanol and distilled water.

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Introduction

Oral and dental health is an integral part of public health. Data by*Word Health Organization* (WHO) in 2008 states that 78% of the population in the world has dental and oral health problems, especially in developing countries. This disorder often occurs in all types of age and 76.5% is experienced by groups of school-age children (Sinatawi, 2009). Data from the 2010 Basic Health Research (Riskesdas) in Indonesia showed complaints of dental pain disrupting 13% of the population per month or the equivalent of 2.62,000 residents per month.[1] Data from the Indonesian Ministry of Health in 2013 showed a national prevalence of dental and oral problems in Indonesia, 9%, with 14 provinces having a prevalence of dental and oral problems above the national rate.[2]

Caries is one of the diseases in the oral cavity whose prevalence in Indonesia is still high. Caries is a bacterial infectious disease in dental hard tissues such as email, dentine and cementum. The sign of the formation of caries is the formation of tooth demineralization, for the formation of dental caries in addition to the activation of bacteria that interact with each other there must be other trigger factors that increase

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bacterial activation, namely the presence of hots, substrates, and time. These four factors have different levels of influence between one individual and another depending on the tissue structure of the tooth, the type of bacteria that is more dominant in the oral cavity, the type of food consumed by sweet and sticky ingredients can produce acid, this acid is the result of bacterial metabolism of streptococcus mutans and interactions between bacteria and the remaining food ingredients consumed differently.[3,4,5]

The high incidence of dental caries shows that it is necessary to prevent caries as early as possible, especially in children so that they will be able to know how to maintain teeth and mouth. The best way to deal with caries growth is to reduce the accumulation of good plaque mechanically which is often done in the form of brushing teeth twice and the use of dental flossing where hygiene level depends on how it is used, besides being carried out chemically using mouthwash that is antibacterial antiseptic that helps improve oral hygiene. Mouthwash circulating in the market contains many active ingredients to increase the results, the active ingredient that is often used is alcohol which is antiseptic. However, it would be better to use mouthwash with natural ingredients which are antiseptic and antibacterial to minimize side effects[6,7,8]

Rice (*Oryza sativa L.)* is a crop that the results can be obtained in almost all parts of the world, this material is a staple food consumed by most of the world's population, especially in Asia. The use of white rice bran as a natural ingredient as an anti-bacterial must be supported by various studies so that the content of chemical compounds, the level of security, and their effectiveness can be known further. To improve the quality, safety, and benefits of white rice bran as an anti-bacterial natural ingredient, standardization of the raw materials needs to be done. One of the factors that influence the good quality of plant extracts is the concentration of solvents used (Gaedcke et al., 2003). The solvents that can be used to make extracts are ethanol and water (POM Agency, 2004). But the ratio of solvents and water to extracts has not been optimized.

Based on this, the researcher was interested in conducting inhibitory tests using white rice bran extract using ethanol and water seamen to compare the effectiveness of streptococcus mutans bacteria.[10]

Materials and methods

The type of research used in this study is laboratory experimental research. The design of this study is a post test only control group design using agar diffusion method / Kirby Bauer using disk paper that has contained flavonoid and polyphenol extracts from white rice bran and then placed into culture media. The Kirby Bauer method is more often done in observing the diameter of the inhibitory zone of a particular extract and producing a good batch to-batch, resulting in satisfactory growth of the most pathogenic bacterial properties. The treatment was carried out 5 times with a concentration of 12.5%, 25%, 50%, 75%. The positive controls used were chlorhexidine and negative control, namely sterile aquades. The research tools used were petri dish, round ose, autoclave, bunsen, erlenmeyer flask, suction pipette, filter tool, rotary evaporator, filter paper, sterile cotton swab, stationery.

The materials used in this study were white rice bran (Oryza sativa L), culture of streptococcus mutans bacteria, sterile aquades, 96% ethanol, paper disk, medium transport, aluminum foil, cotton, Muller hinton agar (mha), brain heart infusion (bhi), and a 0.2% chlorhexidine solution. The sample in the form of white rice bran (Oryza Sativa L.) was filtered to separate it from the husks that might be included. After that, maceration was carried out for 72 hours using 96% ethanol. Then filtered with Bucher Funnel using filter paper. After that it was inserted into the tube and evaporated at a temperature less than 50ºC until thick. For samples extracted using sterile distilled water, samples of white rice bran were oven using a temperature of 120^{o}, after which the sample was blended with a ratio of 2: 1 (2 samples and 1 aquades). After the homogeneous sample will be filtered using filter paper and wait until the liquid extract was separated.

Streptococcus mutans bacteria was extracted using ose needle, then planted in the agar media tilted by scraping. Bacteria that have been scratched on the media were incubated in an incubator at 37ºC for 1 x 24 hours. The incubated bacteria were taken from the media to be tilted using sterile ose needles and then inserted into the BHI-B media until the turbidity was the same as McFarland's standard. Sterile cotton sticks dipped in bacterial suspension until

wet. Cotton wool was squeezed by pressing on the inner tube of the test tube, then scratched evenly on the MHA media until the surface was closed.

The data obtained from this study were collected based on observations on the results of measurements of bacterial growth inhibition zones after the administration of white rice bran extract (Oryza sativa L.) using the calipers to know the inhibition diameter, the data were analyzed using the Kruskal Wallis test and Mann Whitney test with 6 times repetition.

Results

The inhibitory zone of each concentration formed has an increase in diameter in inhibiting the growth of Streptococcus mutans bacteria measured in mm (millimeters).

Table 1 and 2 show that the area of inhibition zones of each concentration formed has an increase in diameter in inhibiting the growth of Streptococcus mutans bacteria measured in mm (millimeters). At a concentration of 12.5%, 25%, 50% and 75%, 6 repetitions were carried out which obtained an average yield of aquades solvents of 13.6 mm, 14.6 mm, 15.2 mm, and 15.5 mm. These results have differences from the average results on positive controls that are equal to 16.2 mm, while the ethanol solvent is 11 mm, 12.4 mm, 13.4 mm, 15.1 mm and the results of the difference from the average results on positive controls are equal to 16 , 4. If the two solvents are compared, the aquades solvent is more effectively used as an antibacterial agent of streptococcus mutas.

No.	Inhibitory Zone Diameter of Ethanol Solvent Bran Extract in Various Concentrations				Positive Control (mm)	Negative Control (mm)
	12,5 % (mm)	25 % (mm)	50 % (mm)	75 % (mm)		
1	11.2	11.8	12.4	12.8	14.2	6.4
2	12.4	13.2	14.2	15.8	16.9	6.2
3	11	12	13.6	16.2	17.2	6,2
4	10.8	12.6	13.8	15.6	17.4	6.2
5	10.4	12.8	13.4	15.5	15.8	6.2
6	10.4	12	13.2	15	17.2	6,2
Average	11	12.4	13.4	15.1	16.4	6.2

Table 1. Diameter of inhibitory zone of White rice bran extract (*Oryza sativa L*) with Ethanol (A) solvent against Streptococcus mutans bacteria.

No.	Diameter of Inhibitory Zone ofAquades Solvent Bran Extract in Various Concentrations				Positive Control (mm)	Negative Control (mm)
	12,5 % (mm)	25 % (mm)	50 % (mm)	75 % (mm)		
1	11.8	12.2	13	13.8	15.4	6,4
2	13.6	15.6	16	16	16.9	6,4
3	14.2	15	15.4	15.6	17.6	6,2
4	16	16.8	16.8	16.4	16	6.2
5	13.8	14	14.2	15	15	6.2
6	12.4	14.2	16	16.2	16.6	6.2
Average	13.6	14.6	15.2	15.5	16.2	6.2

*Diameter of paper disk is 6,2 mm

Table 2. Diameter of Inhibitory Zone of White Rice Bran Extract (*Oryza sativa L*) with Aquades (B) solvent against Streptococcus mutans bacteria.

From the results of the Kruskall Wallis test, the significance results were 0,000 and 0,001 considering the results of <0,05 which stated that there was a significant difference in the test of inhibition on each concentration.

Then the results were processed further using the Mann Whitney Test to see which groups of concentration was the most significant. Because the crucifixion wallis test produces a value of p (0,000 and 0,001) <0.05 then proceed to the Mann Whitney test to find out the comparison between groups with each other The results of the Mann Whitney Test are shown in the following table.

Ethanol Solvent		Mean Difference (I-J)	P Value
12.5%	25.0%	-1.37	0.016
	50.0%	-2.40	0.005
	75.0%	-4.12	0.004
	Positive Control	-5.42*	0.004*
	Positive Control	4.80	0.003
25.0%	50.0%	-1.03	0.020
	75.0%	-2.75	0.008
	Positive Control	-4.05	0.004
	Negative Control	6.17*	0.003*
50.0%	75.0%	-1.72	0.037
	Positive Control	-3.02	0.005
	Negative Control	7.20*	0.003*
75.0%	Positive Control	-1.30	0.065
	Negative Control	8.92*	0.003*
Positive Control	Negative Control	10.22	0.003

*** Mann Whitney Test**

Note :< 0,05 : there is a difference
>0,05 : no difference
* : there is a difference

Table 3. Comparison of diameters at concentrations of 12.5% 25%, 50%, and 75% ethanol extract of white rice bran.

Based on Table 3 shows that a significant group comparison is the ratio between the concentrations of 12.5% with positive controls, 25% with negative controls, 50% with negative, 75% with negative controls and positive controls with negative controls. The highest difference between the concentration groups is 12.5% with positive controls of 5.42.

Aquades Solvent		Mean Difference (I-J)	P Value
12.5%	25.0%	-1.00	0.229
	50.0%	-1.60	0.090
	75.0%	-1.87	0.037
	Positive Control	-2.62	0.013
	Negative Control	7.37*	0.003*
25.0%	50.0%	-0.60	0.421
	75.0%	-0.87	0.335
	Positive Control	-1.62	0.065
	Negative Control	8.37*	0.003*
50.0%	75.0%	-0.27	0.747
	Positive Control	-1.02	0.226
	Negative Control	8.97*	0.003*
75.0%	Positive Control	-0.75	0.261
	Negative Control	9.23*	0.003*
Positive Control	Negative Control	9.98	0.003

*** Mann Whitney Test**
Note :< 0,05 : There is a difference
>0,05 : No difference
* : There is a difference

Table 4. Comparison of diameters at concentrations of 12.5% 25%, 50%, and 75% extracts of white rice bran aquades.

Based on Table 4 shows that a significant comparison of groups is the ratio between concentrations of 12.5% with negative controls, 25% with negative controls, 50% with negative controls, 75% with negative controls and positive controls with negatives. The highest difference between the concentration groups is 12.5% with negative controls of 7.73.

Discussion

This research is an experimental laboratory that aims to know the inhibition of white rice bran extract (Oryza sativa L.) with ethanol and aquades seamen against Streptococcus mutans bacteria. According to Davis and Stout, the criteria for antibacterial strength are as follows: inhibition zone diameter of 5 mm or less is categorized as weak, inhibition zone of 5-10 mm is categorized as medium, inhibition zone of 10-20 mm is categorized strong and inhibition zone of 20 mm or more is categorized as strong.[10]

The factors that influence the formation of inhibition zones include temperature and environmental pH, the concentration of antibacterial compounds, the number of bacteria, the speed of diffusion of antibacterial compounds on agar media and the type of bacteria.

The results of the research have shown that there are inhibitory zones formed at concentrations of 12.5%, 25%, 50%, and 75% in white rice bran extract (*Oryza sativa L.*).

In this study, an anntibacteria of white rice bran extract (Oryza sativa L.) was tested on the growth of Streptoccus mutans showing different results for each treatment given. This shows that positive control and 4 series extract concentrations indicate the presence of different antibacterial activities in the two types of seafarers used.

The activity test of white rice bran extract (Oryza sativa L.) in inhibiting Streptococcusmutans bacteria showed positive results. The results are said to be positive if a inhibitory zone is formed around the paper disk, and vice versa is said to be negative if no inhibition zone diameter is formed around the paper disk. Measuring the inhibition zone was carried out after the media was incubated for 1x24 hours at 37^0C and measured using a caliper in mm.

Table 1 and 2 show that the area of inhibition zones of each concentration formed has an increase in diameter in inhibiting the growth of Streptococcus mutans bacteria measured in mm (millimeters). At concentrations of 12.5%, 25%, 50%, and 75%, 6 repetitions were carried out, the average yield of aquades solvents were 13.6 mm, 14.6 mm, 15.2 mm and 15.5 mm. These results have differences from the average results on positive controls that are equal to 16.2 mm, while the ethanol solvent were 11 mm, 12.4 mm, 13.4 mm, 15.1 mm and the results of the difference from the average results on positive controls was equal to 16 , 4. If the two solvents are compared, the aquades solvent is

more effectively used as an antibacterial agent for streptococcus mutans.

The results of research conducted by Achmad Riwandi, et al (2014) by using various concentrations namely 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and 50%. Effective concentration that can inhibit the growth of Streptococcus mutans are 45% and 50%, which is 15-18 mm.

The formation of inhibition zones around paper discs proves that the extract of white rice bran (Oryza sativa L.) is antibacterial against Streptococcus mutans bacteria. From these statements, (Tables 1 and 2) it can be concluded that the inhibition of white rice bran extract (Oryza Sativa L.) with ethanol solvent at a concentration of 12.5% is categorized as weak because it only forms a zone of inhibition of 4.83, for concentration of 25 %, 50% and 75% categorized as medium, while for aquades solvents 12.5%, 25%, 50%, and 75% categorized as medium. This is in line with the research conducted by Agustie, et al. (2013), the higher the concentration of the extract of the test solution, the greater the diameter of the inhibition zone formed because the greater the concentration of the test solution used, the more active ingredient will inhibit bacterial growth. the use of this type of solvent affects the composition of bioactives contained in bran. Because the solubility of a substance into a solvent is largely determined by the compatibility of the properties between solutes and solvents, namely like dissolves like (Sari et al., 2005).[10]

Based on observations obtained from table 3 the comparison between the groups of white rice bran extract (Oryza sativa L.) concentration and using aquades solvent was more effective than ethanol solvents because aquades had polar properties which dissolved tannins and flavonoids as antibacterial streptococcus mutans which is a type of gram (+) bacteria that has a better antibacterial sensitivity, this is caused by the structure of gram-positive microbial cell walls is relatively simpler, thus antimicrobial compounds are easy to enter into cells and find targets for working activities (Amalia, Wahdaningsih and Untari, 2104).[9] Besides aquades are polar because of differences in charge, high dielectric constants and small sizes, especially for polar ionic and salt compounds. Solvents can dissolve compounds that have the same type of polarity or similar to the type of solvent polarity used, aquades has the same type of polar compound as the type of compound in white rice bran which has polar compounds. Polar compounds can dissolve phenolic compounds better thus the total levels of phenols and flavonoids contained in them can be dissolved to the maximum. This is because phenolic compounds and flavonoids are substances that have aromatic rings with one or more hydroxyl groups, singga is easy to dissolve in polar solvents.[12,14]

This is in line with the research conducted by Abdulmoneim, M and I.E. Zaid, 2011). The results showed that the extracted material using alcohol and distilled water solvents had a inhibitory effect on bacteria. Aquades solvent has superior results in suppressing bacterial growth, especially in gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis), followed by alcohol (methanol). This happens due to more use of water dilution which is used to increase hydrolysis of the active principle to work well against the target organism. In other words, osmotic pressure from solutes and selective permeability. Antimicrobial activity in extracts which have lipophilic compounds that bind internally or internally by cytoplasmic flaring (Body and Beneridge, 1979; 1981). The active ingredients in natural ingredients (bran) are insoluble because they cannot dissolve with alcohol.[13]

From the table, it shows that the concentrations of the four white rice bran extracts (Oeyza Sativa L.) have different inhibitory power to the growth of significant Streptococcus mutans bacteria and the antibacterial effect increases at the concentration of the test solution respectively from 12.5%, 25%, 50 % and 75% which indicate a strong positive relationship between the inhibition zones produced and the use of concentrations both in ethanol and aquades solvents.

Conclusions

Based on the research that has been done, it can be concluded that, extracts of white rice bran (Oryza sativa L.) with ethanol and aquades solvents have inhibition on the growth of Streptococcus mutans bacteria. The inhibition of white rice bran extract on the growth of Streptococcus mutans bacteria at a concentration of 12.5%, 25%, 50%, and 75%

have a slow to moderate inhibition response in ethanol solvents with an average inhibition zone diameter of 11.03 mm, 12.40 mm, 13.43 mm, and 15.15 mm and in aquades solvent with an average diameter of inhibition zones of 13.63 mm, 14.63 mm, 15.23 mm and 15.50 mm.

Suggestions

As for the researcher' suggestions, it is necessary to do further research on the antibacterial effect of white rice bran (Oryza sativa L.) on different bacteria and more varied concentrations and further research to obtain minimum concentrations in inhibiting Streptococcus mutans bacteria.

Declaration of Interest

There is no conflict of interest in this study. This study obtained a label of ethics escaped by the number: 0048 /PL09/KEPKFKG - RSGMUNHAS/2018 and register number UH 17120050 on Oktober 9, 2018.

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