BIOINFORMATICS AND BIOMEDICAL RESEARCH JOURNAL
2021, Vol. 4, No. 2, 48 - 56
http://dx.doi.org/10.11594/bbrj.04.02.02

Research Article

The Antibacterial Activity of Betel (Piper Battle L.) and Basil (Ocimum Sanctum L.) Leaves Infusion as Antiseptic Preparations Against Some Bacteria In Vitro

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Article history:
Submission November 2021
Revised December 2021
Accepted December 2021

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ABSTRACT

Betel leaves (Piper battle L.) and basil leaves (*Ocimum sanctum L.)* are parts of the plant that can be made as antiseptic preparations because they contain antibacterial compounds such as flavonoids, tannins, and saponins. Antiseptic activity can be observed based on inhibiting the colonization of standard test bacteria of the laboratory, namely *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa, and Salmonella* typhi as well as the magnitude of the phenol coefficient value. The study aimed to analyze the infusion activity of betel and basil leaves as antiseptic preparations against some test bacteria. This study in vitro used a posttest-only control group design. The infusion treatment of betel and basil leaves is tested in a single preparation and a combination with concentrations of 50%, 75%, and 100% (w/v). The results showed there was a difference in the infusion activity of betel leaves and basil leaves in a single stock and a combination of the number of bacterial colonies tested. The effect of the treatment produces a small number of bacterial colonies in Gram-positive bacteria; 100% combination treatment (b/v) provides an inhibitory effect that is not significantly different from 70% alcohol control against all test bacteria except *S.typhi*: and obtained the phenol coefficient value from the combination treatment of betel and basil leaves infusion higher against Gram-positive bacteria. The conclusion of this study, the combination of betel and basil leaves infusion has antibacterial activity as an antiseptic preparation that can inhibit the growth of some test bacteria.

Keywords: antiseptic preparations, infusion leaves, Piper battle L, Ocimum sanctum L, number of colonies, phenol coefficient

Introduction

Infectious diseases caused by bacteria are still a public health problem in swamp regions and river environments. This disease is related to a person's hygiene and the consequences of transmission between people. The results of the study that identified the type of bacteria in hand swab samples and feces of elementary school students who lived around the riverbank of Banjarmasin City obtained types of bacteria *Staphylococcus aureus (S. aureus)*, *Staphylococcus epidermidis*

How to cite:
Budiarti, L. Y, Heriyani, F, Medika, G & Fatimah, R. N. (2021). The Antibacterial Activity of Betel (Piper Battle L.) and-Basil (Ocimum Sanctum L.) Leaves Infusion as Antiseptic Preparations Against Some Bacteria In Vitro. *Bioinformatics and Biomedical Research Journal 4*(2): 48 - 56. doi: 10.11594/bbrj.04.02.02

(S.epidermidis), Escherichia coli (E.coli), and *Pseudomonas aeruginosa (P.aeruginosa)* in hand samples and *Salmonella typhi (S.typhi)* in fecal samples [1].

The use of antiseptics to prevent the transmission of infectious diseases is important for everyone. Generally, the transmission of infections often occ due to the use of water, contact with hands that contain normal flora, or the presence of bacterial contamination on the skin surface [2], [3]. Types of pathogens bacteria such as *S.aureus, S.epidermidis, E.coli, P.aeruginosa,* and *S.typhi* cause infectious diseases whose transmission can be prevented by using antiseptics [2].

Alcohol 70% is one type of antiseptic commonly used by various circles of society. Alcohol 70% has broad bactericidal activity against both gram-positive and gram-negative bacteria. Alcohol is an antiseptic that has been shown to reduce the population of bacteria on the skin of the hands [3], as well as in polluting bacteria [4]. However, excessive or prolonged use of alcohol can irritate and even burn the skin [4], [5].

The negative impact of using of antiseptics made from chemicals can be overcome with the use of natural ingredients. Plants contain several phytochemical compounds that are antibacterial. These phytochemical compounds can act as a shield against infectious diseases and can be the foundation for developing alternative antiseptics from antibacterial plants.

Based on this, it can be developed preparations made from natural antiseptics. An antiseptic preparation needs to know its effectiveness in inhibiting bacterial growth colonization and based on the phenol coefficient testThe phenol coefficient test is a standard test used to compare an antibacterial substance with an antiseptic substanceusing 5% phenol as a comparison agent. The test bacteria that can be used in the phenol coefficient test are *S.aureus, S.epidermidis, E.coli, P.aeruginosa,* and *S.typhi* [2].

Among the herbal plants has widely used as alternative herbs are betel leaves (*Piper battle L.*) [6] and basil leaves (*Ocimum sanctum L.*) [7]. The leaves of these two plants are often made into liquid preparations to treat inflammation because they contain antibacterial compounds [8]. The *Ocimum* sp. has pharmacological properties such as antibiotic properties, antidiarrheal, antifungal, antihyperglycemic (diabetes), anesthetic, cicatrizing, and is widely consumed as a food and medicinal plant in the treatment of bacterial infections [9].

Antibacterial content in leaves *Piper battle* L, namely flavonoids, tannins, essential oils, and saponins [6]. While on leaves, *Ocimum sanctum* L essential oils, flavonoids, tannins, saponins, alkaloids, and steroids [8], [9]. Leaves extract was *Piper battle* L able to inhibit *S. aureus* at 20% concentration, *E.coli* at 25% concentration, *Salmonella* sp. at a concentration of 15%, and *P.aeruginosa* at a concentration of 10% [10], [11]. The results of the study stated that extract can inhibit to *S.aureus, E.coli, K.pneumoniae,* and multi-resistant strains of *S.aureus* (MRSA), *Escherichia coli* (ESBL), and *K. pneumoniae* (ESBL) isolated from bacterial specimens in patients [9]. While the leaves of *Ocimum sanctum* L. have antibacterial concentration of 60% against *S.aureus* and *E.coli*, concentration of 10% against *P.aeruginosa*, concentration of 80% against *S.typhi* [12], [13]; the level of 85gr/ml can inhibit *S. epidermidis* [14].

The use of herbal plants, apart from being in a single form, can also be made from a combination [15]. A good herbal combination enhances its antibacterial effect [16]. The study aims to analyze the activity of betel and basil leaves infusion as antiseptic preparations against some test bacteria. Antibacterial activity of betel and basil leaves infusion was observed based on their inhibitory effect on the growth of test bacterial colonies as well as the magnitude of the phenol coefficient value. The method used in this study is the proper experimental laboratories method, with a posttest-only control group design. The standard bacterial isolates tested were Gram-positive *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis ATCC* 35983, and Gram-negative *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhi* ATCC 19430. This study has passed the ethical test based on the letter statement of approval of the ethics committee of the Faculty of Medicine, University of Lambung Mangkurat Number 752/KEPK-FK ULM/EC/VIII/2021 and 767/KEPK-FK ULM/EC/VIII/2021.

Materials and Method

Materials

The material of *Piper battle* Linn and *Ocimum sanctum* Linn plants used by young leaves that are green, good texture, and fresh, which are

processed from Sukamaju street, West of Landasan Ulin District of Banjarbaru City, South Kalimantan Province. Taxonomy of plant samples has been identified in the Biology Laboratory of the Faculty of Science, Lambung Mangkurat University (ULM), Banjarbaru City.

Tested bacterial isolates namely *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis ATCC* 35983, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella typhi* ATCC 19430 are collections of microbiology laboratories of the Faculty of Medicine ULM Banjarbaru City.

The materials and media used for antibacterial activity tests and phenol coefficient tests are nutrient agar (NA) media, Brain Heart Infusion (BHI) media, Nutrient Broth (NB) media, sterile distilled water, 5% phenol solution, 70% alcohol, and standard solution Mc. Farland.

Research Procedure

Preparation of Test Bacteria

Pure isolates of *S. aureus, S.epidermidis, E. coli, P.aeruginosa,* and *S.typhi,* which grew on nutrient agar (NA) at 37℃ (24 hours), were used as test bacteria isolates. 1 ose of bacterial test isolates were taken aseptically and put into the nutrient broth (NB) from several samples of pure test isolates of bacterial. Next, incubation was carried out for 5 hours at 37°C, then homogenized, and the turbidity equalized with Mc Farland 0,5 standard solution ($1,5x10^8$ CFU/ml). Each suspension of the test bacteria in the NB media in this tube was prepared as a test bacterial. Stock solutions of phenol **with** a concentration of 5% were prepared by dissolving 5 grams of phenol crystals with 100 ml of sterile distilled water.

Preparation of Plant Infusion

The prepared betel and basil leaves were washed with running water, then drained and sliced into small pieces. The preparations of betel and basil leaves were weighed as much as 100 grams each. After that, enter 100 ml of distilled water to make liquid preparations in a beaker glass container and obtain a concentration of 100% w/v. Then put into an infusion pan that has been heated to a temperature of 90℃, then heated for 15 minutes. The stew is filtered with filter paper to separate the ingredients and cooking water. If the resulting liquid/water extract is less than the initial volume, it is added with hot distilled water obtained through a dregs filter to obtain a concentration of 75% (w/v). Then make a concentration of 50%. Each preparation is placed in a test tube that labeled according to type and concentration for further use in the combination treatment (ratio 1:1).

Antibacterial Activity Test

The Dilution methods of testing the antibacterial activity against bacterial culture use standard culture methods. Each test treatment has been prepared (infusion of the rind of the test plant at various test concentrations, 70% alcohol, and distilled water) and the bacterial test suspension. 0.1 ml of the bacterial test suspension was taken and added to a tube containing 10 ml of the test treatment and homogenized by shaking slowly for about 5 minutes. Next, 0.1 ml of the test bacteria suspension was taken and poured onto a petri dish, and then a warm NA media was poured in. The petri dish was shaken slowly and evenly until the NA media solidified.After that, each test media was incubated at 37°C for 24 hours. The number of bacterial colonies growing on NA media was calculated using a colony counter.

Phenol Solution Test Dilution Test Preparation from Combination Infusion Specimen and Phenol 5%

Method of dilution of phenol solution: 26 sterile tubes were prepared and numbered 1-26. Tubes 1-13 were given 2 ml of 5% phenol solution each. Then sterile distilled water was added from tubes 1-13 in a row, namely 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 13, 18, and 23 ml. Each tube was homogenized. Next, transfer the 5% phenol solution mixed with distilled water to tubes 14-26 as much as 2 ml each. The dilutions of phenol solution were 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:110, 1:150, 1: 200, 1:250 [17].

Phenol Coefficient Test

Prepared sterile test tubes contain NB and labeled series dilution (1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:110, 1:150, 1:200, 1:250) with contact time (5, 10, 15 minutes). The production of bacterial suspension is conducted by placing the pure isolate of bacteria from NA media into the NB media and then being incubated at 37° C for 24 hours. First, 0.5 ml pipette of bacterial suspension is tested into each sterile tube of each

tested sample (a combination of 5% betel-basil leaves infusion and phenol) starting from the tube with a dilution series of 1:20 to 1:250. Second, homogenize; then, after 5 minutes, take as much as one dispute from each test specimen with various series of dilutions and insert into a test tube containing NB, conducted contact time for 5 minutes. Next, the same way of working with a contact time of 5 minutes and 10 minutes. All tubes containing test specimens are increased at 37ºC for 24 hours, and the tube is filled with turbidity.[2][26].

Positive results (+) indicate the growth of test bacteria, and negative (-) indicates dead bacteria on test specimen treatment. Next, tabulated the data (tables 2 and 3) and calculated the phenol coefficient value of the treatment of each test specimen using the formula below [26]. The formula for the phenol coefficient value can be seeing as follows:

Phenol Coefficient =

$$\frac{\text{The highest dilution of antiseptic is lethal within 10 minutes but not lethal in 5 minutes}}{\text{The highest dilution of lethal phenol solution in time 10 minutes but not turn off in 5 minutes}}$$

Results and Discussion

The results of the treatment of betel leaves (*Piper battle* L.) and basil leaves (*Ocimum sanctum* L.) infusion, both single and combined preparations, as well as 70% alcohol treatment against *S.aureus S.epidermidis, E.coli, P.aeruginosa, and S.typhi* bacteria, obtained varying numbers of colonies as shown in figure 1 and table 1.

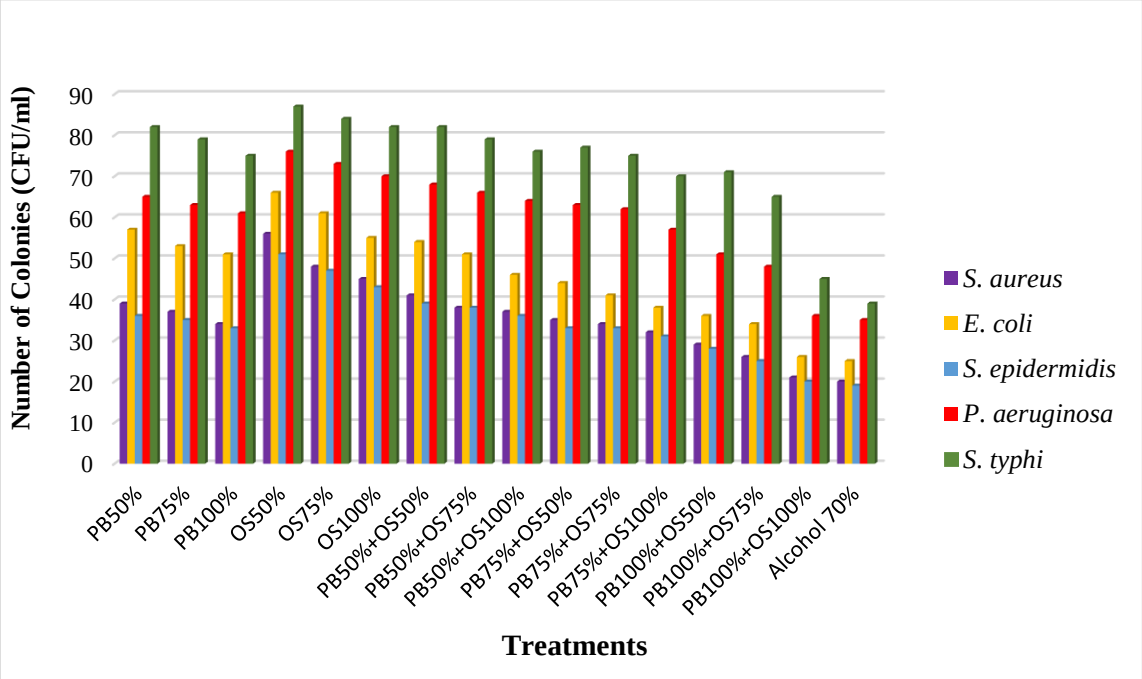

Figure 1. The Average Number of Colonies Combination of Piper battle L (PB) and Ocimum sanctum L(OS) Infusion against S.aureus, S.epidermidis, E.coli, P.aeruginosa and S.typhi

Table 1. The Average Number Colonies and Standard Deviation of Piper battle L (PB) and Ocimum sanctum L (OS) Infusion in Some Test Bacteria

Treatments	Number of bacterial colonies (CFU/ml)				
	S.aureus	***S.epider-midis***	***E.coli***	***P.aeruginosa***	***S.typhi***
PB Infusion 50%	39±2,65^{gh}	37±1,00^{ghi}	57±2,65^{h}	65±2,00^{fg}	82±1,00^{hi}
PB Infusion 75%	37±1,00^{fg}	35±2,00^{efg}	53±1,00^{fg}	63±2,08^{ef}	79±1,00^{fgh}
PB Infusion 100%	34±1,00^{de}	33±3,00^{de}	51±1,00^{f}	61±2,08^{e}	75±1,00^{e}
OS infusion 50%	56±1,00^{k}	52±1,00^{l}	66±3,05^{j}	76±2,00^{k}	87±2,65^{j}
OS infusion 75%	48±1,00^{j}	47±2,00^{k}	61±1,00^{i}	73±2,00^{j}	84±3,06^{ij}
OS infusion 100%	45±1,00^{i}	43±2,65^{j}	55±2,00^{gh}	70±2,00^{i}	82±2,00^{hi}
PB 50%+ OS 50%	41±1,00^{h}	39±1,00^{i}	54±1,00^{fgh}	68±1,15^{hi}	82±1,53^{hi}
PB 50%+ OS 75%	38±1,00^{g}	38±1,00^{hi}	51±1,00^{f}	66±2,65^{gh}	79±1,53^{gh}
PB 50%+ OS 100%	37±1,00^{fg}	36±1,00^{fgh}	46±2,00^{e}	64±1,00^{efg}	76±2,00^{ef}
PB 75%+ OS 50%	35±1,00^{ef}	33±1,00^{de}	44±2,65^{e}	63±1,00^{ef}	77±2,65^{efg}
PB 75%+ OS 75%	34±2,65^{de}	33±1,53^{def}	41±1,00^{d}	62±1,53^{e}	75±1,53^{e}
PB 75%+ OS 100%	32±2,52^{d}	31±1,00^{d}	38±1,00^{c}	57±1,00^{d}	70±1,00^{d}
PB 100%+ OS 50%	29±1,00^{c}	28±1,00^{c}	36±2,00^{bc}	51±1,00^{c}	71±1,00^{d}
PB 100%+ OS 75%	26±1,00^{b}	25±1,00^{b}	34±1,00^{b}	48±1,00^{b}	65±1,00^{c}
PB 100%+ OS 100%	21±1,00^{a}	20±1,00^{a}	26±1,53^{a}	36±1,00^{a}	45±1,53^{b}
Alcohol 70%	20±1,00^{a}	19±1,00^{a}	25±1,53^{a}	35±1,00^{a}	39±1,00^{a}

a, b, c, d, e,f, g, h, i, j superscript in the same column shows no significant difference (P>0.05)

This study found that the average number of colonies tended to decrease with increasing concentration of betel leaves and basil leaves infusion. Table 1 and Figure 1 show that in single preparations, the lowest number of colonies was obtained from 100% betel leaves infusion, while the highest number of colonies was obtained from 50% basil leaves infusion. In the combination preparation, the lowest number of colonies resulted from the 100% betel leaves-100% basil leaves infusion treatment, while the highest number of colonies resulted from the 50% betel leaves-50% basil leaves infusion treatment. 70% alcohol as a positive control resulted in a lower mean number of colonies than the single treatment results or the combination of betel and basil leaves infusion at all concentrations. The lower mean number of colonies is influenced by the solubility properties of the active compounds contained in the infusion treatment, differences in the mechanism of action of secondary compounds compared to alcohol, and differences like bacterial resistance to antimicrobial substances [18].

This study showed the number of colonies of S.aureus, S.epidermidis, E.coli, P.aeruginosa, and S.typhi that grow on the insulating medium (nutrient agar) less and less in line with the increase in the concentration of the combination of betel and basil leaves infusion. These results are from several previous studies which stated that increasing the concentration could strengthen the antibacterial activity [18].

At higher concentrations, it is possible that more active compounds contained in plant preparations can be extracted and work as antibacterial [19]. Antibacterial compounds contained in betel and basil leaves played a role in inhibiting the growth of the test bacteria, and their effectiveness increased according to the increase in treatment concentration [20]. This study obtained a small number of bacterial colonies after giving each treatment concentration.

Antibacterial secondary metabolite compounds can stop the growth of bacterial cells. The types of secondary compounds that are relatively the same and contained in betel leaves and basil leaves are flavonoids, saponins, and tannins. [21], [22]. Some secondary compounds have a relatively simple mechanism of action different. Flavonoid compounds are phenolic compounds that are easily soluble in water and act as antibacterials by inhibiting DNA synthesis and damaging bacterial cell membranes in the phospholipid section [23], [8]. The mechanism of action of saponins is to increase the permeability of the bacterial cell membrane and change the structure and function of the membrane, causing denaturation of membrane proteins so that the cell membrane will be damaged and lysis [24]. Tannins can disrupt peptidoglycan synthesis so that the formation of bacterial cell walls becomes imperfect and able to form complex compounds with proteins so that protein denaturation occurs, which causes enzymes to become inactive and metabolism is disrupted, which causes damage to bacterial cells. Tannins and flavonoids work together to attack the polar groups in the bacterial cell membrane, which causes the cell membrane to leak, and the bacteria will die [8]. Other compounds in basil are eugenol and linalool. Eugenol works by damaging bacterial cell membranes and can stimulate leakage of potassium ions resulting in bacterial cell death and inhibiting the activity of ATPase enzymes so that the energy needed for bacterial cell repair is not formed. Mechanism of activity antibacterial linalool by damaging bacterial cell membranes, suppressing translation of specific gene products, and obstruct bacterial enzymes [25].

Data on the number of colonies of the five test bacteria from the betel leaves infusion and basil leaves infusion were then analyzed for normality. Obtained $p > 0.05$, which indicates the data is normally distributed. Furthermore, the data was tested with *One-way Anava* with a 95% confidence level, and the results were $p = 0.000$ ($p<0.05$), which means that there is a significant difference in effect between the treatments being tested. The analysis results of *Duncan's Post Hoc* can give significantly different and not significantly different effects on the number of tested bacterial colonies compared to 70% alcohol. The combination treatment of 100% betel leaves infusion-100% basil leaves obtained results that were not significantly different compared to 70% alcohol for all test bacteria except for *S.typhi* which the results were significantly different. So, it can be concluded that the antibacterial activity of 100% betel leaves-100% basil leaves was equivalent to the antibacterial activity of 70% alcohol on the number of colonies. *S.aureus, S.epidermidis, E.coli*, and *P.aeruginosa* but against *S.typhi is* still not equivalent to the antibacterial activity of 70% alcohol.

The results of this study (Table 1 and Figure 1) showed that the antibacterial activity of the infusion of betel and basil leaves infusion, both single and in combination, was better against gram-positive bacteria (*S.aureus* and *S.epidermidis*) compared to gram-negative (*E.coli*, *P.aeruginosa*, and *S.typhi*). This is not much different from the previous study; namely, betel leaf infusion at a concentration of 50% was able to inhibit *S. aureus* (inhibition zone 2.87 cm) compared to *E. coli* (inhibition zone 0 cm) [26]. Basil leaf infusion was also more able to inhibit *S. aureus* (23 mm inhibition zone) than *E. coli* (21 mm inhibition zone) and *Salmonella* spp. (20 mm inhibition zone) [27]. Likewise, with the combination preparations where the inhibition zones resulting from the combination of *Cassia fistula* and *Ocimum basilicum* were 24 mm against *S.aureus*, 22 mm against *E.coli*, and 12 mm against *Salmonella spp*. [27]. These results were influenced by differences in the structure and composition of bacterial cells. Gram-positive bacteria consist of high peptidoglycan and teichoic acid, which is polar, and low lipid (non-polar) so that antibacterial compounds can quickly enter cell membranes [28]. Meanwhile, gram-negative bacteria have a complex cell wall consisting of thin peptidoglycan, high phospholipids, and lipopolysaccharide (LPS). Lipopolysaccharides function in limiting cell permeability through the outer membrane of gram-negative bacteria [29], [30].

The number of colonies corresponded to the phenol coefficient value of the combination of *Piper battle* 100% and *Ocimum sanctum* 100% infusion against each of the test bacteria (Table 2, Table 3, and Table 4).

Table 2. Results of Observation Phenol Coefficient Test of 5% Phenol against Some Test Bacteria

Dilutions of each treatment	combination treatment of phenol 5% of test bacteria the average length of contact and repetition per unit time (minutes)														
	S.aurues			*S.epidermidis*			*E.coli*			*P.aeruginosa*			*S.typhi*		
	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:80	-	-	-	-	-	-	-	-	-	+1	-	-	+1	-	-
1:90	+1	-	-	+1	-	-	+2	-	-	+2	+1	+1	+3	+1	+1
1:100	+2	+1	+1	+2	+1	+1	+2	+2	+2	+3	+3	+3	+3	+3	+3
1:110	+3	+2	+2	+3	+2	+2	+3	+2	+2	+3	+3	+3	+3	+3	+3
1:150	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:200	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:250	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3

Table 3. Results of Observation Phenol Coefficient Test of Combination of Piper battle L and Ocimum sanctum L Infusion against Some Test Bacteria

Dilutions of each treatment	combination treatment of betel-basil leaves infusion of test bacteria the average length of contact and repetition per unit time (minutes)														
	S.aurues			*S.epidermidis*			*E.coli*			*P.aeruginosa*			*S.typhi*		
	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'	5'	10'	15'
1:20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:50	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1:60	-	-	-	-	-	-	-	-	-	-	-	-	+1	-	-
1:70	-	-	-	-	-	-	-	-	-	+1	-	-	+2	+1	+1
1:80	-	-	-	-	-	-	-	-	-	+3	+1	+1	+3	+3	+3
1:90	+2	-	-	+2	-	-	+3	-	-	+3	+3	+3	+3	+3	+3
1:100	+2	+2	+2	+2	+2	+2	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:110	+3	+2	+2	+3	+2	+2	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:150	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:200	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3
1:250	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3	+3

\+ = There is the growth of bacteria (cloudy medium)

\- = No growth of bacteria (clear medium)

Table 4. Average Phenol Coefficient of Combination of Piper battle L and Ocimum sanctum L Infusion Against Some Test Bacteria

Treatments	Phenol Coefficient Value				
	S.aureus	*S.epidermidis*	*E.coli*	*P.aeruginosa*	*S.typhi*
Piper battle L and *Ocimum sanctum L* infusion	0,97±0,14	0,97±0,14	0,94±0,10	0,89±0,01	0,79±0,02
Phenol 5%	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00	1,00±0,00

The data from the phenol coefficient after giving the combination treatment of 100% betel leaves-100% basil leaves infusion showed different values for the five test bacteria. The effectiveness of an antiseptic agent is said to be good if it has a phenol coefficient value equal to 1 or equivalent to a 5% phenol solution coefficient [2]. In this study, the mean value of the phenol coefficient of the combination of betel leaves-basil leaves was close to 1, namely against *S. aureus* was 0.97 and against *S.epidermidis* was 0.97. Meanwhile for *E.coli* was 0.94, *P.aeruginosa* was 0.89 and for *S.typhi* was 0.77. From the phenol coefficient value, it can be concluded that the effectiveness of the 100% betel leaves-100% basil leaves infusion combination against *S. aureus* and *S.epidermidis is* close to the effectiveness of 5% phenol.

Based on the results of this study, the infusion treatment of *Piper battle* L and *Ocimum sanctum* L have different activities in inhibiting the growth of gram-positive and gram-negative bacteria. Antibacterial activity is influenced by the composition of the content of antibacterial compounds and their mechanism of working as well as the properties of bacterial cell membranes. Antibacterial activity may increase the use of different combinations of drugs or herbs, but combinations have synergistic effects. The presence of phenol coefficient values from the combination treatment of *Piper battle* L and *Ocimum sanctum* L, which is relatively equivalent to the 5% phenol coefficient value, can be considered the development of *Piper battle* L and *Ocimum sanctum* L infusion as alternative antiseptic preparations.

Conclusions

In conclusion, based on phenol coefficient and several colonies, infusion of betel leaves and basil leaves has antibacterial activity as antiseptic preparations that can inhibit the growth of some test bacteria.

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