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Inhibition of Oral Bacterial Growth (*Streptococcus Mutans* and *Porphyromonas Gingivalis*) using Black Rice Bran Ethanol Extract (*Oryza Sativa L.*) as A Natural Mouthwash

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ABSTRACT

Oral health problems in Indonesia are still relatively high. Indonesian National Health Research 2018 stated that oral health problem's proportion in Indonesia is 57.6%. Diseases in the oral cavity occur due to accumulation of bacteria, including bacteria that cause dental caries (*Streptococcus mutans*) and periodontal disease (*Porphyromonas gingivalis*). Current scientific methods prioritize prevention care to take care of oral health such as teeth brushing regularly using toothpaste and using mouthwash rather than surgical intervention. However, mouthwash contains alcohol, in which can increase the risk of developing oral cancer. Rice bran is a natural comestible containing antibacterial substances. Differences in pigment in rice affect its antibacterial activities. Hence, this research novelty lies in using black rice bran extract (*Oryza sativa L.*) formulated mouthwash. This study was a laboratory experimental study with post-test only with control group design. The study itself was divided into several phases, extract making to inhibition test. Statistical analysis resulted bran extract with concentration of 10, 20, 40, 80, and 100% with ampicillin positive control each have different inhibition zone and are statistically significant (p<0.05) to *Streptococcus mutans* (p=0.00) and *Porphyromonas gingivalis* (p=0.00). Based on study conducted mouthwash A and negative control was used as comparative variables for mouthwash with 10% concentration and shows significance level of 0.00 (p<0.05) which means there is a significant difference between each group. Black Rice Bran extract mouthwash as inhibitor *Streptococcus mutans* and *Porphyromonas gingivalis* growth bacteria.

Keywords: Black rice bran extract, natural mouthwash *Streptococcus mutans*, *Porphyromonas gingivalis*

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BACKGROUNDS

Oral health plays a pivotal part in shaping in overall health. Oral health problem in Indonesia, particularly tooth decay (dental caries) is still relatively high. Dental caries is a disease of tooth hard tissue namely email, dentin, and cementum, caused by microorganism activities in the oral cavity. School-age children, especially elementary school children, are one of the groups that are susceptible to dental and oral diseases, because in general children still have poor behavior or habits in maintaining oral health (Kidd & Fejerskov, 2016). Based on RISKESDAS (Indonesia National Health Research) 2018, dental caries index of average adults with 32 dentitions, on average have 7 decayed teeth. Whereas in young adults aged 15 to 20, each has on average 3 decayed teeth and for children aged below 12, each has 2 decayed teeth (Kemenkes, 2018).

Teeth play a vital role for a child growth and development. Dental decay is the most common oral health problem in children, it can be caused by hereditary factor, race, sex, age, diet, bad habit, host factors i.e. strength of tooth surface, bacterial plaque containing cariogenic bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis*. (Kidd & Fejerskov, 2016; Matsuyama, Fujiwara, Ochi, Isumi, & Kato, 2018).

Plaque usually starts forming on third of the cervical surface of rough and defective tooth. Plaque control can be performed by mechanically removing the plaque by brushing teeth surface and gargling with mouthwash. In general, mouthwash contains 5-25% alcohol. Alcohol in mouthwash acts as an antiseptic, shelf life extending, prevents contamination of microorganisms, and solvents (Rawlinson et al., 2008). However, alcohol content in mouthwash causes certain individuals to not be able to use it, such as children, pregnant / breastfeeding mothers, patients who use certain drugs, and patients with xerostomia (Witt et al., 2005). Alcohol content in mouthwash can increase the risk of oral cancer, especially if it is taken continuously (Quirynen et al., 2005).

Rice bran is a traditional comestible that contains various compounds, including antibacterial compounds. The results of previous studies showed that inhibition of cancer cell growth correlated with a higher total phenolic content of black and red rice bran compared to white rice bran. It was proven that the ethanol extract of white rice bran at concentrations of 10%, 20%, 40%, 80% and 100% could inhibit the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* (Achmad & Singgih, 2020).

Therefore, this research is needed to determine the potential of black rice bran (*Oryza sativa L.*) ethanol extract in inhibiting the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* bacteria.

MATERIALS AND METHODS

This study was a laboratory experimental study with post-test only with control group design. Research was conducted in pharmacognocy-phytochemical laboratory in Pancasakti University Makassar and Microbiology laboratory in Faculty of Medicine Hasanuddin University Makassar from August to September 2019. Samples used were *Streptococcus mutans* and *Porphyromonas gingivalis* bacterial.

Instruments used in this study are Latex examination gloves, Masks, Vial Shelf and Bottles, Evaporator Tubes, Petri Dish, Suction Pipettes, inoculating loop, Buchner Funnels, Stirring rod, Autoclaves and Incubators, Micropipettes, Scales, Aluminum Foil, Small Spoons,

Caliper, Ruler, Writing instruments, Oven, Glass Jar, 10 ml syringe, Bunsen burners, Microscope, Test Tube, Erlenmeyer flask. Materials used: black rice bran (*Oryza sativa L.*), ethanol, alcohol 70%, sterile distilled water, paper dish, Muller Hinton Agar (MHA), DMSO solution, NA medium, NB medium, 0.9% NaCl physiological solution, 10 µg ampicillin, filter paper, denaturated alcohol, labels and bacterial culture (*Streptococcus mutans* and *Porphyromonas gingivalis*), menthol, glycerin, and sodium lauryl sulfate.

Data analysis was performed using SPSS 22.0 software and data was analyzed using Shapiro Wilk, One-Way ANOVA, Kruskal Wallis, Mann Whitney, and LSD test.

RESULTS

From Table 1 descriptively it can be seen that the 10% concentration of black rice bran extract has the smallest inhibition zone that is 9.15 ± 0.19 for *Porphyromonas gingivalis* and 8.05 ± 0.05 for *Streptococcus mutans* when compared to the rest of concentration of black rice bran extract. The zone of inhibition in the positive control group using ampicillin had the greatest inhibition zone of 17.77 ± 0.26 for *Porphyromonas gingivalis* and 14.07 ± 0.95 for *Streptococcus mutans*.

For the observation of the inhibition zone of *Porphyromonas gingivalis* bacteria, the normality value and p value obtained using Shapiro-Wilk statistical test was > 0.05, which means that the data is normally distributed so the test was continued with parametric test namely One-way ANOVA (Table 2). Based on the One-way ANOVA statistical test, significance value of 0,000 (p <0.05) was obtained, which means that there were significant differences among treatment groups.

Shapiro-Wilk statistical test was conducted to determine inhibition zone of *Streptococcus mutans* resulted the normality value and the value of p obtained was <0.05 which means that the data is not normally distributed so the test was continued with non-parametric test namely Kruskal Wallis (Table 2). Based on the Kruskal Wallis statistical test significance value of 0,000 (p <0.05) was obtained, which means there are significant differences between treatment groups. Afterwards, post hoc test was conducted to determine the differences between two variables.

Comparative test results of inhibition zones between groups for both *Porphyromonas gingivalis* and *Streptococcus mutans* on average showed a significant value (p <0.05). At concentration of 10% black rice bran extract when compared with a concentration of 20%, 40%, 80% or 100% black rice bran extract showed there are significant differences, or it has different effects (p value <0.05). Likewise, the zone of inhibition of black rice bran concentration of 10% extract when compared to controls using ampicillin has a significant difference or it has different effects (p value <0.05).

From Table 4 descriptively it can be seen that the mouthwash formula + 10% black rice bran extract has the smallest inhibition zone that is 8.85 ± 0.10 for *Porphyromonas gingivalis* and 10.0 ± 0.47 for *Streptococcus mutans* when compared to the whole group. The zone of inhibition in the positive control group using mouthwash A had the greatest inhibition zone of 9.30 ± 0.23 for *Porphyromonas gingivalis* and 11.17 ± 0.56 for *Sp. mutans.*

The *Porphyromonas gingivalis* bacterial inhibition zone observation with Shaphiro-Wilk statistical test present p value <0.05, which means that the data is not normally distributed so the test was continued with non-parametric test namely Kruskal Wallis (Table 4). Based on the Kruskal Wallis statistical test significance value of 0,000 was obtained (p <0.05), which means there are significant differences between treatment groups.

For the observation of the inhibition zone of *Streptococcus mutans* Shaphiro-Wilk statistical test was performed to determine the normality value and the value of p obtained was >0.05, which means that the data is not normally distributed so the test was continued with parametric test namely One-way ANOVA (Table 4). Based on the One-way ANOVA statistical test significance value of 0,000 was obtained (p <0.05), which means there are significant differences between treatment groups. Afterwards, post hoc test was conducted to determine the differences between two variables.

Comparative test results of inhibition zones between groups for *Porphyromonas gingivalis* and *Streptococcus mutans* on average showed a significant value (p <0.05). Mouthwash formula + 10% bran extract compared to mouthwash A and distilled water has p<0.05 value, which means there are significant differences between groups, or it has different effects. Likewise, differences in inhibition zone of mouthwash A compared to control group using distilled water has p value of <0.05 which means there are significant differences between groups, or it has different effects.

DISCUSSIONS

Based on research that has been conducted, ethanol extract of black rice bran has the ability to inhibit the growth of oral cavity bacteria, namely *Streptococcus mutans* and *Porphyromonas gingivalis*. This is supported by previous research, which states that in antimicrobial screening, rice bran ethyl acetate extracts show good results for antibacterial and antifungal activities. Rice bran ethyl acetate extract showed inhibition zone diameters of up to 20 mm for *S. aureus, A. niger, B. subtilis, Saprophytith* other strains (Yawadio, Tanimori, & Morita, 2007).

Different rice varieties have different levels of total polyphenols compounds and total polyphenols compounds are more abundant in rice bran than rice flour. Different varieties and growing places produce bran with different levels of bioactive components. The process of extracting bioactive components is strongly influenced by several things, one of which is the type of solvent. Because the solubility of a substance is largely determined by the suitability of the properties between the solute and the solvent (Saha, Hossain, Amin, & Bhuiyan, 2014). As in previous study which showed that the white rice bran distilled water extract was more effective in inhibiting the growth of *Streptococcus mutans* bacteria than ethanol extract of white rice bran. The inhibitory capacity of white rice bran ethanol extract on the growth of *Streptococcus mutans* at the concentration of 12.5% was 11.03 mm while that of the distilled water was 13.63 mm (Achmad & Singgih, 2020). The study with using *Porphyromonas gingivalis*, showed that the average inhibition zone size of white rice bran extract with ethanol solvent is relatively larger than that of rice bran extract with distilled water solvent, which at concentration of 12.5% had an average inhibition zone diameter of 12.3 mm while rice bran extract with distilled

water had an average inhibition zone diameter of 10.7 mm (Achmad & Singgih, 2020).

Table 1 shows that the higher the concentration of black rice bran extract, the higher its ability to inhibit bacterial growth, caused by higher active compounds contained in the extract. The formation of inhibition zone diameter is influenced by two things, namely the ability of the extract to diffuse to all parts of the media and the presence of active compounds to inhibit bacterial growth through their respective mechanisms. When compared with previous studies, black rice bran extract is more effective in inhibiting the growth of *Porphyromonas gingivalis* bacteria than white rice bran. Research conducted by Marhamah et al., shows that white rice bran extract of 100% concentration can inhibit the growth of *Porphyromonas gingivalis* bacteria with inhibition zone diameter of 13.22 mm, whereas in studies using black rice 100% of inhibition zone diameter formed is 13.31 mm (Achmad & Singgih, 2020).

Black rice bran extract with a concentration of 100% qualitatively has the same inhibitory power as ampicillin, which is included in the strong category. However, based on statistical tests, it has a p value of <0.05, which means there is a significant difference between black rice bran extract and ampicillin in inhibiting bacterial growth.

Differences in inhibition zone formation are influenced by the composition of the extract and the activity of secondary metabolites contained in the combination of black rice bran extract. The concentration and composition of secondary metabolite activity in the extract has a broad scale. The same type of plant with different composition in each combination treatment can affect secondary metabolite compounds produced. Concentrations and activity of secondary metabolites also vary in different parts of plants (i.e. roots, rhizomes and leaves) and temporally (scale: days, months and years).

Based on research that has been conducted, mouthwash with the addition of 10% black rice bran extract has an inhibitory zone diameter (inhibition) of 8.85 ± 0.10 (medium category) for *Porphyromonas gingivalis* bacteria and 10.0 ± 0.47 (medium category) for *Streptococcus mutans* bacteria. When compared with mouthwash A, qualitatively the black rice bran 10% extract mouthwash both has the same effect for inhibiting *Porphyromonas gingivalis*, which is included in the medium category. As for the bacteria *Streptococcus mutans* both have different effect. Mouthwash A is classified as strong while 10% bran extract mouthwash is classified as moderate. This difference can be caused by the high concentration of alcohol in mouthwash A.

Rice bran contains flavonoids, ferulic acid, phytic acid, oryzanol, vitamin E complex, vitamin B complex, gallic acid, hydroxybenzoic acid, prochate, and phytosterol. The mechanism of action of bran mouthwash to inhibit the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* bacteria is by interfering with the permeability of bacterial cell membranes that causes cell's cytoplasm and cell component with lower molecular weight to penetrate through the membrane and exit the cell wall (Achmad & Singgih, 2020; Leonarto & Heriyanto, 2017).

Mouthwash A, black rice bran 10% mouthwash, and distilled water have different effects in inhibiting bacterial growth. Inhibitory zone of 10% black rice bran mouthwash extract could have the same inhibition zone diameter as mouthwash A if the concentration of bran extract is increased. This is based on the results of research that shows that the higher the concentration of black rice bran extract, the wider the diameter of the inhibitory zone for both *Streptococcus mutans* and *Porhyromonas gingivalis bacteria*.

The results showed that black rice bran mouthwash extract is more effective against *Streptococcus mutans* bacteria than *Porphyromonas gingivalis*. This can be seen by the average inhibition zone diameter for *S. mutans* group is 10mm compared to *P. gingivalis, which* has 8.85 mm. *Streptococcus mutans* is a gram-positive bacterium whereas *Porphyromonas gingivalis* is a gram-negative bacterium. There are differences between each type's cell membrane. Cell walls in gram-positive bacteria do not have lipopolysaccharides while gram-negative bacteria have lipopolysaccharides. Lipopolysaccharides are molecule that is able to hold cationic molecules from mouthwash, which can limit or reduce the effectiveness of the mouthwash. In addition, the outer membrane of gram-negative bacteria, acts as a barrier against cationic anti-bacterial substances as well (Leonarto & Heriyanto, 2017; Xuedong & Yuqing, 2015).

CONCLUSION

Based on the study conducted, black rice bran extract has possibly inhibited the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* bacteria. The higher the black rice bran extract concentration, the higher its ability to inhibit bacterial growth, which can be seen from the wider diameter of the inhibitory zone. A 10% black rice bran mouthwash extract can inhibit the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* with inhibition zone diameter of 8.85 ± 0.10 (moderate category) for Porphyromonas gingivalis bacteria and 10.0 ± 0.47 (moderate category) for Streptococcus mutans.

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Table 1: Black rice bran average inhibition zone of *Porphyromonas gingivalis* and *Streptococcus mutans*

Group	n	*Porphyromonas gingivalis*	*Streptococcus mutans*
		Mean ± SD	Mean ± SD
Back rice bran extract 10%	4	9.15 ± 0.19	8.05 ± 0.05
Back rice bran extract 20%	4	9.93 ± 0.39	9.12 ± 0.15
Back rice bran extract 40%	4	11.61 ± 0.13	10.57 ± 0.32
Back rice bran extract 80%	4	12.52 ± 0.09	11.90 ± 0.14
Back rice bran extract 100%	4	13.31 ± 0.37	12.85 ± 0.05
Ampicillin control	4	17.77 ± 0.26	14.47 ± 0.95

Table 2: Statistical analysis results of black rice bran inihibition zone for *Porphyromonas gingivalis* and *Streptococcus mutans* bacteria

Concentration	n	*Porphyromonas gingivalis*		*Streptococcus mutans*	
		*Normality test**	*Comparison test***	*Normality test**	*Comparison test****
Back rice bran extract 10%	4	0.272*	0.000	0.024	0.000
Back rice bran extract 20%	4	0.499*		0.224	
Back rice bran extract 40%	4	0.123*		0.041	
Back rice bran extract 80%	4	0.272*		0.161	
Back rice bran extract 100%	4	0.224*		0.024	
Ampicillin control	4	0.123*		0.001	

**Shapiro Wilk test: p>0.05; data distribution normal*
***One-Way ANOVA: p<0.05; significant*
****Kruskal Wallis: p<0.05; significant*

Table 3: Post hoc statistic results of black rice bran inhibition zone for *Porphyromonas* gingivalis and *Streptococcus mutans*

Groups (i)	Comparison(j)	*Porphyromonas gingivalis*		*Streptococcus mutans*	
		Mean difference (i-j)	p-value*	Mean difference (i-j)	p-value**
10% Concentration	20% Concentration	-0.78*	0.001	-1.07*	0.019
	40% Concentration	-2.46*	0.000	-2.52*	0.019
	80% Concentration	-3.37*	0.000	-3.85*	0.019
	100% Concentration	-4.16*	0.000	-4.80*	0.018
	Ampicillin control	-8.62*	0.000	-6.42*	0.017
20% Concentration	40% Concentration	-1.67*	0.000	-1.45*	0.019
	80% Concentration	-2.58*	0.000	-2.77*	0.019
	100% Concentration	-3.37*	0.000	-3.72*	0.019
	Ampicillin control	-7.83*	0.000	-5.35*	0.017
40% Concentration	80% Concentration	-0.91*	0.000	-1.32*	0.019
	100% Concentration	-1.70*	0.000	-2.27*	0.019
	Ampicillin control	-6.16*	0.000	-3.90*	0.017
80% Concentration	100% Concentration	-0.78*	0.001	-0.95*	0.019
	Ampicillin control	-5.25*	0.000	-2.57*	0.017
100% Concentration	Ampicillin control	-4.46*	0.000	-1.62*	0.017

***LSD: p<0.05; significant*
****Mann Whitney: p<0.05; significant*

Table 4: Average value of black rice bran inhibition zone for *Porphyromonas gingivalis* and *Streptococcus mutans*

Groups	n	*Porphyromonas gingivalis*	*Streptococcus mutans*
		Mean ± SD	Mean ± SD
Mouthwash formula + 10% bran extract	4	8.85 ± 0.10	10.0 ± 0.47
Mouthwash A	4	9.30 ± 0.23	11.17 ± 0.56
Distilled water	4	6.15 ± 0.05	6.12 ± 0.09

Table 5 : Statistic results of Black rice bran mouthwash inhibition zone for *Porphyromonas gingivalis* and *Streptococcus mutans*

Concentration	n	*Porphyromonas gingivalis*		*Streptococcus mutans*	
		*Normality test**	*Comparison test***	*Normality test**	*Comparison test****
Mouthwash formula + 10% bran extract	4	0.001	0.000	0.130*	0.000
Mouthwash A	4	0.024		0.155*	
Distilled water	4	0.024		0.272*	

**Shapiro Wilk test: p>0.05; data distribution normal*
*** Kruskal Wallis: p<0.05; significant*
**** One-Way ANOVA: p<0.05; significant*

Table 6 : Post hoc statistic results of black rice bran inhibition zone for *Porphyromonas gingivalis* and *Streptococcus mutans*

Groups (i)	Comparison (j)	*Porphyromonas gingivalis*		*Streptococcus mutans*	
		Mean difference (i-j)	p-value*	Mean difference (i-j)	p-value**
Mouthwash formula + 10% bran extract	Mouthwash A	-0.45*	0.029	-1.15**	0.021
	Distilled water	2.70*	0.029	3.90**	0.000
Mouthwash A	Distilled water	3.15*	0.029	5.05**	0.000

**Mann Whitney: p<0.05; significant*
***LSD: p<0.05; significant*