Mouthwash Product Development Based on Ethanol Extract of White Rice Bran (Oryza sativa L.) as Antibacterial of Streptococcus mutans and Porphyromonas gingivalis

by Marhamah 02

FILE	MOUTHWASH PRODUCT DEVELOPMENT BASED ON ETHANOL EXTRACT OF WHITE RICE BRAN (ORYZA SATIVA L.) AS ANTIBACTERIAL OF STREPTOCOCCUS MUTANS AND PORPHYROMONAS GINGIVALIS.PDF (394.98K)		
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Mouthwash Product Development Based on Ethanol Extract of White Rice Bran *(Oryza sativa L.)* as Antibacterial of *Streptococcus mutans* and *Porphyromonas gingivalis*

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Abstract

Oral health issues are being an endless problem it classified to be the highest prevalence in the world and has the extensive impact on local health, oral and systemic. Oral diseases occur due to bacterial accumulation, including the bacteria that cause dental caries (Streptococcus mutans) and causes of periodontal disease (P. gingivalis). The current scientific approach puts forward prevention such as maintaining oral hygiene by regular brushing with toothpaste and mouthwash rather than surgical intervention. However, today the use of antiseptics in mouthwash is thought to have a carcinogenic effect. So, innovation in this research is to use rice bran extract (Oryza sativa L.) which will be formulated into dosage form mouthwash. It is also based on the potential of rice bran around 2.5 million tons per year.

This type of research is a laboratory experimental study with post test design only with control group design. This research is divided into 5 stages of research, making of bran extract through maceration process, the test of bran extract content, preliminary test, mouthwash formulation formulation, stability test, comparative power inhibition test. Then the measurement and data analysis using SPSS version 22.0 for windows.

The statistical analysis of bran extract with concentration of 10%, 20%, 40%, 80% and 100% with positive control of ampicillin respectively showed significant difference of drag zone ($p < 0,05$) to Streptococcus mutans ($p = 0.00$) and Porphyromonas gingivalis ($p = 0.001$). From the test results of the content, the positive bran extract contains polyphenols. Formulation of mouthwash using 10% concentration with comparative variable mouthwash A and B and negative control showed significant effect in mouthwash extract of bran at Porphyromonas gingivalis bacteria ($p = 0,018$) and not significant on Streptococcus mutans ($p = 0,058$).

Mouthwash Bran extract effectively inhibits the growth of Streptococcus mutans bacteria and Porphyromonas gingivalis.

Experimental article (J Int Dent Med Res 2019; 12(3): 985-990)

Keywords: Mouthwash Bekatul, Bran extract of white rice, Streptococcus mutans, Porphyromonas gingivalis.

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Introduction

Oral health problems in Indonesia is still an unresolved problem. The high rate of dental caries and the low status of oral hygiene are dental and oral health problems that are often found in some age groups. Oral diseases occur due to the accumulation of bacteria, including bacteria that cause dental caries (Streptococcus mutans) and causes of periodontal disease (*P. gingivalis*). [1,2]

Caries is a process of local dental hard tissue destruction by bacteria. Caries is classified as a part of chronic diseases that have the highest prevalence in the world and have a wide impact on local health, oral cavity and even system health.[2,3,4] WHO data shows that in the world, 60-90% of children and nearly 100% of adolescents are affected by caries. The results of the 2013 RISKESDAS data analysis showed an

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increase in caries prevalence in Indonesia. the highest increase in prevalence occurred in South Sulawesi (29.1%) which was twice as high as the national prevalence.[5]

The impact caused by oral cavity disease causes interference in biting, chewing, smiling, talking, and psychosocial well-being.[6,7] Besides having an impact on oral and systemic health, caries also has a strong impact on the economy. In the industrial world, the latest information states that treatment of oral diseases, including the fourth most expensive in the world and caries, can weaken the productivity of community work especially children.[2,8]

Seeing the impact caused by oral diseases such as caries and periodontal disease is very large, optimal prevention efforts are needed because until now various efforts have been made to reduce the prevalence of caries and periodontal diseases including fluoridation, fissure sealants, toothpaste and mouthwash. One way to maintain effective oral and dental hygiene is by gargling using mouthwash. The use of mouthwash is one of the earliest prevention methods for caries due to reduced plaque attachment. The use of mouthwash is very effective because of its ability to reach places that are difficult to clean with a toothbrush and can damage plaque formation. The use of herbal and nonherbal mouthwash has become a routine that is trusted by the public to overcome caries.[9]

Recent research states that caries prevalence continues to increase. This proves that the efforts that have been made are still less effective that further innovation in caries prevention is needed. The current scientific approach promotes precautions such as maintaining oral hygiene by brushing teeth regularly using toothpaste and mouthwash rather than surgical interventions (e.g. fillings on teeth) as a normal response to dental caries management.[9]

The use of antiseptics in adult mouthwash is thought to have carcinogenic effects on users. This fact is supported by the results of research by McCullough and Farah (2008) which stated that the use of mouthwash with an antiseptic content in the form of alcohol can lead to oral cancer. Therefore, one of the innovations to make mouthwash products safer to use is the use of mouthwash formulations made from herbs.[10] One of the natural ingredients that has various ingredients and is able to be used as an antibacterial and safe ingredient to use which is already known empirically by the people of Indonesia is rice. Rice is used as a staple food both in Indonesia and in the world. Indonesia is one of the largest rice producing countries in the world. Rice production in Indonesia is around 47 million tons per year, or equivalent to about 32 million tons of rice. Bran is a byproduct of the rice milling process and rice seeding. Of the 32 million tons of rice obtained by the form of bran around 2.5 million tons. Bran is usually only used as a component of animal feed and poultry. Rice bran is considered as a less useful ingredient because rice bran is a waste in the processing of grain into rice. The rest of the rice pounding or grinding is called bekatul (bran).[11,12]

In this study, rice bran extract was used to inhibit *Streptococcus mutans* and *Porphyromonas gingivalis.Streptococcus mutans* has a major role in the etiology of caries, because Streptococcus mutans is attached to the enamel pellicle and other bacterial plaques. In this attachment, S. mutans produce strong acids and cause acidic environment[5] to create the risk of cavities while Porphyromonas plays a major role as the etiology of periodontal disease.[13] Looking at these problems one of the breakthroughs carried out in this study was to use white rice bran ethanol extract (Oryza sativa L.) which will be formulated into a mouthwash dosage form that will be tested against bacteria *Streptococcus mutans* and *Porphyromonas gingivalis* as the main cause of periodontal disease and caries.

Materials and methods

The type of research used in this study is laboratory experimental research. Research design is *post test only with control group design*. This research was conducted in the hytochemical Laboratory of Hasanuddin University Faculty of Pharmacy, Pharmacetic Laboratory of Hasanuddin University Faculty of Pharmacy and Microbiology Laboratory of Faculty of Medicine, Hasanuddin University. This research was carried out into several research stages, namely sample extraction, making the concentration of white rice bran extract, bacterial culture procedure *Streptococcus mutans* and *Porphyromonas gingivalis,* Inhibitory Power Test, Inhibitory Zone Measurement, Making Mouthwash, Mouthwash Stability Test, Inhibitory Test of Mouthwash and Data Analysis.

The tools used in this study were Handschoen, Masks, Vital Bottles and Bottles,

Evaporator Tubes, Petri Dishes, Suction Pipettes, Round Ose, Buchner Funnels, Stirrers, Autoclaves and Incubators, Micropipets, Scales, Aluminum foil, Small Spoons, Caliper, Ruler, Writing Tool, Evaporator, Oven, Glass Jar, 10ml Spoit, Bunsen, Microscope.

The material used in this study were white rice bran (*Oryza sativa L.)* ethanol, alcohol 70%, sterillized aquades, *paper dish*, *Muller Hinton Agar* (MHA), DMSO solution, medium NA, medium NB, NaCl physiological 0,9%, ampicillin 10µg, filter paper, methylated paper, label paper, and bacterial culture (*Streptococcus mutans*dan *Porphyromonas gingivalis*) menthol, methyl salicylate, Thymol, Benzoic acid, sorbitol, obtained from the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University, and Phytochemical and Pharmacetic Laboratory Faculty of Pharmacy, Hasanuddin University.

Results

This research was conducted at the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University. This study used a sample of Streptococcus mutans and Porphyromonas gingivalis bacteria with sampling method and fulfilled the requirements for inclusion criteria. The number of samples used in this study were 8 treatments, each divided into 2 groups consisting of 4 samples from each group. This research was an experimental laboratory study. This study used the intervention material of white rice bran extract (Oryza sativa L.) 10%, 20%, 40%, 80%, and 100% and ampicillin 10µgas a positive control which is an oxoid standard antibiotic often used as an intervention. Bran is made in bran extract preparations at the Fitiochemical Laboratory, Faculty of Pharmacy, Hasanuddin University. Whereas for positive control Ampicillin 10µg was obtained at the Microbiology Laboratory, Faculty of Medicine Hasanuddin University.

The test of inhibitory power was carried out using the disk diffusion method placed on a petri dish that had bacterial colonies *Streptococcus mutans* and *Porphyromonas gingivalis* then the test substance used was bran extract at concentrations of 10%, 20%, 40%, 80%, 100% and positive control. Then the mouthwash formulation was made using 10% rice bran extract. In making the Moutwash formulation 7 reps were performed against the moutwash formula. In the process of making formulations there were 2 formulations, namely: Formulation of Gargle 1 (Table 1) and Formulasi Obat Kumur 2 (Table 2).

Name of Materials	Formula
Rice bran extract (%)	10
Menthol (%)	0.04
Methyl Salicylate (%)	0.06
Thymol (%)	0.06
Alcohol (%)	10
Benzoic Acid (%)	1
Aquadest ad (%)	78.84

Table 1. Formulation of Gargle 1 (3 times repetition).

Name of Materials	Formula
Rice bran extract (%)	10
Menthol (%)	0.0025
Sorbitol (%)	0.05
Alcohol (%)	0.02
Aquadest ad (%)	89.9275

Table 2. Formulasi Obat Kumur 2 (4 times repetition).

The inhibitory test results were carried out by various statistical tests based on observations of the bacteria Streptococcus mutans and Porphyromonas gingivalis by doing 4 times of reprocessing of each treatment. After that the results of the study were recorded and data management was carried out using SPSS version 22 for windows data.

The results of the study are as follows: From the statistical test, the normality test of the concentration of bran extract showed that the concentration of Streptococcus mutans was normally distributed because the sig value of shapiro wilk was more than 0.5 while for Porphyromonas gingivalis was not normally distributed. For the value of Streptococcus mutans which was normally distributed, the statistical test continued to the One Way Anova test to see the differences in each treatment of bacterial death. Based on *One Way Anova* test it was known that all treatments showed a significant relationship to the death of bacteria in various concentrations where p value is 0,000 (p value $\leq$ 0,05). Because the results of the One-Way Innovative test were followed by a post hoc test using LSD to see the difference in each

treatment for the control group (ampicillin).

Based on the Post-Hoc test using LSD it was found that after testing the bacteria had a significant difference to the positive control group (ampicilin). Whereas for Porphyromonas gingivalis using krusskal wallis due to abnormal distribution. For the test results using krusskal wallis was significant thus it was continued to post hoc using mann whitney.

For the results of statistical tests, the normality test of the influence of bran moutwash and other brands of moutwash on the market against the bacteria Streptococcus mutans and Porphromonas gingivalis. The results of the normality test showed that both were not normal thus further testing used krusskal wallis. For the results of material testing of Streptococcus mutans was not significant so it was not continued to the post hoc test. Whereas for Porphyromonas gingivalis bacteria have a significant test results thu it was continued to post hoc using mann whitney.

	S. mutans	Value of P*	P. gingivalis	P value
10%	9.9500±2.28108	0.00	9.3500±0.44347	0.001
20%	12.1000 ±0.86023	0.00	10.7500±0.55076	0.001
40%	13.1750±1.00789	0.00	11.5250±0.62915	0.001
80%	14.7250±2.26918	0.00	12.0250±0.94296	0.001
100%	16.0000±2.11818	0.00	13.2250±0.51881	0.001
Control+	22.3750±2.09980	0.00	22.5000±0.38297	0.001

Paired sample t-test P< 0.05; Significant (*Anova,Kruskal Wallis Test)

Table 3. Table of Average Value of Standard Deviations Concentration of Deviation of Bran Extract to Bacteria *Streptococcus mutans* and *Porphyromonas gingivalis*.

Materials	Means±Std S.Mutans	P Value	Means±Std P.gingivalis	P Value
A	18.4000±1.21244	0.058	11.0000±0.98489	0.018
B	17.2000±0.51962	0.058	12.7000±1.11355	0.018
C	17.5000±1.21655	0.058	15.0500±0.70534	0.018
Control -	0.0000±0.0000	0.058	0.0000±0.0000	0.018

Paired sample t-test P< 0.05; Significant (Kruskal Wallis Test)

Table 4. Table of Average Value of Standard Deviations Concentration of Mouthwash Deviation of Bran and Mouthwash on the Market Against Bacteria *Streptococcus mutans* and *Porphyromonas gingivalis*.

Concentration	Concentration	Average Range	Standard Deviation	P Value
10%	20%	-2.15000	1.32301	0.122
	40%	-3.22500*	1.32301	0.025
	80%	-4.77500*	1.32301	0.002
	100%	-6.05000*	1.32301	0.000
	AMP	-12.42500*	1.32301	0.000
20%	10%	2.15000	1.32301	0.122
	40%	-1.07500	1.32301	0.427
	80%	-2.62500	1.32301	0.063
	100%	-3.90000*	1.32301	0.009
	AMP	-10.27500*	1.32301	0.000
40%	10%	3.22500*	1.32301	0.025
	20%	1.07500	1.32301	0.427
	80%	-1.55000	1.32301	0.257
	100%	-2.82500*	1.32301	0.047
	AMP	-9.20000*	1.32301	0.000
80%	10%	4.77500*	1.32301	0.002
	20%	2.62500	1.32301	0.063
	40%	1.55000	1.32301	0.257
	100%	-1.27500	1.32301	0.348
	AMP	-7.65000*	1.32301	0.000

Table 5. Table of Post Hoc test of *Streptococcus mutans* and *Porphyromonas gingivalis* Konsentrasi Deviasi Ekstrak Bekatul Terhadap Bakteri *Streptococcus mutans* dan *Porphyromonas gingivalis*.

Discussion

Rice bran consists of a layer outside the grain of rice with a number of seed institutions. In the grain milling process, there are several levels which were first obtained by broken rice with the result of joining the husk and coarse bran. Rice bran consists of pericarp, testa, and aleurone layers. This percentage varies, depending on the variety and age of rice and the degree of sosoh (Grist, 1965). Rice bran yield is influenced by several factors, including degree of siltation, rice cooking rate, grain moisture content, type of slurry device, and separator hole (Soemardi, 1975)[14,15]

Qualitative test results of bran extract at the Laboratory of Phytochemical Faculty of Pharmacy Hasanuddin University showed positive results, meaning that the white rice bran ethanol extract had positive phenolic compounds, namely polyphenols. This study is in line with the research of Devi and Arumughan (2006) who found the content of phenolic compounds in defatted rice bran extracted with methanol.47 Hodzic et al. (2009) said that in cereals, phenolic compounds are mainly present in the pericarp. Phenolic compounds have biological properties such as: antioxidant, antiapoptosis, anti aging, anticarcinogen, antiinflammatory, antiatherosclerosis, cardiovascular protection, improvement of endothelial function, inhibit angiogenesis and cell proliferation activity.48 The results showed that bran contains high bioactive components or phytochemical compounds such as tocopherol, tootrienol, oryzanol (Chen and Bergman, 2005), phenolic antioxidants (Chanphrom 2007; Sompong *et al.,* 2011), β-carotene (Chanphrom, 2007), and anthocyanin in black rice bran and black sticky rice (Yawadio et al., 2007). Garcia et al. (2007) reported that each rice variety has a total level of polyphenols that are different and the total polyphenols more abundant in the bran compared to its rice flour.[16,17,18,19,20] Based on the results of the qualitative test, the bran extract was continued to the next stage, namely the preliminary test of the effectiveness of bran extract on the streptococcus mutans and porphyromonas gingivalis bacteria to determine the inhibitory KHM extract.

In this study showed that each concentration of bran extract had antibacterial effects in inhibiting the growth of Streptococcus mutans and porphyromonas gingivalis. This is due to the presence of active compounds, namely polyphenols which have antibacterial activity. This study used bran extract with concentrations of 10%, 20%, 40%, 80% and 100%, respectively. The results of each concentration in this study showed that the results of a significant different test, the higher the concentration, the higher the result of the diameter of the inhibitory power caused by bacteria. *Streptococcus mutans and porphyromonas gingivalis*.

The effectiveness of bran mouthwash on S. mutans and P.gingivalis bacteria occurs because the mechanism of action of the Bekatul Mouthwash will cause changes in the permeability of the bacterial cell membrane causing the cytoplasm to exit cells and low molecular weight cell components from inside the cell through the cell membrane causing death bacteria. The work effectiveness of bran Mouthwash is more effective against Gram positive bacteria (S. mutans) compared to Gram negative bacteria (*P. gingivalis*). This can be seen from the mean diameter of the bacterial inhibition zone in S. mutans study group of 17,500 mm compared to P. gingivalis study group of 15,0500 mm. This happened because there were differences in cell wall types in gram-positive bacteria where gram-positive bacteria do not have lipopolysaccharide while gram-negative bacteria have lipopolysaccharide. Lipopolysaccharide is able to work the mechanism of flavonoids as antibacterial to form complex compounds with extracellular proteins and dissolved thus it can damage the bacterial cell membrane and followed by the release of intracellular compounds.[53] The cytoplasm in cells all lives is limited by the cytoplasmic membrane, which acts as a selective barrier permeability, carries an active transport function and then controls the internal composition of the cell. If the integrity function of cytoplasmic membrane cells is damaged, macromolecules and ions come out of the cell, then the cell is damaged or death occurs. The antibacterial effect of bran extract can increase in inhibiting *Streptococcus mutans* and *porphyromonas gingivalis* similar to positive control of ampicillin 10µg if an increase in the concentration of rice bran extract was carried out. This study is in accordance with the research conducted by IndoBIC et al, namely in his study there was an average diameter of the bacterial inhibition zone in the S. mutans study group of 16.0833 mm compared to the P. gingivalis study group of 13.2250 mm.[21,22]

Effectiveness analysis of bran Moutwash given C code compared with Mouthwash code A, Mouthwash code B, and negative controls showed significant results on Porphyromonas gingivalis bacteria. This means that the difference in inhibitory power of the treatment has a significant difference. There is a significant difference, p = 0.018. In this test analysis of the effectiveness of bran mouthwash (15.05) which only uses alcohol with a fairly small proportion of 0.02% had the highest effectiveness compared to moutwash code A (11.0) which contained alcohol quite high around 0.5% or moutwash code B

(12.7) containing herbal ingredients. This showed that mouthwash had a high effectiveness on Porphyromonas gingivalis bacteria that caused periodontal disease.

In contrast to the analysis of the effectiveness of bran moutwash on Porphyromonasgingivalis. The effectiveness of bran mouthwash on Streptococcus mutans bacteria had a value of p = 0.058 which means insignificant. This means that there was no significant difference in the amount of bran mouthwash inhibitory power compared to other mouthwash. This happened because the effectiveness of bran moutwash and comparator which were code A and B had almost the same inhibitory zone. In this test, analysis of the effectiveness of bran mouthwash (17.5) had greater effectiveness compared to moutwash code B (17.2) which was made from herbs. However, the inhibition zone of code A (18.4) was greater than bran moutwash. This could happen because alcohol formulations from mouthwash A code were high that it can kill more Streptococcus mutans bacteria causing caries disease.

Conclusions

Mouthwash product of Bran extract effectively inhibits the growth of baterial *Streptococcus mutans* and *Porphyromonas gingivalis*.

Declaration of Interest

The authors report no conflict of interest and this research obtains ethical pass information number: 777/H4.8.4.5.3.1/PP36-KOMETIK/2017 and registered number UH170 60386 on October 10, 2017.

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