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Effectiveness of betel and kaffir lime combination form of leaf infusion as an in-vitro antiseptic candidate

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Original Article

ABSTRACT

Background: Natural antiseptics can be alternatives to reduce the adverse effects of alcohol. An antiseptic is classified as effective if it has an inhibitory ability and a phenol coefficient value ≥ 1. Betel plants (*Piper betle* L.) and kaffir lime (*Citrus hystrix* DC.) contain a variety of antibacterial compounds and have the potential to act as an antiseptic.
Objective: To analyze the in vitro effectiveness of a combined leaf infusion preparation of betel (PB) and kaffir lime (CH) as potential antiseptic candidates.
Methods: This quasi-experimental research employed a post-test-only control group design. We utilized diffusion (measuring inhibition zone) and dilution (determining phenol coefficients) methods. The treatments, performed in triplicate, included PB+CH infusion at concentrations 6.25%, 12.5%, 25%, 50%, 75%, and 100%, with 70% alcohol and 5% phenol as control. They were tested against five types of ATCC standard bacterial isolates.
Results: Phytochemical screening of the tested infusion revealed the presence of phenolic compounds, flavonoids, alkaloids, tannins, saponins, and terpenoids. The inhibition zone area increased with concentration, with PB+CH 100% producing the most significant effect. ANOVA and post-hoc Duncan test analysis ($p < 0.05$) showed significant differences between treatment groups. PB+CH infusion at 75-100% produced antibacterial effects exceeding the control for all bacteria tested, except *S. typhi*. Phenol coefficient tests (dilution 1:20-1:250) showed the effectiveness of PB+CH infusion as a potential antiseptic. The infusion had a phenol equivalent coefficient of 5% against *S. aureus, S. epidermidis, E. coli,* and *P. aeruginosa* but only 0.99 ± 0.07 for *S. typhi*. The 70% alcohol coefficient value was < 1.
Conclusion: The combined infusion of betel leaves and kaffir lime demonstrates significant antibacterial activity and shows a potential candidate as an effective natural antiseptic.

INTRODUCTION

Environment-based infectious diseases are still frequently found in the community. Factors that influence the transmission of infectious agents are a person's behavior and hygiene. Frequent hand washing is the primary prevention method for infectious diseases. Hand washing using antiseptic and clean water can reduce pathogen colonization on hands. Synthetic antiseptics are generally alcohol-based and have a bactericidal effect, but their long-term use results in dry and irritated skin.[1–3] Natural antiseptics are currently under development to reduce the adverse effects of alcohol-based antiseptics. Phytochemical content is known to have effective and non-

toxic herbal antimicrobial properties. Several natural and environmentally friendly antiseptics have been developed, offering the advantage of reduced toxicity compared to alcohol. The use of water as a solvent in extract preparations (herbal) has been used to treat wounds (such as boils and blisters) and systemic diseases of the digestive tract (such as diarrhea and dysentery).[4]

The effectiveness of an antiseptic substance is observed *in vitro* through the phenol coefficient test with 5% phenol. A good phenol coefficient value is equivalent to 1; effectiveness is better when the phenol coefficient value is ≥ 1.[5] Laboratory standard bacterial isolates that can be used in the phenol coefficient test are *S. aureus, S. epidermidis, E. coli, S. typhi,* and *P. aeruginosa*. These bacteria are often found as pathogens transmitted through hand skin or water media contact.[6] The effectiveness of routinely used antiseptics needs to be evaluated due to the development of bacterial strains resistant to various antimicrobials.[7]

Phenol coefficient testing can also be applied to herbal preparations that have antibacterial activity.[8] The concept of synergistic effects in combination preparations for hand washing is recommended because it provides a more beneficial effect.[9,10] Previous reports produced better infusion phenol coefficient values from the *Piper betle* combination and *Ocimum basilicum,* as well as from the fruit of *Averrhoe blimbi* and the flowers of *Cananga odorata*. Infusions in both studies gave effective results, so water can be used as a solvent in making natural antiseptics.[8,11] Water is a universal solvent that can attract phytochemical compounds, and its availability is relatively easy for the community to obtain. Therefore, in this study we used the water extract/infusion method.

The type of plant utilized as an antiseptic material is betel (*Piper betle* Linn.), an important species of the *Piperaceae* family.[12] The betel leaves contain phytochemicals as therapeutic agents[13] and act as stimulants, antiplatelets, anti-inflammatory, anti-gastroprotective, and antidiabetic.[12] Empirically, betel leaves are used to treat mouth ulcers, cough, and astringent.[14] The type and concentration of betel leaf bioactives depend on the diversity of plant species. The antibacterial power betel leaf extract produces in a single preparation is relatively low.[12] The test results contain many secondary plant metabolites, which have been demonstrated to possess therapeutic value.[13] The bioactive contents in the aqueous extract of betel leaves are alkaloids, phenolics, tannins, saponins, and flavonoids.[13,15] Single preparations of betel leaf extract show relatively low antibacterial activity. For example, a hand sanitizer containing 10-25% betel leaf infusion can inhibit *E. coli* bacteria, but its inhibition zone is less effective than the positive control.[14]

Potential antiseptic ingredients can also be obtained from citrus leaves with a strong and distinctive aroma. Kaffir lime, or *Citrus hystrix DC (C. hystrix)*, belongs to the Rutaceae family and is found in various countries. The benefits of *C.hystrix* are generally more in the leaves.[16,17] The bioactive content in the leaves of this citrus species is very important for humans to be utilized as cooking and medicinal ingredients. Besides containing essential oils, folic acid, and vitamin C, citrus leaves contain potassium, flavonoids, coumarin, and pectin.[17] The quantity and quality of essential oils in the leaves of *C. hystrix* are influenced by the growth location.[18] The herbal content of *C.hystrix* helps treat flu, fever, high blood pressure, digestive disorders, diarrhea in babies, heart disease, dizziness, and as a nutritious food. Fresh *C. hystrix* leaves can help maintain dental health by regularly applying leaf powder (crushed leaves) to the teeth and gums.[16] The antibacterial phytochemicals in *C. hystrix* leaves are flavonoids, phenolics, terpenoids, alkaloids, and tannins.[19] The results of the *E.coli* inhibition test showed that the activity of kaffir lime leaf infusion is better than lime leaves.[20] The *C. hystrix* leaf extract 10-100% in a single preparation produced a lower effect than the positive control against *E. coli*.[21] Hand sanitizer treatment containing 75% *Ocimum basilicum* leaf extract and 25% *C. hystrix* fruit peel has been demonstrated to effectively reduce the bacterial numbers in comparison to other formulae.[22]

The abundant availability of betel leaves and kaffir lime leaves in Indonesia can be utilized more widely for public health aspects. Antiseptics made from infusions can be made simply by utilizing local herbal wealth. The study aims to determine the ability of betel leaves or *Piper betle* (PB) and kaffir lime leaves or *C. hystrix* (CH), as effective alternative antiseptic ingredients; based on the phenol coefficient test on its inhibitory power. Furthermore, this study aims to evaluate

the effectiveness of betel leaves and kaffir lime leaves in a combined preparation, specifically a PB + CH leaves infusion with 70% alcohol as an antiseptic control.

METHODS

This experimental study used a post-test-only with a control group design and used two testing methods. This study was conducted from October to December 2023 at the Microbiology Laboratory of the Faculty of Medicine and Health Sciences, Lambung Mangkurat University. The diffusion method was used to analyze the inhibitory power produced by the PB+CH infusion treatment group (6.25%, 12.5%, 25%, 50%, 75%, and 100%) and positive control (70% alcohol). The inhibitory parameter was the area of the inhibition zone diameter measured in millimeters (mm). At the same time, the dilution method was used to assess the effectiveness as an antiseptic based on the results of the phenol coefficient test. The coefficient values of the PB+CH infusion group and the positive control were compared with the phenol coefficient value as a comparison (5% phenol solution). The negative control used was sterile aquadest.

Infusion preparation

The leaves of CH and PB used were young, fresh green, free from pests or diseases, and picked from the plants in the morning from Banjarmasin, South Kalimantan. The collected leaves were washed, sliced thinly, and dried in an oven at 60°C. After cooling, they were grounded, weighed 40 g, and dissolved in 100 mL of hot distilled water.[23] The mixture was heated for 15 minutes at 90°C, then filtered through flannel while still hot. Infusions were made in concentrations of 3.125%, 6.25%, 12.5%, 25%, 3.75%, and 50%. To assess the comparative effect of the infusion, a single preparation of betel leaves and kaffir lime leaves was made at a concentration of 50%. A combination infusion preparation was made by mixing 2 mL of betel leaf infusion and 2 mL of kaffir lime leaf infusion (PB+CH) into a sterile tube. The infusion combinations tested were 6.26%, 12.5%, 25%, 50%, 75%, and 100% (w/v).[19]

Bacterial preparations

The test bacterial isolates were collected from the Microbiology Laboratory of the Faculty of Medicine, Lambung Mangkurat University, namely *S. aureus* ATCC 25923 *S. epidermidis* ATCC 35983, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. typhi* ATCC 19430. Other research materials used were 5% phenol, 70% alcohol, Nutrient Agar (NA) media, Brain Heart Infusion (BHI) media, Mannitol Salt Agar (MSA) media, MacConkey agar media, sterile distilled water, and 0.5 Mc Farland solution.

Each test bacteria were rejuvenated in a sterile NA medium in an incubator at 37°C for 24 hours. The bacterial colonies obtained were identified microscopically and macroscopically tested according to microbiology laboratory procedures. Microscopic examination by Gram staining demonstrated the presence of Gram-positive cocci, specifically *S. aureus* and *S. epidermidis*. In contrast, *E. coli, P. aeruginosa*, and *S. typhi* were identified as Gram-negative bacilli. Macroscopically, *S. aureus* formed colonies with a yellow appearance, while *S. epidermidis* produced colonies with a red appearance on MSA media. Meanwhile, *E. coli* formed colonies with a reddish appearance, and *P. aeruginosa* produced colonies with a clear appearance on MacConkey media; *S. typhi* grew with blackish colonies on Salmonella Shigella Agar (SSA) media.

Each bacterial strain was cultured in BHI media at 37°C for 24 hours, until the media reached the optimal cloudiness for the assay. Bacterial cells were prepared as a suspension by adding 0.8% NaCl, and the suspension was standardized to a concentration of 0.5 MacFarland (equivalent to 1.5 x 10^8 CFU/mL). From each test, bacterial cell suspension 1,5 x 10^8 CFU/mL was taken with a 1 mL micropipette, and the culture was wiped on the surface of MHA media.

Antibacterial assay

In the tube containing the treatments, the PB+CH infusion and the controls, several paper disks (6 mm in diameter) were added, and each tube was left to stand for 30 minutes.

Diffusion test by placing each paper disk containing the infusion treatment and control on the surface of the MHA media, and the plate was incubated for 24 hours at 37°C. The mean diameter of the inhibitory zone was interpreted in terms of inhibitory activity based on Davis and Stout (1971) category (Table 1).

Table 1. Categorization interpreted of inhibitory activity [19,24]

No	Diameter (mm)	Categorization
1	< 5	Less as weak
2	5 - 10	Moderate
3	10 - 20	Strong
4	> 20	Very strong

Phenol coefficient test

A 5% w/v phenol stock solution was prepared using sterile distilled water. This stock was then diluted to create a series of concentrations: 1:20, 1:30, 1:40, 1:50, 1:60, 1:70, 1:80, 1:90, 1:100, 1:110, 1:150, 1:200, and 1:250 (labeled A-M respectively). Each dilution was prepared in 5 mL volumes in sterile tubes and homogenized by gentle inversion. These phenol dilutions serve as a standard reference for comparing the antibacterial activity of the PB+CH infusions. For each dilution, three aliquots were prepared to allow for different contact times (5, 10, and 15 minutes) in the subsequent antibacterial assay. All solutions were freshly prepared before each experiment and handled with appropriate safety measures due to phenol's toxicity.

The test employs a dilution method using 13 concentrations (A-M) for each treatment: PB+CH infusion, 70% alcohol, and 5% phenol. A standardized bacterial suspension (0.5 McFarland standard) was prepared. For each treatment, 0.5 mL of bacterial suspension is added to tube A and left for 30 seconds. Subsequently, 0.5 mL from tube A was transferred to tube B, and this process was repeated through tube M. From each dilution tube, a 10 µL loop of suspension was transferred to corresponding tubes containing 5 mL of sterile Nutrient Broth (NB), labeled A1-M1. This process was repeated for contact times of 5, 10, and 15 minutes. All tubes were homogenized and incubated at 37°C for 24 hours. Bacterial growth was assessed by observing turbidity: (+) indicated growth (cloudy suspension), while (-) indicated no growth (clear suspension). The experiment was performed in triplicate for statistical validity. Positive (untreated bacteria) and negative (sterile media) controls were included. Turbidity was quantified using a spectrophotometer at 600 nm for objective comparison. The phenol coefficient value was calculated using the following formula.[5]

$$\text{Phenol coefficient} = \frac{\left\{\frac{\textit{The lowest phenol dilution that is lethal to bacteria}}{\textit{The lowest antiseptic dilution that kills bacteria}}\right\}}{\left\{\frac{\textit{Highest phenol dilution that killed bacteria}}{\textit{Highest antiseptic dilution that kills bacteria}}\right\}}$$

Phytochemical screening

Phytochemical screening was carried out using procedures according to Harborne. The compounds in the PB leaf infusion were tannins, phenols, saponins, anthraquinones, steroids, and terpenoids, while in the CH leaf infusion were flavonoids, alkaloids, phenols, saponins, steroids, and terpenoids.

Statistical analysis

Data on inhibition zone diameter and phenol coefficient values were obtained from three independent experiments. Before analysis, data were tested for normality using the Shapiro-Wilk test and for homogeneity of variances using Levene's test. One-way Analysis of Variance (ANOVA) was conducted to compare the effects of different concentrations of PB+CH infusion, as well as the positive controls (70% alcohol and 5% phenol), on the inhibition zone diameter and phenol coefficient values. When significant differences were detected ($p < 0.05$), post hoc comparisons were performed using Duncan's Multiple Range Test. This test was chosen due to its power to detect differences between means while controlling for Type I errors. All statistical analyses were

performed using Statistical Package of Social Science (SPSS) version 25.0 at a 95% confidence level (α = 0.05). Results were presented as mean ± standard deviation (SD).

Ethics

This experimental research involved the use of potentially pathogenic bacterial isolates. All procedures were conducted in a Biosafety Level 2 laboratory, adhering to institutional biosafety guidelines. Researchers involved in the study received appropriate training in handling potentially hazardous biological materials. Aseptic techniques were rigorously employed throughout the experimentation process. All equipment and materials were sterilized before use, and work was conducted in a laminar flow hood to maintain sterility and prevent contamination. After experimentation, all biological materials were decontaminated by autoclaving before disposal by institutional waste management protocols. This research has been reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Lambung Mangkurat University (Reference number: 237/KEPK-FK ULM/EC/IX/2023). The study complied with all relevant ethical guidelines and regulations for research involving potentially hazardous biological materials.

RESULTS

This research investigated the antibacterial effectiveness of a combined infusion of PB and CH leaves. The infusion method was chosen for its simplicity and accessibility, offering an alternative approach to obtaining antibacterial properties. The diameter of the inhibition zone for each treatment of each PB+CH infusion combination treatment and control on the tested bacteria are listed in Figure 1.

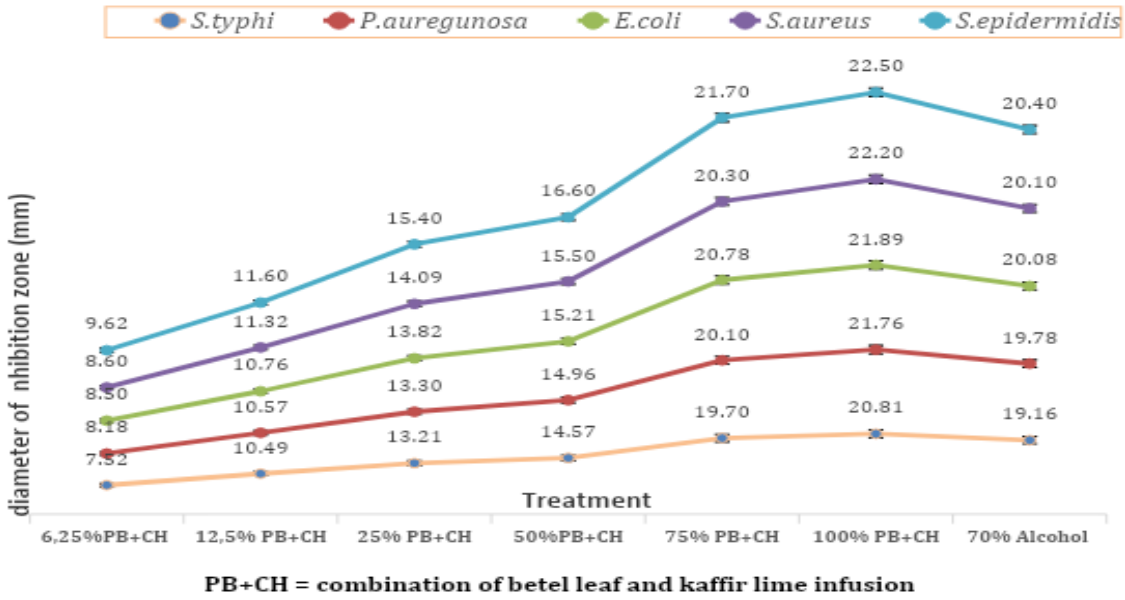

Figure 1. The average zone of inhibition against bacteria; CH: *C. hystrix;* PB: *Piper betle*

The negative control (distilled water) showed no inhibitory effect. The inhibitory effect of PB+CH infusion increased proportionally with concentration, ranging from 12.5% to 100%. On average, the PB+CH infusion demonstrated a more significant inhibitory effect against Gram-positive bacteria than Gram-negative bacteria. Based on Davis and Stout criteria, we found that the inhibition strength produced by PB+CH infusion 12.5-50% was in the strong category (> 10 mm) and PB+CH 75-100% in the very strong category (> 20 mm) in all bacteria except PB+CH 75% on *S. typhi* (19.70 ± 0.14 mm). The 70% alcohol control produced strong inhibition (> 20 mm) against *S. epidermidis, S. aureus, E. coli*, and *P. aeruginosa*, but not against *S. typhi*. These results indicate that the antibacterial efficacy of the PB+CH infusion is influenced by both the

concentration of the infusion and the type of bacteria tested.

The data of this study were normally distributed and homogeneous. The results of ANOVA and post-hoc Duncan tests showed significant effects ($\alpha < 0.05$) (Table 1). Comparison of activity between single preparation infusions (50% betel leaf infusion and 50% kaffir lime leaf infusion) with PB+CH combination infusion was significantly different at various concentrations tested. The inhibition of PB+CH infusion (6.25-100%) significantly differed between each bacterium. The inhibition effect of alcohol on *S. epidermidis, S. aureus, E. coli,* and *P. aeruginosa* was below the inhibition effect of 75-100% PB+CH infusion. Except for *S. aureus*, the inhibition effect of 70% alcohol was equivalent to that of 75% PB+CH infusion.

Table 1. Comparison of the inhibitory effects of infusions and control against bacteria

Treatment	Mean zone of inhibition ± standard deviation				
	S. epidermidis	***S. aureus***	***E. coli***	***P. aeruginosa***	***S. typhi***
50% PB	8.65 ± 0.15^{h}	8.34 ± 0.17^{f}	7.27 ± 0.25^{h}	7.67 ± 0.23^{h}	7.64 ± 0.12^{g}
50% CH	8.51 ± 0.21^{h}	8.21 ± 0.25^{f}	7.30 ± 0.20^{h}	7.64 ± 0.23^{h}	7.56 ± 0.29^{g}
6.25% PB+CH	9.62 ± 0.18^{g}	8.64 ± 0.08^{f}	8.50 ± 0.18^{g}	8.18 ± 0.32^{g}	7.52 ± 0.27^{g}
12.5% PB+CH	11.60 ± 0.18^{f}	11.32 ± 0.37^{e}	10.76 ± 0.16^{f}	10.57 ± 0.30^{f}	10.49 ± 0.20^{f}
25% PB+CH	15.35 ±0.07^{e}	14.09 ± 0.37^{d}	13.82 ± 0.22^{e}	13.30 ± 0.23^{e}	13.21 ± 0.19^{e}
50% PB + CH	16.60 ± 0.20^{d}	15.54 ± 0.85^{c}	15.21 ± 0.08^{d}	14.96 ± 0.21^{d}	14.57 ± 0.43^{d}
75% PB + CH	21.67 ± 0.19^{b}	20.34 ± 0.04^{b}	20.78 ± 0.29^{b}	20.61 ± 0.36^{b}	19.70 ± 0.14^{b}
100% PB +CH	22.48 ± 0.37^{a}	22.24 ± 0.48^{a}	21.89 ± 0.94^{a}	21.76 ± 0.31^{a}	20.81 ± 0.36^{a}
70% alcohol	20.35 ± 0.81^{c}	20.17 ± 0.60^{b}	20.08 ± 0.42^{c}	19.78 ± 0.18^{c}	19.16 ± 0.25^{c}

CH: *C. hystrix;* PB: *Piper betle;* The same letter notification in the column indicates that no significant difference. While the different letter notification indicates that there is a significant difference ($p > 0.05$).

The phenol coefficient test results show that the PB+CH infusion coefficient value is effective as an antiseptic (Table 2). PB+CH infusion has a 5% phenol equivalent coefficient on *S. aureus*. *S. epidermidis. E. coli* and *P. aeruginosa,* except on *S. typhi* (0.99 ± 0.07). The coefficient of 70% alcohol was <1. The effectiveness of PB+CH infusion based on phenol coefficient value on gram-positive bacteria was greater than that on gram-negative.

Table 2. Average phenol coefficient value of treatment on each test bacteria

Treatments	Phenol coefficient value				
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***	***S. typhi***
PB+CH infusion	1.02 ± 0.01	1.10 ± 0.00	1.00 ± 0.01	1.00 ± 0.04	0.99 ± 0.07
70% alcohol	0.99 ± 0.00	0.99 ± 0.00	0.99 ± 0.00	0.89 ± 0.00	0.89 ± 0.00
5% phenol	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00

CH: C. hystrix; PB: Piper betle

DISCUSSION

In this study, we confirmed that the infusion of betel leaf (*Piper betle L.*) and kaffir lime (*Citrus hystrix* DC) has antibacterial properties. The infusions exhibited varying antibacterial strength, ranging from moderate to very strong. The infusions of both plants also yielded effective phenol coefficient values (≥ 1), except for *S. typhi*, which was below 1. The inhibition of 70% alcohol, which served as a control in this study, was found to be strong to very strong, while the phenol coefficient value was < 1. Therefore, the infusion of betel leaf and kaffir lime has the potential to serve as an alternative antiseptic. However, the infusion's concentration and the type of bacteria inhibited influence the effectiveness.

Antiseptics are mostly made from alcohol. Alcohol, as an antibacterial, works by damaging the membrane and denaturing proteins. The process of inhibition or release of mRNA and protein synthesis occurs through its effect on ribosomes and RNA polymerase. Alcohol is bactericidal against vegetative bacteria that undergo metabolism and binary division but does not affect

microbial spores.[25] The effectiveness of alcohol may decrease in spore- and biofilm-forming bacteria. Routine use of antiseptics has also been reported to cause resistant bacteria. These limitations highlight the need for alternative antiseptic solutions, such as the PB+CH infusion.

Generally, the phytochemical content in plants of the *Rutaceae* family is saponins, steroids, alkaloid, glycosides, and flavonoids.[26] Flavonoids are the main chemical compounds present in the citrus genus.[27] Bioactive components in betel leaves, such as alkaloids, polyphenols, flavonoids, and tannins, have yielded positive results in their pharmacological activities.[28] The results of phytochemical screening of betel leaf and kaffir lime leaf infusions in this study contain a class of compounds that are not different from other studies. Water solvents can distill polar and slightly semipolar phytochemical compounds.[29] The bioactive content of betel leaf and kaffir lime leaf has potential as an antibacterial and antiseptic alternative. Bioactive compounds of flavonoids, terpenoids, saponins, and phenolics are beneficial as anti-sore, anti-cough, astringent, and antiseptic.[14.31]

This study found that the inhibitory effect produced by the combination of infusions was better and produced a very strong category inhibition zone at 75-100% infusion concentration. In contrast to Pohan & Djojosaputro's research, the single effect of 100% kaffir lime leaf extract against *E.coli* formed an inhibition zone of 13.25 mm (strong).[21] Betel extract can inhibit the growth of *E. coli*. *S. pyogenes* and *S. aureus* at lower activity.[12] A plant's habitat affects its nutrients and metabolite compounds.[31] Differences in location can affect the type of secondary compounds in the extract, such as the yield of essential oils.[19]

Combining the two infusions allows more bioactive compounds to dissolve, complement, and produce a better effect. The results of this study are similar to the combination of *Stenochlaena palustris* and *Sauropus androgynous* leaf infusions, in which the antibacterial and anti-candida effects produced are more potent than those of the antibiotic-positive control.[32] The similar results in testing the combination of infusions against gram-positive and negative bacteria produced more significant effects than the antiseptic control (alcohol 70%), such as basil and betel leaves;[11] and starfruit and ylang-ylang.[33] The antiseptic power of the appropriate combination formula (75% *Ocimum basilicum* leaf extract and 25% *C. hystrix* DC extract) was effective in reducing bacterial colonization.[22]

This study resulted in the combination of betel leaf and kaffir lime infusion exhibiting a phenol coefficient value equivalent to 5% phenol, which is superior to that of 70% alcohol. The same results are obtained in the phenol coefficient test for the combination of betel leaf infusion and basil, which inhibits bacterial colonization with greater effectiveness in inhibiting gram-positive bacteria.[11] Based on the phenol coefficient value, this combination of infusions has potential as an antiseptic. Concentration and contact time factors affect the effectiveness of antiseptics. The higher the concentration of the extract, the longer the exposure will increase its effectiveness.[34] In combination preparations, phenolic compounds, flavonoids, alkaloids, and terpenoids play a greater role. However, their effects may increase or decrease, depending on the amount of compound content and mechanism of action.[35]

These results can be explained by understanding how phytochemical components work to inhibit or kill bacteria. Antibacterial power is more potent against Gram-positive bacteria (*S. aureus, S. epidermidis*) than Gram-negative (*E. coli, P. aeruginosa, S. typhi*), due to differences in peptidoglycan components in bacteria.[36-38] Gram-positive bacteria have a thicker peptidoglycan layer on their cell walls than Gram-negative bacteria. The antibacterial effectiveness of phytochemicals can penetrate the cell wall to the inner membrane and disrupt cell metabolism. Lack of bacterial adhesion leads to bacterial cell death.[36]

The alkaloid compounds inhibit metabolic processes, damage cell membranes and walls, and modify cell membrane permeability.[38] Phenolic groups denature cell proteins, affecting cell wall permeability, and cytoplasmic membranes. Hydrogen bond formation between phenols and proteins causes damage to protein structure, disturbed permeability of cell walls, and cytoplasmic membranes can cause macromolecular and ionic imbalances in cells so that cells become lysed.[39] Flavonoid compounds have antibacterial properties because these compounds are able to interact directly with bacterial DNA. DNA structure plays an important role in

bacteria's transcription and replication process.[40] Flavonoids work by inhibiting cell membrane function by forming complex compounds with extracellular proteins, causing the cell membrane to be damaged and intracellular compounds to escape. Flavonoids inhibit energy metabolism by preventing energy formation in the cytoplasmic membrane and inhibiting bacterial motility, which plays a role in antimicrobial activity and extracellular proteins.[41]

Tannins can attack cell wall polypeptides, which eventually disrupt cell permeability and inhibit growth or cause bacterial cell death.[36] Antibacterial mechanisms of tannins, e.g. inhibition of extracellular microbial enzymes and oxidative phosphorylation, and cellular disruption of membrane permeability.[42] The mechanism of action of steroids as anti-bacterial is related to lipid membranes. Their sensitivity to steroids can cause liposomes to leak, membrane integrity to decrease, and cell membrane morphology to change so that cells become fragile and lysis. Terpenoids are elements of essential oils. Terpenoid compounds act reactively on cell membranes by hydrogen bonding with the active sites of cell target enzymes and inactivating them. The result is dysfunction or rupture of the cell membrane.[39.44] Saponins have detergent-like properties and contain molecules with hydrophilic and lipophilic properties that affect cell membrane permeability.[37] Saponins reduce bacterial cell wall surface tension and disrupt bacterial cell membrane permeability. Saponins can diffuse through the cytoplasmic membrane so that the stability of the membrane is disrupted, cytoplasmic leakage occurs, leading to cell death.[39]

Plants have been used for their medicinal properties since ancient times. The high antimicrobial potential of phytochemicals and the lack of any apparent emergence of bacterial resistance make plant products valuable research subjects. Beneficial properties produced by bioactive compounds of therapeutic potential and developed as drug candidates.[44] The future outlook is for the increased use of herbs in treating various conditions.[12] The study demonstrates that a combination infusion of betel leaf (*Piper betle*) and kaffir lime leaves (*Citrus hystrix*) is an effective antiseptic. The plants are well-known and easily accessible in local communities, making them potential for development as health products. However, this research has limitation and needs to be equipped with accurate, stable, and safe levels that meet Indonesian National Standards (SNI). Future studies should also focus on product stability, organoleptic properties, and toxicity testing to support their application as antiseptic and phytopharmaceutical products.

CONCLUSION

The combination of betel leaf and kaffir lime infusion has antibacterial activity, and they are effective as an antiseptic candidate. Further study needs to be done for antiseptic development to make a more accurate, stable, and safe product for public use. Several tests can be done on various antiseptic formulations (gel, spray) with phytopharmaceutical safety and toxicity according to health standards.

CONFLICT OF INTEREST

The research team and the conduct of this research have no conflict of interest.

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AUTHOR CONTRIBUTION

The research team worked together on the concept, research implementation, and manuscript writing. LYB created the concept/idea, design, definition of intellectual content, literature search, manuscript preparation, manuscript editing, and manuscript review. Co-author I performed pharmacological method preparation, manuscript editing, and manuscript review. The author and co-authors, NGN, DEP, FY, and F, conducted experimental studies on different bacteria, followed by data acquisition, data analysis, and statistical analysis respectively.

LIST OF ABBREVIATIONS

SNI: Standar Nasional Indonesia; PB: Piper betle L; CH: Citrus hystrix; MHI: Mueller Hinton Agar

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