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Soaked Water of Rice Straw Ash and *Ziziphus mauritiana* Leaf Extract as Disinfectant: A Microbiological Assessment

Yulia[1]*, Nurul Amaliyah[2]

[1] Department of Environmental Health, Politeknik Kesehatan Kemenkes Pontianak, Pontianak, West Kalimantan, Indonesia
[2] Department of Environmental Health, Politeknik Kesehatan Kemenkes Semarang, Semarang, Central Java, Indonesia

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**Corresponding author*
Email: *yuliaamok@gmail.com*

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ABSTRACT
Commercial dishwashing liquids contain chemicals that are extremely toxic to humans and the environment, hence alternative dishwashing using natural compounds is urgent. The purpose of this study was to evaluate the effect of a combination formula of rice straw ash and Ziziphus mauritiana leaf extract (JEBI) in reducing germs colonies per food equipment. This experimental study has a pre-post-test design with a control group. The variations of the JEBI combination used are 1:1, 1:2, 1:3, 2:1, and 3:1. Another treatment is using a commercial dishwasher brand "X" and only running water. A total of 210 cutleries (plates and bowls) from 30 restaurants in six districts were taken randomly—calculation of bacterial colonies using the Total Plate Count Agar method. The results were analyzed using one-way ANOVA to compare the effect between treatment groups on germ counts. Overall, a significant difference between the JEBI combination and the number of germs on food equipment was present in this study ($p<0.001$). The 1:3 JEBI ratio reduced the highest number of germs (average 24.6 colonies/cm2), slightly better than using "X" dishwashing liquid (average 25.6 colonies/cm2). Additionally, all treatments gave a significant reduction of germs compared to washed cutleries without any disinfectant ($p<0.001$). These findings strengthen the potential disinfectant function of rice straw ash and Ziziphus mauritiana leaf extract (JEBI) against germs on cutleries. Further studies with different ratio formulas and better study designs are required to obtain better and more precise results.

Keywords: Rice Straw Ash, Ziziphus mauritiana Leaf Extract, Germs, Cutleries.

ABSTRAK
Cairan pencuci piring komersial mengandung bahan kimia yang sangat beracun bagi manusia dan lingkungan, sehingga, diperlukan alternatif pencuci piring dengan menggunakan senyawa alami. Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh formula kombinasi abu jerami padi dan ekstrak daun bidara (JEBI) dalam mengurangi koloni kuman per peralatan makanan. Penelitian eksperimental ini memiliki desain pre-post-test dengan kelompok kontrol. Variasi kombinasi JEBI yang digunakan adalah 1:1, 1:2, 1:3, 2:1, dan 3:1. Perlakuan lainnya adalah menggunakan mesin pencuci piring komersial merek "X" dan hanya air mengalir. Sebanyak 210 alat makan (piring dan mangkuk) dari 30 restoran di enam distrik diambil secara acak—perhitungan koloni bakteri menggunakan metode Total Plate Count Agar. Hasilnya dianalisis menggunakan ANOVA satu arah untuk membandingkan pengaruh antar kelompok perlakuan terhadap jumlah kuman. Secara keseluruhan, terdapat perbedaan yang signifikan antara kombinasi JEBI dan jumlah kuman pada peralatan makanan dalam penelitian ini ($p<0,001$). Rasio JEBI 1:3 menurunkan jumlah kuman tertinggi (rata-rata 24,6 koloni/cm2), sedikit lebih baik daripada menggunakan cairan pencuci piring "X" (rata-rata 25,6 koloni/cm2). Selain itu, semua perlakuan memberikan penurunan kuman yang signifikan dibandingkan dengan peralatan makan yang dicuci tanpa disinfektan ($p<0,001$). Temuan ini memperkuat potensi fungsi disinfektan abu jerami padi dan ekstrak daun bidara (JEBI) terhadap kuman pada peralatan makan. Penelitian lebih lanjut dengan formula rasio yang berbeda dan desain penelitian yang lebih baik diperlukan untuk mendapatkan hasil yang lebih baik dan lebih tepat.

Kata Kunci: Abu Jerami Padi, Ekstrak Daun Ziziphus mauritiana, Kuman, Alat Makan.

INTRODUCTION

Foodborne disease remains challenging for public health. CDC estimated that 48 million people suffered from foodborne illness each year, and 3,000 of them die (Centers for Disease Control and Prevention, 2022). In Indonesia, Public Health Emergency Operation Center reported 163 outbreak events of food poisoning with a Case Fatality Rate of 0.1% (Kementerian Kesehatan Republik Indonesia, 2018). Some factors may cause foodborne diseases, including food hygiene and sanitation.

It is well documented that foodborne disease frequently occurs neglect of hygiene and sanitation practices in food preparation (Lubis et al., 2019). Prevention of food contamination caused by pathogenic microorganisms during their manufacture, processing, and packaging has considerable importance to the occurrence of the disease. Dishwashing is a part of food processing that needs attention because it can cause cross-contamination. Thus, the use of detergent for washing dishes is very important to ensure the sanitation of food equipment and reduce or eliminate bacteria by no more than 100 colonies/cm2 on the surface (Menteri Kesehatan Republik Indonesia, 2011).

Many antibacterial products are specifically manufactured for the reduction of bacteria in dishwashing. These products include solid dish soap and dishwashing liquids. Additionally, in modern lifestyles, some people often use dishwashers. However, previous studies have caused increasing concern about the effect of chemical surfactants in dishwashing liquids can potentially harm the environment and be related to climate change (Pandey et al., 2014; Ramirez-Martinez et al., 2014). Therefore, new findings regarding the use of environmentally friendly compounds to sanitize cutleries and kitchen equipment are an urgent need.

Despite using detergent, Indonesian ancestors were familiar with using rice straw ash as a dishwasher for the past decades, as it is cheap and straightforward. Evidence supports that the high silica content in rice straw helps remove dirt on the cutlery surface (Kharel, Sharma, & Kandel, 2016; Hemalatha et al., 2018; Murdiono et al., 2022). Removing dirt does not necessarily remove germs. The presence of germs on utensils can be due to the non-optimal washing of cutlery. An antibacterial is needed to ensure cutleries are sanitized.

Several studies have investigated the antibacterial activity of Ziziphus mauritiana leaf extracts (Abalaka et al., 2010; Palejkar et al., 2012; Yahia et al., 2020). Ethanol extract from Ziziphus mauritiana leaves is proven to have antibacterial activity against bacteria with strong inhibition at concentrations of 50% and 70% (Mardhiyani et al., 2021). Similar study has shown an increase in antibacterial effectiveness in liquid soap preparations wi.th the addition of Ziziphus mauritiana leaf extract (Khoirunnisak et al., 2020). However, it still uses chemical compounds, such as sodium sulfate and detergent which may harm the environment.

As far as we know, no studies investigating the combination of two natural resources as an alternative dishwashing detergent have been carried out. In this study, the effects of a combination of rice straw ash and Ziziphus mauritiana leaf extract as an antibacterial dishwashing liquid were investigated under laboratory conditions. The efficacy of the antibacterial of this formula was assessed by counting the number of germs on the surface of cutleries in different restaurants.

RESEARCH METHODS

This randomized experimental study was conducted in Pontianak, West Kalimantan, from March to September 2022. A total of 30 restaurants located across six sub-districts in the area were selected based on their availability and the owners' willingness to participate. From each restaurant, 210 clean plates and bowls—ready for customer use—were randomly collected, amounting to a total sample used in the experimental procedures. The samples were divided into seven treatment groups, each with five repetitions, and appropriately labeled for identification and further analysis.

The treatments in this study involved five different combinations of soaked water of rice straw ash and *Ziziphus mauritiana* leaf extract (JEBI), with the following ratios: 1:1, 1:2, 2:1, 1:3, and 3:1. For example, the 1:1 ratio refers to 100 ml of soaked rice straw ash water combined with 100 ml of *Ziziphus mauritiana* extract. In addition to the herbal combinations, two control groups were included: one where dishes were rinsed using running water and another using a

commercial dishwashing liquid labelled "X." All washing treatments followed a consistent method, where each item was rinsed for approximately two minutes under running water.

Plant materials were prepared with care. Fresh *Ziziphus mauritiana* (bidara) leaves were harvested from Pontianak, washed, and dried before being ground into a fine powder using a food processor. The extraction was performed using 10 grams of leaf powder in 100 mL methanol, centrifuged at 350 rpm for 6 hours. The resulting solution was evaporated with a rotary evaporator to obtain a concentrated extract, which was then stored in a brown bottle at -4°C. Meanwhile, rice straw was collected from farmers in Pemangkat District, Sambas Regency, and dried in the sun for three days. It was then burned in a large pan to produce ash. Ten grams of this ash were soaked in one liter of distilled water for 24 hours, filtered through a cotton cloth, and stored in dark bottles for later use.

Samples were processed in the laboratory where the inner surfaces of the plates and bowls were swabbed using sterilized cotton sticks dipped in normal saline. Swabbing was performed by crossing two strokes at an angle from the edge of each item. After use, each cotton swab was placed back into a saline bottle, pressed against the wall to release liquid, and then reused for the next sample. All samples were labeled and stored properly for microbial analysis following protocols by Nahar & Mahyudin (2018).

Microbiological testing began by preparing six dilution tubes (10^{-1} to 10^{-6}) and seven Petri dishes (six for test groups and one for control). Each test tube was filled with 9 mL of saline, into which the specimens were serially diluted. One millilitre from each tube was poured into a corresponding Petri dish and combined with 15–20 mL of heated Plate Count Agar (PCA). The dishes were gently swirled to mix and then cooled until solidified. Incubation was conducted at 37°C for 48 hours in an inverted position to promote bacterial growth. The control group was prepared using phosphate-buffered saline and PCA alone.

Bacterial colony counting was carried out manually by two independent investigators. Each Colony Forming Unit (CFU) was marked on the Petri dish lid with a pen to distinguish counted colonies. The total number of colonies was then calculated by multiplying the count in one sector by the total number of sectors on the plate. This study only measured total colony numbers and did not identify specific pathogens.

For statistical analysis, the Kolmogorov-Smirnov test was used to assess the normality of the data. The One-Way ANOVA test was applied to determine the significance of differences between treatment groups, with a 95% confidence interval. A p-value of ≤ 0.05 was considered statistically significant. This analysis evaluated the antibacterial effectiveness of the various ratios of rice straw ash water and *Ziziphus mauritiana* extract on the cleanliness of dishware.

RESULTS

Table 1 shows that the germs were seen in all the sample groups but at different levels. The presence of germs was identified as higher among plates and bowls washed with running water only than in other treatments. In contrast, consistently lower germs have been shown in samples washed with a 1:3 JEBI combination (100 ml of soaked water of rice straw ash and 300 ml of *Ziziphus mauritiana* leaf extract).

Table 1. The average of germs identified on the surface of plates and bowls in 30 randomized restaurants in Pontianak, West Kalimantan

Sample Location	Running water	Detergent "X"	JEBI Combination				
			1:1	1:2	1:3	2:1	3:1
North Pontianak	223.6	43	56.8	46.8	37.6	63	76.9
East Pontianak	202.4	40.2	61.4	52.4	42.6	65	79.5
West Pontianak	193.6	33	54.6	45.4	35.6	58	71.9
Pontianak City	120	7.8	24.6	15.4	5.6	28	44.1
South Pontianak	126	13.8	30.6	21.4	11.6	34	50.1
Southeast Pontianak	128	15.8	33.6	24.4	14.6	37	53.7
Mean	165.6	25.6	43.6	34.3	24.6	47.5	62.7

***Description**: *JEBI: soaked water of rice straw ash and Ziziphus mauritiana leaf extract)*

Table 2. The effect of a combination formula of rice straw ash and *Ziziphus mauritiana* leaf extract on the number of germs

	Sum of squares	Mean	df	F	p-value
Between groups	439287,9	73214,649	6	45,670	<0.001*
Within groups	325435,6	1603,131	203		
	764723,5		209		

Description: **significant at p-value ≤ 0.05*

Table 2 shows that the data met the normality assumption, allowing for the use of one-way ANOVA. A one-way ANOVA was performed to compare the effect of combination formulas on the number of germs. The result revealed that there was a statistically significant difference in the mean of the germs (p<0.001).

Table 3. Comparison effect between combination formulas of rice straw ash and *Ziziphus mauritiana* leaf extract against the number of germs

Treatment A	Treatment B	p-value
Running water	Dishwashing liquid "X"	<0,001*
	JEBI combination of 1:1	<0,001*
	JEBI combination of 1:2	<0,001*
	JEBI combination of 1:3	<0,001*
	JEBI combination of 2:1	<0,001*
	JEBI combination of 2:1	<0,001*
Dishwashing liquid "X"	JEBI combination of 1:1	1,000
	JEBI combination of 1:2	0,748
	JEBI combination of 1:3	0,009
	JEBI combination of 2:1	1,000
	JEBI combination of 3:1	1,000
JEBI combination of 1:1	JEBI combination of 1:2	1,000
	JEBI combination of 1:3	1,000
	JEBI combination of 2:1	1,000
	JEBI combination of 3:1	1,000
JEBI combination of 1:2	JEBI combination of 1:3	1,000
	JEBI combination of 2:1	1,000
	JEBI combination of 3:1	0,585
JEBI combination of 1:3	JEBI combination of 2:1	0,138
	JEBI combination of 3:1	0,006*
JEBI combination of 2:1	JEBI combination of 3:1	1,000

Description: **significant at p-value ≤ 0.05; Bonferroni test*

Table 3 shows that the Bonferroni test for multiple comparisons found that the mean value of germs was significantly different between running water and detergent "X", and between running water and all other combinations (p<0.001). There was no statistically significant difference in mean germ number between detergent "X" and all combined formulas of rice straw ash and *Ziziphus mauritiana* leaf extract (p>0.05) or between one formula and another (p>0.05), except between JEBI combination 3:1 and 1:3 (p= 0.006).

DISCUSSION

In the present study, a parameter of germs' number was evaluated through a laboratory test with Total Plate Count Agar (PCA) as a microbiological growth medium. Few studies have previously measured the parameter for different reasons as a microbiological method used to assess the overall quality of foods (Mendonca, Juneja, & Daraba, 2014; Hazan et al., 2012). The

results of the present study showed that high bacterial numbers were observed in cleaning utensils washed using running water only (>100 colonies/cm^2). This figure exceeds the threshold value for the germ rate on cutlery that comes in direct contact with served food (Menteri Kesehatan Republik Indonesia, 2011). The presence of these bacteria in plates and cutleries could create health hazards when they are ingested through microbial cross-contamination. Microbial cross-contamination is a term that refers to the transfer, direct or indirect, of microorganisms (bacteria, viruses, parasites, or fungi) from a contaminated item to a non-contaminated item. However, many people have less awareness that food contact surfaces and utensils are contributing factors to the occurrence of foodborne illness.

A number of factors may contribute to influencing microbial contamination with food-contact surfaces, mainly due to poor hygiene and sanitation (Birawida et al., 2019; Minnesota Department of Health, 2023). Contaminated food, improperly hand-washed, and sanitized evidence increase the bacterial number (Suryani & Wibowo, 2019). Poor hygiene and sanitation of cutlery may be a growth medium and spread bacteria. Also, equipment washing has a significant vulnerability to bacterial contamination in food processing equipment (Akwila et al., 2022). It clearly indicates the necessity for proper cleansing of all eating and drinking utensils. This supported the result that the number of germs on samples washed using all different treatments reduced significantly compared to those washed with running water without any treatment as a disinfectant ($p<0.001$).

In this study, the lowest germ count was seen in cutlery washed using the JEBI 1:3 combination with a mean of 24.6 CFU, this result was even slightly lower than the use of commercial detergents. Although dishwashing liquid is one of the most effective stain busters, specifically catered to remove grease, hazard issues rise. Most dishwashing liquids contain several toxic chemicals, such as formaldehyde and phosphates which can have a serious impact on the skin and environment safe (Gholami et al., 2016; Kusumaningrum et al., 2002). In addition, the fragrance can be extremely harmful in concentrated forms. In short, people can never be sure how efficiently the detergent components are cleaned from the eating and drinking equipment. All these factors can determine that even though there is a slight difference in the average total germ count between the two groups, it can be assumed that the alternative dishwashing liquid used is feasible to compete in terms of the hygiene quality produced.

An interesting finding was the combination formula with a higher ratio of *Ziziphus mauritiana* leaf extract will have the potential to reduce the number of germs continuously. This result aligns with previous studies indicating that the leaves extract contains significant amounts of phenolic and flavonoid compounds, which are believed to contribute to the effectiveness of silver nanoparticles in against germs (Asimuddin et al., 2020). In contrast, with the higher ratio of soaked water of rice straw ash, the number of germs observed increased. It is supported by the previous antimicrobial studies that a fraction of *Ziziphus mauritiana* leaf extract was active against *E. coli* and *S. aureus* (Abalaka et al., 2010; Haeria, Dhuha, & Habra, 2018; Yahia et al., 2020). Meanwhile, there have not been published studies proving the antibacterial activity in rice straws yet. This should be due to the fact that rice straw ash contains silica and cellulose which are usually used as a raw component of detergent and soap, hence, its function is assumed not to remove bacteria but rather to remove the remaining dirt on cutlery (Hemalatha et al., 2018; Murdiono et al., 2022).

Many dishwashing products easily make eating and drinking equipment clean, yet bacteria may be present in large numbers and consequently cause foodborne disease due to cross-contamination (National Institute for Public Health and the Environment (RIVM), The Netherlands, Iulietto, & Evers, 2020). Antibacterial activities against *E.coli* and *S. aureus* are essential, more importantly, using an eco-friendly compound. The antibacterial test showed that 40% was the optimal concentration of ethanol extract of *Ziziphus mauritiana* leaves against *S. aureus*, while insignificant results were in *E. coli* (Muharrami et al., 2019). However, different results of bacterial growth on utensils' surface may appear due to some factors, including surface material properties, contact pressure, and contact time (Purohit, 2009).

Given that the components of the JEBI formula are environmentally friendly, non-toxic, and inexpensive, it could be used as an alternative to commercial dishwashing liquid. The primary limitation of this experimental study is that it does not explain the efforts to control the

confounding factors that have the potential to cause biased results. Moreover, this study was performed using a laboratory scale, therefore, the true application may need further investigation. Additionally, the analysis of antibacterial activities in the formula is required to strengthen the findings based on the reduction of germs.

CONCLUSION

All formulas combination of soaked water of rice straw ash and *Ziziphus mauritiana* (JEBI) leaf extract had proven to a significant reduction of germs on eating equipment, in this study were plates and bowls ($p<0.001$). The lowest germs were counted on samples using JEBI combination 1:3, with a mean of 24.6 colonies/cm^2, slightly lower than dishwashing liquid "X". More ratio of *Ziziphus mauritiana* leaf extract in this experimented solution (JEBI 1:3) had a better effect against germs than more ratio of soaked water of rice straw ash. Overall, the findings strengthen the potential function of the JEBI product as an alternative dishwashing liquid. Further studies need to conduct in different formula ratios to find a more effective germs reduction and a better study design.

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