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Antimicrobial Effectiveness of Betel Leaf Extract (*Piper betle Linn*) compared to *Triclosan*, *Chlorhexidine gluonate*, on *Staphylococcus aureus* and *Streptococcus pyogenes* as Hand Washing Soap

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ABSTRACT

Hand washing is an important key factor to prevent transmission of infectious agents to patients. Antiseptics like *triclosan* and *chlorhexidine gluconate* are frequently used for both skin antisepsis. Natural products like betel leaf *(Piper Betle Linn*) have emerged with fewer side effects so that they can be considered safer, which has antiseptic properties. All Parts have antiseptic properties, including the essential oil whose main component consists of *bethel phenol* and several of its derivatives including *cavibetol, alkaloids, flavonoids, tripenoids or steroids, saponins, terpenes, phenylpropane, terpinene, diastase, tannins*, etc. The purpose of this research is to examine the effectiveness of betel leaf extract soap as an alternative to natural hand soap with *chlorhexidine* and *triclosan*. This research was conducted on oral and maxillofacial surgery residents who will become operators at the oral surgery clinic of Faculty of Dental Medicine of Airlangga University Surabaya. The operator's hands is swabbed before and after handwashing using three different kind of soap. Based on the Kruskal wallis overall there was no significant difference in the status of the number of bacteria between the *chlorhexidine, triclosan*, and betel leaf groups (p-0.368 or p>0.05). The significance value of the difference in the Mann Whitney test which was bigger than 0.05 (p>0.05) which is no difference colony count bacteria in every compared group of research. Conclusion of this research is betel leaf extract in the form of hand soap is as effective as the other two soaps which are *chlorhexidine* and *triclosan* which are exist already in market.

1. INTRODUCTION

Every procedure that involves the potential for the entry of pathogens into the patient's body uses aseptic

technique as a way of care. Medical aseptic technique and surgical aseptic technique are the two different types of aseptic technique. Medical asepsis is a technique or process used to lessen the amount of bacteria in an object and lessen the likelihood that these microorganisms will spread. One example of medical aseptic is hand washing. Hand washing is an important key factor to prevent transmission of infectious agents to patients. While sterile or aseptic surgical techniques also involve methods for removing microorganisms from a space. If nurses fail to perform aseptic measures, one of the repercussions that may occur is a nasocomial infection [1], [2]. According to WHO standards, using the five-moment rule for hand hygiene whenever working with patients in hospitals is the most effective strategy to prevent nosocomial infections [3]. By washing hands the operator can eliminate hand interference, eliminate unpleasant odors that stick to the hands, prevent the spread of cross infection, keep hands sterile, provide a fresh and clean feeling [4]. Washing hands are more productive when they are washed with soap and water because doing so kills infections chemically as well as mechanically [5]. WHO recommends 7 steps for washing hands. The first step is rubbing the palms with soap, the second rubbing the back of the palms alternately from the right then the left, the third between the fingers also using soap, the four fingertips are washed clean, the fifth rub and rotate the thumb alternately, the sixth is to place all the fingertips on the palm and then clean by rubbing gently using running water, and the seventh holding the left wrist with the right hand and vice versa in a circular motion [6]. The research by [7] said that there are 5-step technique which included (1) between fingers, (2) backs of hands, (3) backs of fingers, (4) fingertips and (5) thumbs that students with intellectual disabilities can more easily follow and comprehend the five steps to washing their hands properly by using this method.

In healthcare settings, antiseptics like *triclosan* and *chlorhexidine gluconate* are frequently used for both skin antisepsis and *Staphylococcus aureus* decolonization [8]. Several natural products have emerged with fewer side effects so that they can be considered safer, one of which is a plant that grows in Indonesia or Southeast Asia, which has antiseptic properties, namely betel leaf (*Piper Betle Linn*). Bacteria cannot grow and die, while the betel leaf contains approximately 4.2% of the essential oil present in the betel leaf which is suitable for use as an antiseptic and anti-inflammatory because the essential oil in betel leaf contains *kavikol* and *cavibetol* compounds which are phenol derivatives which have antibacterial power is five times that of phenol, while tannins are compounds that clinically have antibacterial abilities that can be used for the treatment of skin infections [9].

Staphylococcus aureus can be moderately inhibited from growing by an ethanol extract of green betel leaves [10]. At doses of 15%, 20%, and 25%, the ethanol extract of betel leaf demonstrated a growth inhibition of *Streptococcus mutans* bacterium [11]. Green betel leaf extract has the potential as an antibacterial *Staphylococcus aureus* ($P<0.05$). The optimal concentration of betel leaf ethanol extract as an antibacterial for *Staphylococcus aureus* was 40%, which resulted in an average inhibition zone of 3.01 mm (weak inhibition) [12]. Leaf extracts and purified compounds of *P. betel* have antiseptic, antibacterial, antioxidant, anti-inflammatory, anti-cancer, and immunomodulatory efficacy [13]. The number of virus colonies that had developed on the bacterial growth medium was reduced by 50% as a result of using gel with a concentration of 15%. The 20 percent concentration of gel extract exhibited the same antiviral activity as *triclosan*, which served as the positive control. but there was no evidence of microbial development in the 25% gel extract concentration [14]. An opportunistic bacterium called *Staphylococcus aureus* typically colonizes the human anterior nares. However, this bacterium is also one of the main culprits of sepsis and endocarditis, two life-threatening bloodstream infections [15]. While the gram-positive bacterial pathogen that affects humans is *Streptococcus pyogenes*. Asymptomatic carriers, mild, superficial infections of the skin and mucous membranes, and signs of systemic purulent invasive toxic illness are all examples of interactions with humans [16], [17]. Therefore, this journal assesses whether there is a difference in the antimicrobial effectiveness of betel leaf soap (piper betle linn) 25%, triclosan 0.3%, and chlorhexidine 4% on *Staphylococcus aureus* and

Streptococcus pyogenes bacteria as a means of washing hands.

The purpose of this study was to determine the antimicrobial effectiveness of soap containing 25% betel leaf extract (*piper betle linn*) against *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria as a means of hand washing soap and to obtain an alternative type of antimicrobial soap that is effective as hand scrub against *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria. So that the benefits are obtained, it is found that there is an alternative hand scrub that can be used as a substitute for standard soap, namely 4% *chlorhexidine gluconate* or 0.3% *triclosan*.

2. Research Method

This research is a quasi-experimental clinical research with the pre and post test control group design. In this research, we used participants of the Resident of Faculty of Dental Medicine Airlangga University Oral and Maxillofacial Surgery with a 11 of minimum samples using the Lemeshow formula according to Steel & Torrie. This research using 3 groups consists of liquid antimicrobial hand soap agents containing 4% *chlohexidine gluconate*, 0.3% *triclosan*, and 25% betel leaf extract (*piper betle linn*) with the hand washing method (WHO). The number of bacterial colonies calculated based on their density and isolated, namely *Staphylococcus Aureus* and *Streptococcus sp* after washing hands. The place for washing hands in the minor operating room of the Faculty of Dental Medicine University of A oral surgery clinic, hand washing time, antiseptic application techniques, sampling and storage techniques, *Staphylococcus aureus* bacteria isolation techniques, and colony counting techniques germs can affect the result of this research.

The tools used in this research include UV-1201 spectrophotometer, sterile cotton stick, incubator, brander spirit, and test tubes. The materials used in this research included 4% *chlorexidine glutamate* liquid soap, 0.3% *triclosan* liquid soap, liquid soap containing 25% betel leaf extract, 5 cc Brain Hearth Infusion (BHIB), and Blood Agar (BA). The production of betel leaf extract was carried out using the soxhletation method by UPT Materia Medica, Batu, Health Office of East Java province. Meanwhile, soap is made by the Faculty of Pharmacy, Widya Mandala Catholic University, Surabaya with ingredients consisting of betel leaf extract, *sodium lauryl sulfate*, NaCl solution, and *Oleum mentha piperithae*. After the betel leaf extract soap was made, the Resident of Oral and Maxillofacial Surgery, totaling 12 people, were asked to wash their hands (WHO) with 3 types of soap with 1 type of soap every day (*chrolexidine gluconate* 4% the first day, *triclosan* 0.3% the second day, leaf extract betel 25% third day). Each sample was swab in the hands before and after washing hands with a cotton stick which was then swabbed on agar media and implanted in BHI liquid media. Then the BHI liquid media was stored in an incubator for 1 x 24 hours at 37 degrees Celsius. Then the culture results were identified macroscopically and microscopically with salt staining by viewing under a microscope with 100-1000 times magnification with the coloni counting unit method in duplicate for each sample before and after treatment.

This research was conducted in the minor operating room of the Department of Oral and Maxillofacial Surgery Faculty of Dental Medicine Airlangga University as specimen collection, the Microbiology Laboratory of Faculty of Dental Medicine Airlangga University as a place for isolating Staphylococcus aureus bacteria, and the Microbiology Laboratory of medical analysts of Faculty of medicine Airlangga Univeristy for counting the number of bacterial colonies (Colony Forming Unit).

3. Result

Based on the results of the observation on the effectiveness of antimicrobials which were divided into groups of 25% betel leaf soap (*piper betle linn*), *triclosan* 0.3%, and *chlorhexidine* 4% against *Staphylococcus aureus* and *Streptococcus pyogenes* bacteria as a means of washing hands, with 12 samples each, the following results

were obtained.

Table 5.1 distribution of the status of the number of bacteria in the study group.

Group	Status after washing hands	Frequency	Percentage
Chlorhexidine 4%	Decrease	8	66,7%
	Increase	4	33,3%
	Total	12	100%
Triclosan 0,3%	Decrease	5	41,7%
	Increase	7	58,3%
	Total	12	100%
Betel leaf soap 25%	Decrease	8	66,7%
	Increase	4	33,3%
	Total	12	100%

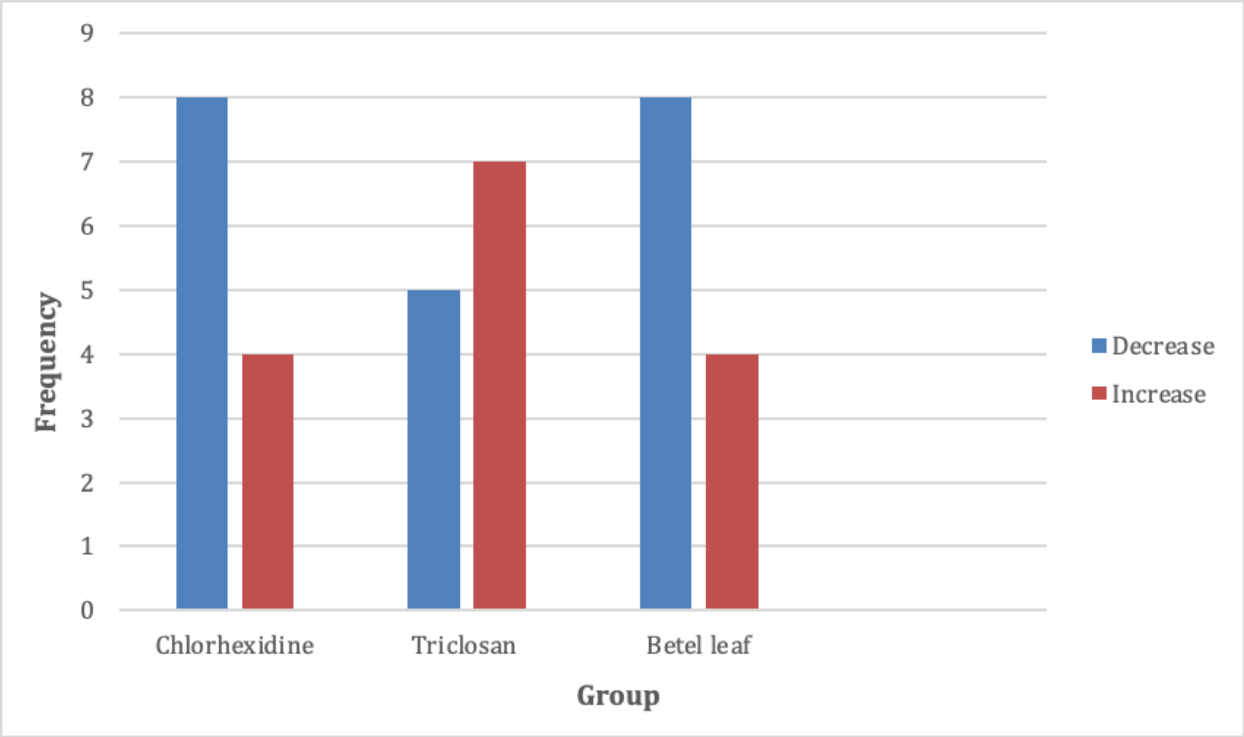

Figure 5.1 Distribution of the status of the number of bacteria in each study group.

From the table, it can be seen that the frequency of bacterial count status was higher in the decreased status in the *chlorhexidine* and betel leaf groups. Meanwhile, in *triclosan*, the status is increased which is higher than decreasing.

Table 5.2 Test of different bacterial count status between each research group using Mann Whitney and Kruskal Wallis tests.

	Chlorhexidin	*Triclosan*	Betel leaf	Significance test Kruskal wallis
Chlorhexidin	-	0,229	1,000	p = 0,368
Triclosan		-	0,229	
Betel leaf			-	

In the table, the Kruskal Wallis difference test has been carried out in order to see a comparison of the status of the number of bacteria. However, overall there was no significant difference between the status of the number of bacteria in the *chlorhexidine*, *triclosan*, and betel leaf groups (p=0.368 or p>0.05), then it can be seen that the significance value of the Mann Whitney test difference is greater than 0.05 which can it was concluded that there was no difference in the status of the number of bacteria in each comparison of the study groups.

4. Discussion

Betel leaf (*Piper betle Linn*) is one of the plants used by the Indonesian people for traditional herbal medicine. Green betel leaf ethylacetate extract contains antibacterial components consisting of phenolic compounds and their derivatives. Betel leaf extract contains betel oil, namely phenol betel and kavikol which functions as natural antiseptics that can ward off bacteria [18]. Piper Betel leaf and chlorhexidine are considered effective in reducing the number of bacteria, while *triclosan* is still less effective because bacteria tend to be resistant, triclosan itself is already present in many soap products and so on. The limitations of this study are only for *Streptococcus* and *Staphylococcus aureus* bacteria, while many bacteria found on hands are Pseudomonas spp, Enterobacteriaceae and *Enterococcus* spp. [19]. In a study conducted by [14], Piper betel leaf was effective in being antibacterial and reducing the number of *Staphylococcus aureus* bacteria on hands. This is indicated by research which has the result that the inhibition of growth of S. aureus by betel nut extract with a concentration between 0.625% to 0.75% is 14.67.

In accordance with the results of research and analysis that has been carried out, it is found that there are similarities between the results of *chlorhexidine* and betel leaf. While in *triclosan* obtained relatively unfavorable results. The test results showed that there was no significant difference in the status of the number of intermediary bacteria in the *chlorhexidine*, *triclosan*, and betel leaf groups (p=0.368 or p>0.05). Furthermore, from the significance value of the difference in the Mann Whitney test, which is greater than 0.05 (p>0.05), it states that there is no difference in the status of the number of bacteria in each comparison between the study groups.

Liquid soap on the market mostly contains synthetic ingredients such as *Sodium Lauryl Sulfate* (SLS), and *triclosan* which have a negative effect on human skin. The *ethanol* extract of red betel leaf (P. *crocatum* Ruiz & Pav.) has been shown to have antibacterial activity against Gram-positive bacteria, namely *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacteria, namely *Escherichia coli* and *Pseudomonas aeruginosa*, which have a minimum kill concentration (MBC) of 25%. This is because red betel leaf contains antibacterial compounds such as *tannins, flavonoids*, essential oils, *polyphenols*, and *saponins* [20]. In this study, 95% *ethanol* was used, because in the research of [21], the solvents usually used were *chloroform, ether, acetone, hexane, alcohol*, and *ethanol*. Hand sanitizer with active betel leaf extract is more effective in inhibiting the growth of *Staphylococcus aureus bacteria* [22]. This is because essential oils are volatile at room temperature, smell good according to the smell of the producing plants, generally dissolve in organic solvents, and do not dissolve in air [23]. The research of [11], also proved that the *ethanol* extract of betel leaf at concentrations of 15%, 20%, and 25% showed an inhibition of growth against *Streptococcus mutans* bacteria.

The test results on *chlorhexidine* soap showed a decrease in the number of bacteria by 66.7%. This substance is bacteriostatic against gram positive and negative bacteria and is sporostic. However, *chlorhexidine* is not sporicidal against bacterial spores and exhibits antifungal activity.

The results of the *triclosan* test were unfavorable because it showed a 41% decrease in the number of bacteria. This can be caused by *triclosan* which causes bacterial resistance because it has been obtained by many things, namely soap, toothpaste, and others. *Triclosan* works by blocking the active site of an enzyme in the bacterial wall that functions to synthesize fat and its reproduction called *enocyl-acyl carrier protein reductase enzyme* (ENR). ENR is an enzyme found in the synthesis of type II fatty acids that can catalyze the elongation cycle. *Triclosan* is difficult to destroy by aquatic biota so that it will bioaccumulate and be brought back to clean water consumed by humans. When *triclosan* reacts with chlorine, it can cause adverse effects, including mutating into *dioxins*. This substance will become toxic when exposed to UV radiation and can cause obstacles to the endocrine system. In this study, it was proven that the culture of water used for washing hands in agar was proven to contain fungi and affect the work of the soap agent used.

5. Conclusion

From the research above, it can be concluded that there is no significant difference between the three hand soaps, namely *chlorhexidine*, betel leaf extract and *triclosa*n. So that soap containing betel leaf extract is proven to be an alternative material for anti-bacterial hand soap with the advantage that it is easy to obtain in the natural environment, production is not too complicated, but has anti-bacterial power that is parallel to soap agents that have been circulating in the market.

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