DETERMINATION OF THE DIFFERENCE HANDRUB BOILED BETEL LEAF (*PIPER BETLE*) COMPARED ALCOHOL ON THE PERCENTAGE OF REDUCING THE NUMBER OF MICROORGANISM COLONY

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Abstract

Betel leaf (Piper Betle Linn) is one of the natural ingredients that can be used as a substitute for alcohol in hand sanitizers. This study aims to analyze the differences in handrubs from boiled betel leaves (Piper betle) compared to 70% alcohol on the percentage of reduction in the number of microorganism colonies. The type of research conducted was experimental research with a Pre Post Test Group Design research design. Sampling was carried out by Concutive Sampling from Students of the Faculty of Medicine, Sultan Agung Islamic University, Semarang through the glove juice method with a total of 20 samples per group. Data on the percentage of reduction in the number of microorganism colonies between the handrub group boiled betel leaves (Piper betle) compared to 70% alcohol were analyzed using Mann Whitney. The results of the study found that 70% alcohol handrub was better than handrub boiled betel leaves (Piper betle Linn) with a difference in handrub boiled betel leaves of 39.46% and handrub 70% alcohol of 59.18%. So it is concluded that there is a significant difference between handrub boiled betel leaves (Piper betle) compared to 70% alcohol.

Keywords: handrub; betel leaves; 70% alcohol

1. INTRODUCTION

Hand hygiene is one of the most crucial and effective measures in preventing the transmission of infections. Infections can enter the body through the respiratory tract, gastrointestinal tract, urinary tract, eyes, genitals, or direct skin contact. The spread of infection occurs through direct or indirect contact, airborne transmission, droplets, vectors (such as insects or animals), and contaminated food and beverages (Kemenkes RI, 2020b). According to Ratmaja *et al.*, (2023), the most common mode of infection transmission in humans is through direct hand contact, as hands are frequently used in daily activities. Hand hygiene can be maintained by washing hands with soap and running water or using alcohol-based products (Menteri Kesehatan Republik Indonesia, 2017). The COVID-19 pandemic has heightened the urgency of preventing disease transmission. Disease transmission occurs when a person's hands come into contact with contaminated surfaces, increasing public awareness of the importance of hand hygiene (Yatmo *et al*, 2020). Various methods can be employed to maintain hand hygiene, including washing hands with running water and antiseptic soap. Additionally, hand hygiene can be maintained using alcohol-containing solutions (alcohol-based hand rubs) and natural antiseptics such as betel leaves (Piper betle) and aloe vera (Kemenkes RI, 2020).

Handwashing not only cleanses the hands but also reduces microorganisms, including pathogenic and non-pathogenic bacteria that commonly colonize the hands, such as *Staphylococcus aureus*, *Salmonella sp,* and *Bassilus cereus* (Hillier, 2020).

Alcohol-based solutions or gels containing 70% ethanol (hand rubs) are considered effective in preventing infection transmission on hands compared to soap and water. However, alcohol-based hand rubs do not eliminate certain bacteria, such as *Clostridium difficile*, which is a causative agent of diarrhea in humans. Therefore, in such cases, handwashing with soap and water is preferred (Hillier, 2020). Alcohol-based hand rubs with 70% ethanol concentration are more effective against emerging pathogens, including *SARS-CoV-2, Candida auris*, and other coronaviruses. Alcohol-based hand sanitizers with ethanol concentrations ranging from 70% to 95%, combined with specific active ingredients, can inhibit the transmission of norovirus and adenovirus (Boyce, 2023). Ethanol concentrations exceeding 80% may reduce bacterial protein denaturation processes, thereby disrupting bacterial cell metabolism (Ratmaja *et al*., 2023).

Washing hands with soap and antiseptic solutions is considered effective in removing dirt and eliminating disease-causing pathogens present on the hands (Hillier, 2020). However, the availability of sinks and handwashing stations in public spaces and residential areas is often limited, reducing the effectiveness of this method and lowering compliance with hand hygiene practices. Innovative waterless hand sanitizing products are now widely available on the market and are commonly known as hand sanitizers (Herawati *et al*., 2023). Unbranded hand sanitizers are sold freely, with some products not registered on Badan Pengawas Obat dan Makanan (BPOM) while others have undergone safety testing and are officially registered on BPOM (Herawati *et al*., 2023).

Hand sanitizers and hand rubs predominantly contain synthetic and chemical ingredients, which may pose environmental health risks. Ingredients such as alcohol and triclosan, commonly found in hand sanitizers, may cause skin irritation and a burning sensation in individuals with sensitive skin when used frequently. Green betel leaf (*Piper betle Linn*) is a natural ingredient that can serve as an alternative to alcohol in hand sanitizers (Hapsari *et al*., 2015). Betel leaves (*Piper betle Linn*) is a climbing plant from the Piperaceae genus, originally found in central and eastern Malaysia, before spreading to East Africa and other tropical Asian countries, including Indonesia. Betel leaves have been used in traditional medicine for treating conditions such as colds, bronchial asthma, coughs, stomach aches, rheumatism, bad breath, body odor, gum swelling, mouth ulcers, conjunctivitis, and wound healing. Betel leaves contain alkaloids, flavonoids, glycosides, tannins, terpenes, chavicol, safrole, and essential oils, which are believed to have antimicrobial properties against various microorganisms.

2. METHOD

The study was conducted at the Microbiology Laboratory of the Faculty of Medicine, Sultan Agung Islamic University (UNISSULA). The study population consisted class of 2021 medical students from Sultan Agung Islamic University.

This study employed the *glove juice* method, in which participants immersed their hands in sterile gloves containing 50 mL of 0.9% NaCl solution. A tourniquet was applied to the wrist, and the hand was massaged for one minute. The tourniquet was then released, and 1 mL of the NaCl solution was aspirated using a syringe and transferred into a sterile tube. A calibrated loop (10 µL) was used to streak the sample onto a solid nutrient agar medium in a biosafety cabinet (BSC). The plates were labeled accordingly as post-hand rub with alcohol or boiled betel leaf solution. The samples were subsequently incubated at 37°C for 24 hours, after which the number of microorganism colonies was counted.

Microorganism Sample Collection

All equipment used in this study was sterilized using an autoclave at 121°C for 20 minutes. The study involved 20 participants, with separate testing days for the post-hand rub conditions using 70% alcohol and boiled betel leaf-based hand rub. Before handwashing, participants immersed their hands in sterile gloves containing 50 mL of 0.9% NaCl solution. A tourniquet was applied to the wrist, and the hand was massaged for one minute. The tourniquet was then released, and 1 mL of the solution was aspirated using a syringe and transferred into a sterile tube. The sample was streaked onto a solid nutrient agar medium in the BSC using a calibrated loop (10 µL). The plates were labeled accordingly as pre-hand rub with alcohol or boiled betel leaf solution (Qothrunnadaa, 2024).

Either 70% alcohol-based hand rub or boiled betel leaf-based hand rub was applied to the participant's palms (approximately 3–5 cc) and rubbed thoroughly over the entire hand surface for 20–30 seconds, following the WHO hand hygiene procedure. Once the hands were dry, participants were instructed to wear sterile gloves again and immerse their hands in 50 mL of 0.9% NaCl solution. The hand was massaged for one minute, after which 1 mL of the solution was aspirated using a syringe and transferred into a sterile tube. The tubes were labeled as post-hand rub with alcohol or boiled betel leaf solution (Qothrunnadaa, 2024).

The Measurement of Microorganism Colony Reduction Percentage

The number of microorganism colonies growing on petri dishes was measured using Colony-Forming Units (CFU), with the formula namely percentage reduction in colony count = (colony count before treatment – colony count after treatment / colony count before treatment x 100%.

Preparation of Boiled Betel Leaf-Based Hand Rub

The boiled betel leaf-based hand rub was prepared by collecting all necessary materials and equipment. A total of 10 grams of thoroughly washed green betel leaves were chopped into small pieces. The chopped leaves were added to a pot containing 100 mL of hot distilled water and boiled for 15–30 minutes at 100°C. The solution was then allowed to stand for 30 minutes before being filtered into a spray bottle.

The hand rub was tested for microbial contamination by taking a sample using an inoculating loop and culturing it on nutrient agar medium daily until contamination was observed. This allowed for the determination of the shelf life of the hand rub.

3. RESULTS AND DISCUSSION PERCENTAGE REDUCTION IN MICROORGANISM COLONY COUNT

The number of microorganism colonies was counted before and after the application of hand rubs. Measurements were performed using the glove juice method. Data on the reduction in microorganism colony count pre- and post- intervention, along with microorganism identification, are presented in the tables 1.

Table 1. Percentage Reduction in Microorganism Colony Count in the Boiled Betel Leaf Hand Rub Group

Number	pH	Number of Microorganisms (CFU/ml)		Bacterial Microorganisms		Effectiveness (% Reduction)
		Pre	Post	Pre	Post	
1.	5.0	47	36	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	23,40
2.	5.0	64	40	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	37,50
3.	5.0	51	48	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	5,88
4.	5.0	38	30	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	21,05
5.	5.0	64	59	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	7,81
6.	5.0	72	53	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	26,39
7.	5.0	64	47	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	26,56
8.	5.0	67	5	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	92,54
9.	5.0	52	40	*Coagulase negative Bacillus sp*	*Staphylococcus Coagulase negative*	23,08
10.	5.0	61	24	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	60,66
11.	5.0	60	31	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	48,33
12.	5.0	53	31	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	41,51
13.	5.0	53	8	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	84,91

14.	5.0	35	13	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	62,86
15.	5.0	45	40	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	11,11
16.	5.0	114	59	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	48,25
17.	5.0	35	21	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	40,00
18.	5.0	125	51	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	59,20
19.	5.0	60	55	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	8,33
20.	5.0	105	42	*Bacillus sp Coagulase negative*	*Staphylococcus Coagulase negative*	60,00
Average Effectiveness						39,46%

The boiled betel leaf (*Piper betle Linn*) hand rub group exhibited an average effectiveness in reducing microorganism colony count by 39.46%. The bacterial species identified on the agar media were *Bacillus sp*. and Coagulase-negative *Staphylococcus* (CoNS).

Table 2. Percentage Reduction in Microorganism Colony Count in the 70% Alcohol Hand Rub Group

Number	**pH**	**Number of Microorganisms (CFU/ml)**		**Bacterial Microorganisms**		**Effectiveness (% Reduction)**
		Pre	**Post**	**Pre**	**Post**	
1.	6.0	217	64	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	70,51
2.	6.0	80	40	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	50,00
3.	6.0	185	90	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	51,35

4.	6.0	63	27	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	57,14
5.	6.0	172	82	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	52,33
6.	6.0	216	87	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	59,72
7.	6.0	109	48	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	55,96
8.	6.0	142	57	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	59,86
9.	6.0	188	69	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	63,30
10.	6.0	127	39	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	69,29
11.	6.0	70	32	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	54,29
12.	6.0	99	43	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	56,57
13.	6.0	134	64	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	52,24
14.	6.0	160	69	*Coagulase Negative*	*Staphylococcus Coagulase Negative*	56,88
15.	6.0	108	50	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	53,70
16.	6.0	98	36	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	63,27
17.	6.0	105	40	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	61,90
18.	6.0	190	68	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	64,21
19.	6.0	218	54	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	75,23
20.	6.0	127	56	*Bacillus sp Coagulase Negative*	*Staphylococcus Coagulase Negative*	55,91
				Average Effectiveness		59,18

The 70% alcohol hand rub group demonstrated an average effectiveness in reducing microorganism colony count by 59.18%. The bacterial species identified on agar media were *Bacillus sp*. and Coagulase negative *Staphylococcus* (CoNS).

Hypothesis testing using the Mann-Whitney test was conducted to determine the difference in effectiveness between boiled betel leaf hand rub (*Piper betle Linn*) and 70% alcohol hand rub in reducing microorganism colony count.

Table 3. Mann-Whitney Test Results

	Mann whitney
Assymp. Sig. (2-tailed)	0,003

Based on Table 3, the Mann-Whitney test yielded a p-value of 0.003 ($p < 0.05$), indicating statistically significant difference between the boiled betel leaf hand rub and the 70% alcohol hand rub in reducing microorganism colony counts. The 70% alcohol hand rub was found to be more effective than the boiled betel leaf hand rub (*Piper betle Linn*) in reducing microorganism colonies.

This study obtained a p-value of 0.003 ($p < 0.05$), indicating a statistically significant difference in reducing microorganism colony counts. The 70% alcohol hand rub was more effective than the boiled betel leaf hand rub (*Piper betle Linn*), as demonstrated by statistical data showing an average microorganism colony reduction of 59.18% for the alcohol hand rub, compared to 39.46% for the boiled betel leaf hand rub.

In this study, the 70% alcohol-based hand rub had a higher average percentage reduction in microorganism colony count. This finding aligns with the study by Ratmaja *et al*., (2023) which stated that hand sanitizer containing 70% alcohol is the most effective for eliminating bacteria on hands. The alcohol concentration used in this study falls within the optimal antiseptic range (60–80%), known for its bactericidal properties, which act rapidly against Gram-positive and Gram-negative bacteria, certain fungi, and non-enveloped viruses. Alcohol exerts its antimicrobial effect by denaturing bacterial proteins and disrupting the bacterial cytoplasmic membrane (Ratmaja *et al*., 2023).

The evaluation of the hand rub formulation in this study revealed that boiled betel leaf hand rub, composed of 10 grams of betel leaves mixed with 100 mL of distilled water, maintained its antibacterial properties for four days when stored in a refrigerator. The betel leaf hand rub was found to be non-sticky, smooth on the skin, and did not cause irritation among study participants, demonstrating its potential as a natural hand rub alternative.

The boiled betel leaf hand rub demonstrated a microorganism colony reduction effectiveness of 39.46%, which aligns with the study conducted by Ekawati *et al* (2021) who reported that hand rub or hand sanitizer containing boiled betel leaf extract effectively reduced bacterial counts on the palms. Similarly, a study by Go *et al* (2022) confirmed the antibacterial potential of boiled betel leaf extract, supporting its use as a natural hand rub ingredient. An ideal hand rub formulation should have a balanced viscosity—neither too thick nor too watery—as this significantly influences its antibacterial effectiveness (Sutiswa *et al*., 2022).

Betel leaves contain 1–4.2% essential oils, along with several bioactive compounds such as saponins, alkaloids, tannins, flavonoids, chavicol, glycosides, and phenols, which contribute to their antibacterial properties (Hapsari *et al*., 2015). The antibacterial mechanism of betel leaf extract involves damaging the bacterial plasma membrane, coagulating nucleotides, and significantly reducing bacterial acid production. This mechanism depends on the specific bioactive compounds present in the boiled betel leaf extract (Widiyastuti dkk et al., 2020).

The pH of the boiled betel leaf extract in this study was measured at pH 6, which is consistent with Sutiswa *et al*., (2022) who reported that boiled betel leaf extract had a pH of 5.8, meeting the ideal pH range for hand rub formulations (4.5–7.0). This pH compatibility ensures that the hand rub is comfortable for use and does not cause skin irritation. An optimal hand rub formulation must also maintain appropriate consistency, as this factor influences its antibacterial effectiveness (Sutiswa *et al*., 2022).

4. CONCLUSION

Based on the findings of this study on the differences between boiled betel leaf (*Piper betle*) hand rub and 70% alcohol hand rub in reducing microorganism colony counts, the following conclusions were drawn. The average percentage reduction in microorganism colony count for the boiled betel leaf hand rub (*Piper betle Linn*) was 39.46%, meanwhile for the 70% alcohol hand rub was 59.18%. There was a significant difference in effectiveness between boiled betel leaf hand rub (*Piper betle Linn*) and 70% alcohol hand rub, with 70% alcohol proving to be more effective.

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