Inhibitory Power Test

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Inhibitory Power Test of White Rice Bran Extract (*Oryza sativa L.*) with the Solution of Ethanol and Aquades on *Porphyromonas gingivalis* (In Vitro) Bacteria

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ABSTRACT

Background: Bran is byproduct of the rice milling process and rice waste. Out of 32 million tons of rice obtained by rice bran as a byproduct of around 2,5 million tons. White rice bran (*Oryza sativa L.*) has many bioactive ingredients, one of which is a phenol compound. Phenol compound are known to have antibacterial activity. *Porphyromonas gingivalis* (*p.gingivalis*) is one of Gram negative anaerobic that contribute to the occurrence of periodontal disease, so that materials that have antibacterial properties are very necessary to be given to inhibit the growth of these bacteria.

Objectives: Knowing the inhibitory effect of white rice bran extract (*Oryza sativa L.*) with ethanol and aquadest solvents to *P.gingivalis* bacteria at concentrations of 12,5%, 25%, 50%, 75% and to determine the difference in the effectiveness of white rice bran extract (*Oryza sativa L.*) with ethanol and aquadest solvents to *P.gingivalis bacteria.*

Methods: This research was laboratory experiment with post test only control group design with diffusion method Kirby Bauer. In this research with 6 repetitions with extract at concentrations of 12,5%, 25%, 50%, 75%, positive control (Chlorhexidine), and negative control (aquadest). The measuring instrument in this research was using caliper with millimeters (mm). Analysis data was using Krusskal Wallis continued by Mann Whitney test.

Results: The results showed that white rice bran extract at concentrations of 12,5%, 25%, 50%, 75% with ethanol and aquadest solvents are significantly influence (Krusskal Wallis test showed p value = 0,000 (p<0,005) to inhibit the growth of *Porphyromonas gingivalis.*

Conclusions: white rice bran extract at concentrations of 12,5%, 25%, 50%, 75% with ethanol and aquadest solvents can inhibit the growth of *Porphyromonas gingivalis* bacteria and inhibit zone that ethanol result is more then aquadest solvent. The inhibition zone produced by white rice bran extract with ethanol solvent is almost the same as the chlorhexidine inhibition zone.

Keywords: White rice bran extract *(Oryza sativa L),* Antibacterial, Ethanol, Aquadest, *Porphyromonas gingivalis*

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INTRODUCTION

Oral health is important for general health and quality of life. Oral health means free throat cancer, infections and sores in the mouth, gum disease, tooth decay, and other diseases, resulting in disorders that limit one's to bite, chew, smile, talk, and psychosocial well-being.[1,2,3,4] One of the most common dental and oral diseases in the community is periodontal disease. WHO (World Health Organization) also reports that dental and oral diseases such as dental caries, periodontal disease, early tooth loss, oral cancer associated with HIV/ AIDS, trauma to teeth is a global burden in various countries. Periodontal disease that is often encountered is inflammation of the gums or gingivitis and plaque bacteria are the main etiological factors of periodontal disease.[3,5,6]

Porphromonas gingivalis is a Gram negative anaerobic type involved in the pathogenesis of periodontitis and other inflammatory diseases that destroy tooth supporting tissues.[7,8] Porphyromonas gingivalis is an anaerobic gram-negative bacterium which is a normal flora in the human oral cavity found in the area of gingival sulcus, subgingival plaque, tongue and tonsils.[9]

The use of natural ingredients for dental and oral health has been widely used because it has a therapeutic effect namely controlling plaque, gingivitis, halitosis and preventing tooth decay.[10] The use of natural ingredients as antibacterial must be higher, given the side effects caused by the use of chemicals on dental and oral health, such as the appearance of brown on the teeth, ulceration, pain and parasthesia.[11] In knowing the effectiveness of an antibacterial extract it is necessary to note the type of solvent used. The type of solvent used during extraction can affect the inhibitory power or antibacterial activity of the extract.[8]

In this study, we will see the comparison of the effectiveness of white rice bran extract macerated using ethanol 96% with macerated bran extract using aquades solvent. By using two types of solvents in making bran extract, a comparison of the effectiveness between the two solvents that will be used to make extracts to test the inhibition of the growth of Porphyromonas gingivalis bacteria will be obtained.

RESEARCH METHOD

The type of research used is experimental laboratory.

a. The cause variable in this study is white rice bran extract obtained by filtering the smoothest part of the remaining rice mill (bran), then maceration with ethanol 96% solvent and sterile aquades solvent. The extract is diluted according to the required concentration.

b. Porphyromonas gingivalis bacteria that are stored in agar media, then measured inhibition zones after being given treatment given from the independent variable and control with paper disk.

Tools and materials :

Tools: petri dishes, Erlenmeyer flasks, bunsen burner, glass jars, suction pipettes, round oases, incubators, autoclaves, vial bottles, rotary evaporators, ovens, rack and test tubes, blenders, filter devices, reaction cups.

Ingredients: white rice bran (Oryza sativa L.), culture of Porphyromonas gingivalis bacteria, transport medium, Muller Hinton Agar (MHA), filter paper, sterile cotton swab, cotton, 0.2% chlorhexidine solution, 96% ethanol, paper disk, sterile aquades, spiritus, aluminum foil, masks, gloves.

The inhibitory test was carried out by using empty Disc Diffusion Method placed on the petri which has bacteria, then the test substance was given to the disc. Petri dishes were divided into 6 quadrants, namely each quadrant containing disc paper containing white rice bran extract (Oryza sativa L.) 12.5%, 25%, 50%, 75% and chlorhexidine solution as K (+) and aquades positive control sterile as a negative K (-) control in each quadrant carried out on 12 petri dishes. Petri dishes were incubated at 37°C for 24 hours. After 24 hours, the number of bacteria that grew with measuring the clear zone was seen by using a caliper.

RESULTS

The inhibitory test was carried out in the Microbiology Laboratory of the Faculty of Medicine, Hasanuddin University. In this study the sample used was Porphyromonas gingivalis bacteria. Testing the inhibitory test was carried out by disc diffusion method placed on a petri dish which had Porphyromonas gingivalis bacteria then the test material used was white rice bran extract at a concentration of 12.5%, 25%, 50%, 75%, positive control in the form of chlorhexidine, and negative control in the form of sterile aquades. The measurement results of inhibitory zones of bran extract with ethanol (table 1) and aquades (table 2) solvents on Porphyromonas gingivalis bacteria can be characterized by the presence of an inhibitory zone (looks clearer) around the paper disk).

In table 1 it shows that the area of inhibitory zone of ethanol solvent bran extract from each concentration formed has an increase in diameter in inhibiting the growth of Porphyromonas gingivalis bacteria measured in millimeters (mm) at a concentration of 12.5%, 25%, 50%, 75% carried out 6 repetition which obtained an average yield of 12.3 mm, 13.8 mm, 14.7 mm, 16.2 mm.

Table 1: Diameter of inhibitory zone of white rice bran extract (Oryza sativa L.) with ethanol (A) solvent against Porphyromonas gingivalis bacteria

No.	Inhibitory Zone Diameter of Ethanol Solvent Bran Extract				Positive Control (mm)	Negative Control (mm)
	12,5 % (mm)	25 % (mm)	50 % (mm)	75 % (mm)		
1	13,6	14,2	14,6	16,2	14,8	7
2	14	14,6	16,7	18,2	17,4	6,2
3	10,8	12,5	12,6	14,2	15,1	6,2
4	10,2	12,6	13,2	15,4	18,5	7
5	13,4	14,4	16,2	17,4	18,4	7
6	12,3	14,6	15,3	16,2	17,2	6,2
Average	12.3	13.8	14.7	16.2	16.9	6.6

*Diameter of paper disk : 6,2 mm

In table 2 it shows that the area of inhibitory zone of distilled water bran extract from each concentration formed has an increase in diameter in inhibiting the growth of Porphyromonas gingivalis bacteria measured in millimeters (mm). at a concentration of 12.5%, 25%, 50%, 75% with 6 repetition which obtained an average yield of 10.7 mm, 11.8 mm, 12.6 mm, 12.9 mm. The largest diameter of the inhibitory zone can be seen in Petri dish number 2, which is 12.5% extract concentration with 11 mm diameter, 25% extract concentration with 12.6 mm diameter, 50% extract concentration with 13.2 mm diameter, extract concentration 75% with a diameter of 13.2 mm. positive control 17,4 mm, and negative control 6.2 mm.

Table 2: Inhibitory zone diameter of white rice bran extract (Oryza sativa L) with Aquades (B) solvent against Porphyromonas gingivalis bacteria

No.	Inhibitory Zone Diameter of Aquades Solvent Bran Extract				Positive control (mm)	Negative control (mm)
	12,5 % (mm)	25 % (mm)	50 % (mm)	75 % (mm)		
1	11	12,6	13,2	13,4	17,4	6,2
2	11	11,7	12,7	13,4	18,2	6,2

3	10,5	11,9	13,2	13,2	16	6,2
4	10,2	11,2	11,8	12,4	18	6.4
5	10,8	12,2	12,8	12,9	15,2	6.2
6	10,8	11,2	11,9	12,2	17	6.2
Average	10.7	11.8	12.6	12.9	16.9	6.2

*Diameter of paper disk: 6,2 mm

Based on the Normality Test, the distribution of data is not normal. It can be seen from the significance values at Shapiro-Wilk for bran extract with ethanol and aquades solvents that both have p values below 0.05. Hypothesis test was done by using non-parametric statistics, namely Krusskal Wallis. With the Krusskal Wallis test, significant results were then followed by Mann Whitney. In table 3 it can be seen that each treatment of ethanol solvent bran extract and bran solvent bran extract showed a significant relationship to bacterial death in various groups where the significance value or p value was 0,000 (p value ≤ 0.05). Groups that had significant differences were followed by Mann Whitney (tables 4 and 5)

In table 4, the Mann Whitney test results of bran extract with ethanol solvent showed that there were significant differences between groups of concentration 12.5% with groups 75%, groups 12.5% with positive controls, groups 12.5% with negative controls, groups 25% with groups 75% , groups 25% with positive control group, groups 25% with negative control, groups 50% with control group, groups 75% with negative control group, positive control group with negative control group.

In table 5 the results of Mann Whitney bran extract with aquades solvent showed a significant difference between groups 12.5% with groups 25%, groups 12.5% with groups 50%, groups 12.5% with groups 75%, groups 12, 5% with positive control group, groups 12.5% with negative control group, groups 25% with groups 50%, groups 25% with groups 75%, groups 25% with positive control group, groups 25% with negative control group, groups 50% with positive control group, groups 50% with negative control group, groups 75% with positive control group, groups 75% with negative control group, positive control group with negative control group.

Table 3: Kruskall wallis test results of white rice bran extract with ethanol (A Pg) and aquades (B Pg)

	A Pg		B Pg	
Group	**Mean**	**SD**	**Mean**	**SD**
12.5%	12.38	1.58	10.72	0.31
25.0%	13.82	0.99	11.80	0.55
50.0%	14.77	1.63	12.60	0.62
75.0%	16.27	1.42	12.92	0.52
Positive control	16.90	1.60	16.97	1.17
Negative control	6.60	0.44	6.23	0.08
P Value	0.000		0.000	

Table 4: Mann whitney test results of ethanol solvent bran extract

(I) Group (A Pg)		Mean Difference (I-J)	P Value
12.5%	25.0%	-1.43	0.054
	50.0%	-2.38	0.055
	75.0%	-3.88	0.004*
	Positive control	-4.52	0.004*
	Negative control	5.78	0.003*
25.0%	50.0%	-0.95	0.170
	75.0%	-2.45	0.020*
	Positive control	-3.08	0.004*
	Negative control	7.22	0.003*
50.0%	75.0%	-1.50	0.147
	Positive control	-2.13	0.055
	Negative control	8.17	0.003*
75.0%	Positive control	-0.63	0.470
	Negative control	9.67	0.003*
Positive control	Negative control	10.30	0.003*

*Significant difference (p<0,05)

Table 5: Mann whitney test results of white rice bran extract with aquades solvent

(I) Group (B Pg)		Mean Difference (I-J)	P value
12.5%	25.0%	-1.08	0.004*
	50.0%	-1.88	0.004*
	75.0%	-2.20	0.004*
	Positive control	-6.25	0.004*
	Negative control	4.48	0.003*
25.0%	50.0%	-0.80	0.044*
	75.0%	-1.12	0.013*
	Positive control	-5.17	0.004*
	Negative control	5.57	0.003*
50.0%	75.0%	-0.32	0.258
	Positive control	-4.37	0.004*
	Negative control	6.37	0.003*
75.0%	Positive control	-4.05	0.004*
	Negative control	6.68	0.003*
Positive control	Negative control	10.73	0.003*

*Significant difference (p<0,05)

DISCUSSION

This study used white rice bran extract (Oryza sativa L.) with ethanol and aquades solvents with different concentrations of 12.5%, 25%, 50%, and 75% respectively. The test bacteria used were Porphyrmonas gingivalis bacteria. The results of statistical tests showed that there was p value = 0,000 which means that white rice bran extract in concentrations of 12.5%, 25%, 50% and 75% with ethanol and aquades solvents significantly affected the Porphyromonas gingivalis bacteria (Table 3). Dwipriastuti et al (2017) used a concentration of 12.5%, 25%, 50% and 75% to test the difference in effectiveness of 0.2% Chlorhexidine gluconate with green tea (Camellia sinensis) on the amount of Porphyromonas gingivalis, and 75% and 25% extract has an effectiveness equal to 0.2% chlorhexidine gluconate in killing Porphyromonas gingivalis bacteria.[9]

Observation and processing of data was carried out after the incubation period carried out for 24 hours at 37°C by looking at and measuring the diameter of the resistance zone formed around the paper disk in the petri dish. The measurement results of inhibition zone bran extract with ethanol solvent (table 1) and aquades solvent (table 2) have an average increase in each concentration group. Thus the greater the diameter of the inhibition zone, the greater the antibacterial activity.[12,13,14] This is due to the increasing number of active compounds contained in the extract. [15,16] The concentration of the extract the more the content of the antibacterial active ingredients. The addition of the concentration of antibacterial compounds is thought to increase the penetration of antibacterial compounds into the interior of microbial cells which will damage the cell's metabolic system and can result in cell death. Most bacterial growth will decrease with increasing antibacterial concentration added. The higher the concentration of extract, the greater the number of antibacterial compounds released, there by facilitating the penetration of these compounds into cells. [16,17]

From the phytochemical test, the results of white rice bran extract were found to contain flavonoids. Flavonoids played an active role in inhibiting bacterial growth by damaging cell walls, deactivating enzyme action, binding to adhesin, and damaging cell walls. Beta rings and OH groups on flavonoids are thought to be responsible structures as antibacterial activity.[18] The presence of antibacterial activity in bran due to the content of phenolic compounds.[15]

Phenol compounds have antibacterial activity that works by interacting with bacterial cells through an absorption process that involves hydrogen bonds, interfering with work within the cytoplasmic membrane including disrupting active transport and the strength of protons.[17] The phenol compound is able to break the peptidoglycan cross link in an effort to break through the cell wall. After breaking through the cell wall, phenol compounds will cause cell nutrient leakage by damaging the hydrophobic bonds of cell membrane components (such as proteins and phospholipids) and dissolving hydrophobic binding components resulting in increased membrane permeability. Damage to cell membranes results in the inhibition of activities and biosynthesis of specific enzymes needed in metabolic reactions.[16]

In table 1 the inhibitory zone formed is the largest in the petri dish No. 2, and in table 2 the inhibitory zone formed is the largest in the petri dish No. 1. The size of the area of resistance affected by the growth rate of microorganisms, the ability and rate of diffusion of active ingredients in the medium, the sensitivity of microorganisms to the active substance and the thickness and viscosity of the medium.[19,20,21,22,23]

In tables 1 and 2 there are differences in the inhibitory zone between white rice bran extract in concentrations of 12.5, 25, 50, and 75% with positive control, wherein the inhibitory zones of various concentrations are lower than the inhibitory zone produced by positive control in the form of chlorhexidine[16], 9 mm. This is because chlorhexidine has a

biguanid content with a large cation charge (positive charge) while most bacteria have anion loads (negative charge). Thus chlorhexidine can bind well with bacteria and cause changes in permeability of bacterial cell membranes which subsequently occur cell death due to the release of bacterial cell cytoplasm. [14]

If it is associated with the provisions of the criteria for inhibitory activity that inhibitory zones formed ≥ 20 mm are considered to have very strong inhibitory activity, 10-20 mm stated to have strong inhibitory activity, 5-10 mm stated to have moderate inhibitory activity and ≤ 5 mm are said to have weak inhibitory activity.[12,13] Criteria for inhibitory activity of white rice bran extract with ethanol and aquades solvents at concentrations of 12.5%, 25%, 50%, and 75% are considered to have a weak inhibition to moderate. However, for the average size of the inhibitory zone of white rice bran extract with ethanol solvent has a higher average than bran extract with aquades solvent, ie the concentration of 12.5% has an average diameter of the inhibitory zone 12.3 mm, concentration 25% has an average inhibitory zone diameter of 13.8 mm, a concentration of 50% has an average diameter of inhibitory zone of 14.7 mm, a concentration of 75% has an average diameter of inhibitory zone of 16.2 mm, while bran extract with solvent aquades in concentration 12.5% has an average diameter of inhibitory zone of 10.7 mm, a concentration of 25% has an average diameter of inhibitory zone of 11.5 mm, a concentration of 50% has an average diameter of inhibitory zone of 12.5 mm, and a concentration of 75% has an average diameter of inhibitory zone of 12.9 mm .

In tables 1 and 2 show the inhibitory zone of white rice bran extract with ethanol solvent greater than the resistance zone formed from white rice bran extract with aquades solvent. Inhibitory zone formed by white rice bran extract (Oryza sativa L.) with ethanol solvent larger than aquades solvent. This is determined by the polarity of the type of solvent. The solvent can extract compounds which have the same polarity or similar to the polarity of the solvent used, in this case ethanol which is polar in nature.[24,25] Ethanol is a solvent that has polar properties that can extract active compounds that are soluble in extracellular and intracellular fluids.[24] Ethanol solvents can extract flavonoids belonging to polar compounds thus they will be more soluble in polar solvents.[25,26,27] Polar solvents are capable of dissolving phenolic compounds better thus the total phenol and flavonoid levels in the extract are high. This is because both phenolic compounds and flavonoids are substances that have an aromatic ring with one or more hydroxyl groups, that they are soluble in polar solvents.[19] Phenolic is acidic because of the nature of the easy to escape H^+ group.[26,28,29,30] Flavonoids are the largest group of polyphenol compounds . Flavonoids are very effective for use as antioxidants.[20] Flavonoids are non-polar compounds, but flavonoids have sugar groups that cause solubility in polar or semi-polar. The flavonoid compounds are nonpolar and found in plant stems.[26]

CONCLUSION

Based on the research that has been done, it can be concluded as follows:

1. White rice bran extract (Oryza sativa L.) with both ethanol and aquades solvents at a concentration of 12.5%, 25%, 50%, 75% with positive control of chlorhexidine and control negative aquades is able to inhibit the growth of Porphyromonas gingivalis bacteria.
2. White rice bran extract ethanol and aquades at a concentration of 12.5%, 25%, 50%, 75% form a zone of resistance which is categorized as a weak to moderate inhibition power.
3. The inhibitory zone produced is positive control (chlorhexidine 0.2%) greater than the white rice solvent extract ethanol concentration of 75%, with a difference of 0.9 mm. The chlorhexidine inhibitory zone is higher than the inhibitory zone with 75% white rice bran solvent extract with a difference of 4 mm.

CONFLICT OF INTEREST

There is no conflict of interest in this study. This study obtained a label of ethics escaped by the number: 0047/PL09/KEPK FKG - RSGM UNHAS /2018 and register number UH 17120049 on Oktober 9th, 2018.

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