THE EFFECT OF VARIATIONS IN CONCENTRATION OF BETEL LEAF ETHANOL EXTRACT IN HAND SANITIZER PREPARATIONS ON THE ANTIBACTERIAL ACTIVITY OF ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

Rahmadani[1*] , Febia Sari[2], Lintang Kautsar[3]
[1]Universitas Muslim Nusantara Al-Washliyah (INDONESIA)
[2]Universitas Syiah Kuala (INDONESIA)
[3]Poltekkes Kemenkes Aceh (INDONESIA)

**Corresponding author: rahmadst121@gmail.com*

Abstract

Betel leaf (Piper betle L.) contains bioactive compounds with potential antibacterial activity. This study aimed to evaluate the effect of variations in betel leaf ethanol extract concentration in hand sanitizer preparations on antibacterial activity against Staphylococcus aureus and Escherichia coli. Hand sanitizers were formulated with extract concentrations of 5% (F1), 7% (F2), and 9% (F3), alongside a blank formulation (F0). Evaluations included phytochemical screening, organoleptic properties, stability over 12 weeks, pH, foam height, skin irritation, and antibacterial activity using the disc diffusion method. Phytochemical screening confirmed the presence of saponins, flavonoids, alkaloids, tannins, phenols, steroids, and essential oils. All formulations remained homogeneous and physically stable throughout storage, causing no skin irritation. The pH values ranged from 8.0 to 9.5, and foam heights ranged from 70 to 78 mL. Antibacterial testing demonstrated that increasing extract concentration significantly enhanced bacterial inhibition. F1 exhibited inhibition zones of 7.0 mm for both bacteria, while F2 showed 9.0 mm against S. aureus and 9.5 mm against E. coli. The highest antibacterial activity was recorded in F3 (9%), with inhibition zones of 12.9 mm against S. aureus and 12.7 mm against E. coli. Consequently, F3 was identified as the optimal formulation, demonstrating effective antibacterial activity while maintaining good physical stability and skin safety.

Keywords: Betel Leaf, Hand Sanitizer, Ethanol Extract, Antibacterial Activity, Piper Betle

1. INTRODUCTION

Hand hygiene is an important measure for preventing the transmission of pathogenic microorganisms through direct and indirect contact. Hands can become a medium for transferring microorganisms from contaminated surfaces to the body, making proper hand hygiene an important component of infection prevention. Hand sanitizer provides a practical alternative for maintaining hand hygiene when soap and running water are not readily available. The development of hand sanitizer preparations containing natural antibacterial ingredients has therefore received increasing attention, particularly through the utilization of medicinal plants with established antimicrobial potential [1].

One medicinal plant with considerable potential as a natural antibacterial ingredient is betel leaf (*Piper betle* L.). Betel leaf has been traditionally used for various health-related purposes and contains diverse bioactive constituents. Recent reviews have reported that *P. betle* contains phytochemical compounds associated with antimicrobial activity, including phenolic compounds, flavonoids, tannins, alkaloids, saponins, and other secondary metabolites. These constituents contribute to the broad biological activity of betel leaf extracts and support their potential development into pharmaceutical and topical preparations [1].

The antibacterial properties of *P. betle* are associated with the presence of these bioactive compounds, which may interfere with bacterial cell integrity and essential cellular processes. Recent phytochemical and antibacterial investigations demonstrated that alcoholic extracts of *P. betle* contain detectable levels of flavonoids, tannins, saponins, alkaloids, and other phytochemical constituents and exhibit antibacterial activity against several pathogenic bacteria. Ethanol extracts of *P. betle* have also demonstrated antibacterial effects in experimental studies, supporting their potential application as natural antimicrobial agents[2].

The extraction solvent is an important factor in obtaining bioactive compounds from plant materials. Ethanol is commonly used for extracting phytochemicals because it can dissolve a broad range of polar and semi-polar compounds. Studies conducted on *P. betle* have demonstrated that ethanol extracts contain bioactive constituents and possess antibacterial activity. A recent study reported antibacterial activity of ethanol extracts of *P. betle* against several bacterial strains, while another study demonstrated that ethanol leaf extract exhibited antimicrobial and antibiofilm effects. These findings support the selection of ethanol extract as the active botanical ingredient in the present hand sanitizer formulation [2].

The antibacterial activity of *P. betle* is particularly relevant against *Staphylococcus aureus* and *Escherichia coli*, which represent Gram-positive and Gram-negative bacteria, respectively. A 2022 study specifically evaluated extracted *P. betle* leaf materials against *S. aureus* and *E. coli* using the disc diffusion method and demonstrated antibacterial activity against both organisms. A comprehensive review published in 2021 likewise reported that *P. betle* preparations and extracts can inhibit both Gram-positive and Gram-negative bacteria, including *S. aureus* and *E. coli*. These findings provide a scientific basis for selecting both bacteria as test organisms in evaluating the antibacterial activity of the developed hand sanitizer [3].

The concentration of plant extract is an important factor that can influence the antibacterial activity of a formulation. Increasing the concentration of an extract may increase the amount of bioactive compounds available to interact with bacterial cells and may consequently enhance bacterial growth inhibition. However, changes in extract concentration can also affect the physical and chemical characteristics of a topical preparation, including color, odor, pH, homogeneity, stability, and other formulation characteristics. Therefore, formulation studies need to evaluate antibacterial activity together with relevant quality parameters to identify an appropriate concentration of the active extract. Previous studies have demonstrated the feasibility of incorporating *P. betle* ethanol extract into topical preparations, including hand sanitizer formulations [4].

In addition to antibacterial activity, the quality and safety of a hand sanitizer preparation should be evaluated through physical and stability parameters. Organoleptic characteristics, homogeneity, pH, stability, foam characteristics, and skin irritation are relevant parameters for assessing the quality of a topical preparation. Stability evaluation is particularly important because changes during storage may influence the appearance and performance of the formulation. Previous research on *P. betle*-based topical preparations has demonstrated the feasibility of formulating the extract into topical products while maintaining acceptable physical characteristics and antibacterial activity [5].

Previous research has established the antibacterial potential of *P. betle* extract and its application in topical preparations; however, further evaluation of different extract concentrations in hand sanitizer preparations remains relevant to identify a concentration that provides antibacterial activity while maintaining acceptable formulation characteristics. A 2021 literature review specifically highlighted the potential development of *P. betle* extract and essential oil in hand sanitizer products, while subsequent formulation research demonstrated the application of betel leaf ethanol extract in hand sanitizer preparations [6].

Based on these considerations, this study formulated hand sanitizer preparations containing different concentrations of betel leaf ethanol extract, namely F1 (5%), F2 (7%), and F3 (9%). The formulations were evaluated through phytochemical screening, organoleptic examination, homogeneity testing, stability testing during 12 weeks of storage, pH measurement, foam-height evaluation according to the applicable Indonesian National Standard (SNI), and skin irritation testing. Antibacterial activity was evaluated using the diffusion method against *S. aureus* and *E. coli*. This study aimed to determine the effect of variations in the concentration of betel leaf ethanol extract on the antibacterial activity of

hand sanitizer preparations and to identify the best formulation based on antibacterial activity and formulation characteristics.

2. METHODOLOGY

3.1 Research Design

This study used a laboratory experimental method to investigate the effect of variations in the concentration of betel leaf (Piper betle L.) ethanol extract in hand sanitizer preparations on antibacterial activity against Staphylococcus aureus and Escherichia coli. Three formulations were prepared using different concentrations of betel leaf ethanol extract, namely F1 (5%), F2 (7%), and F3 (9%). The experimental approach included preparation of the ethanol extract, formulation of the hand sanitizer, evaluation of physical characteristics and stability, skin irritation testing, and antibacterial activity testing. Similar experimental approaches have previously been applied to the development and evaluation of P. betle ethanol extract hand sanitizer preparations. [7,8]

3.2 Materials and Instruments

The materials used in this study consisted of betel leaves (Piper betle L.), ethanol as the extraction solvent, and other excipients required for the hand sanitizer formulation. The test microorganisms were S. aureus and E. coli. The instruments included laboratory glassware, analytical balance, measuring cylinders, pH meter, stability storage equipment, microbiological culture equipment, sterile Petri dishes, micropipettes, incubator, and measuring instruments for determining the inhibition zone. The selection of S. aureus and E. coli as test organisms was based on their representation of Gram-positive and Gram-negative bacteria and their established use in evaluating the antibacterial activity of P. betle extracts. [8]

3.3 Preparation of Betel Leaf Ethanol Extract

Fresh betel leaves were collected, cleaned, and processed into simplicia before extraction. The plant material was dried and subsequently powdered. The powdered betel leaves were extracted using ethanol as the solvent. The extraction process was continued until the active constituents were sufficiently extracted, after which the extract was separated from the solvent and concentrated to obtain a concentrated ethanol extract of betel leaves. Ethanol extraction is appropriate for obtaining a range of bioactive compounds from P. betle, including phenolic, flavonoid, tannin, and other phytochemical constituents associated with antibacterial activity. [8]

3.4 Phytochemical Screening

Phytochemical screening was performed to identify the major secondary metabolite groups present in the betel leaf ethanol extract. The screening included tests for flavonoids, alkaloids, tannins, saponins, and phenolic compounds using appropriate qualitative reagents. The results were recorded based on characteristic changes in color, precipitate formation, or foam formation. Phytochemical screening was conducted to provide preliminary information regarding the chemical constituents potentially contributing to the antibacterial activity of the extract. Previous research has demonstrated the presence of several bioactive phytochemical groups in P. betle leaves and their association with antibacterial activity. [8]

3.5 Preparation of Hand Sanitizer Formulations

The concentrated betel leaf ethanol extract was incorporated into the hand sanitizer base at three different concentrations: F1 (5%), F2 (7%), and F3 (9%). Each formulation was prepared using the same base composition, while the concentration of betel leaf ethanol extract was varied. The ingredients were mixed gradually until a homogeneous preparation was obtained. The formulations were subsequently transferred into suitable containers and stored under predetermined conditions for further evaluation. Previous formulation studies have demonstrated the feasibility of incorporating ethanol extract of P. betle leaves into hand sanitizer preparations at different concentrations. [7,9]

3.6 Organoleptic and Homogeneity Evaluation

Organoleptic evaluation was performed by observing the physical appearance, color, odor, and consistency of each formulation. The evaluation was conducted immediately after preparation and during the stability study. Particular attention was given to changes in color, odor intensity, and physical

appearance during storage. Homogeneity was evaluated by observing whether the formulation showed uniform distribution of the extract without visible aggregates or phase separation. These parameters are commonly included in the evaluation of topical preparations containing plant extracts. [7,9]

3.7 Stability Testing

Stability testing was conducted by observing changes in the physical characteristics of the hand sanitizer preparations during 12 weeks of storage. The parameters evaluated included organoleptic characteristics, homogeneity, and physical appearance. Observations were performed periodically throughout the storage period to determine whether the formulations remained stable or showed changes such as separation, precipitation, or other visible alterations. The stability evaluation was important to determine the ability of the formulation to maintain its physical characteristics during storage. Previous studies of P. betle hand sanitizer formulations have also included stability evaluation as an important quality parameter. [7]

3.8 pH Evaluation

The pH of each hand sanitizer formulation was determined using a calibrated pH meter. A sufficient amount of sample was prepared according to the measurement procedure, and the pH value was recorded for each formulation. The pH evaluation was performed to characterize the formulation and assess its suitability for topical application. In this study, the highest pH value obtained among the formulations was 10. Changes in pH may occur as a consequence of differences in extract concentration or interactions between the extract and formulation components; therefore, pH was evaluated as one of the important quality parameters. [7,9]

3.9 Foam Height Test

The foam height of each hand sanitizer formulation was evaluated according to the applicable Indonesian National Standard (SNI) procedure for the relevant preparation. A predetermined amount of the formulation was subjected to the specified agitation procedure, after which the height or volume of the resulting foam was measured. The foam characteristics were compared among F1, F2, and F3 to determine the effect of extract concentration on the foaming characteristics of the preparation. The highest foam height obtained in this study was 85 mL.

3.10 Skin Irritation Test

A skin irritation test was conducted to evaluate the safety of the hand sanitizer preparations for topical application. The formulations were applied according to the predetermined irritation-testing procedure, and the skin was observed for signs of irritation, including redness, swelling, itching, or other visible reactions. The results were recorded for each formulation. The irritation evaluation was included because hand sanitizer is intended for repeated contact with the skin and because changes in the concentration of plant extract may influence the tolerability of the preparation. Previous P. betle hand sanitizer formulation research has also included skin irritation testing as part of formulation evaluation. [7]

3.11 Antibacterial Activity Test

The antibacterial activity of the hand sanitizer formulations was evaluated using the diffusion method against S. aureus and E. coli. Bacterial cultures were prepared and inoculated onto appropriate agar media. Each formulation was subsequently applied to the test medium using the selected diffusion technique. After incubation under appropriate microbiological conditions, the diameter of the clear inhibition zone surrounding each test sample was measured in millimeters. Antibacterial activity was determined based on the diameter of the inhibition zone produced by each formulation. The diffusion method has been used in previous studies to evaluate the antibacterial activity of P. betle extracts against S. aureus and E. coli. [8]

3.12 Data Analysis

The results of the physical evaluation, stability testing, irritation testing, and antibacterial activity testing were recorded and presented descriptively. The antibacterial activity of F1, F2, and F3 was compared based on the diameter of the inhibition zones produced against S. aureus and E. coli. The formulation showing the greatest antibacterial activity while maintaining acceptable physical characteristics and stability was designated as the best formulation. In this study, F3 containing 9%

betel leaf ethanol extract was subsequently identified as the best formulation based on the experimental results.

3. RESULTS

a. Phytochemical Screening of Betel Leaf Ethanol Extract

The phytochemical screening of the betel leaf (Piper betle L.) ethanol extract showed positive results for saponins, flavonoids, alkaloids, tannins, phenols, steroids, and essential oils. The presence of these phytochemical constituents indicates that the extract contains several groups of secondary metabolites with potential biological activity, including antibacterial activity. The results of phytochemical compounds can be seen in the table 1

***Table 1**. Phytochemical compounds*

Phytochemical compound	Result
Saponin	Positive
Flavonoid	Positive
Alkaloid	Positive
Tannin	Positive
Phenol	Positive
Steroid	Positive
Essential oil	Positive

b. Organoleptic Evaluation and Stability

Four preparations were evaluated, consisting of F0 as the blank formulation and F1, F2, and F3 containing 5%, 7%, and 9% betel leaf ethanol extract, respectively. Organoleptic evaluation showed that F0 had a yellow color and no characteristic odor, whereas the formulations containing betel leaf ethanol extract showed colors ranging from greenish-brown to brown and had the characteristic odor of betel leaves. During 12 weeks of storage, all preparations maintained their physical form and remained stable, with no observable separation or major changes in the physical characteristics of the preparations. However, a decrease in the intensity of the characteristic odor of betel leaf was observed during storage.

c. pH Evaluation

The pH evaluation showed that F0 had a pH of 9.0, while the pH values of the formulations containing betel leaf ethanol extract were 8.0, 9.5, and 8.8 for F1, F2, and F3, respectively. The results indicate that the variation in betel leaf ethanol extract concentration produced differences in the pH values of the preparations. The pH values of all formulations remained relatively stable during the observation period.

d. Foam Height Evaluation

The foam-height evaluation showed that F0 produced a foam height of 78 mL. Among the formulations containing betel leaf ethanol extract, F1 produced a foam height of 76 mL, F2 produced 70 mL, and F3 produced 72 mL. The highest foam height was observed in the blank formulation F0, while the lowest foam height was observed in F2. The differences in foam height indicate that the addition and variation of betel leaf ethanol extract affected the foaming characteristics of the hand sanitizer preparations.

e. Skin Irritation Test

The skin irritation test showed that neither the blank formulation nor the formulations containing betel leaf ethanol extract produced observable allergic reactions or skin irritation during use. No visible signs of irritation were observed following application of F0, F1, F2, or F3. These results indicate that the tested hand sanitizer preparations did not demonstrate an observable irritating effect under the conditions of the study.

f. Antibacterial Activity

The antibacterial activity of the hand sanitizer formulations was evaluated against Staphylococcus aureus and Escherichia coli using the diffusion method. All formulations containing betel leaf ethanol extract demonstrated antibacterial activity, as indicated by the formation of inhibition zones around the test samples. F1 (5%) produced inhibition zones of 7.0 mm against both S. aureus and E. coli. F2 (7%) produced inhibition zones of 9.0 mm against S. aureus and 9.5 mm against E. coli. The greatest antibacterial activity was observed in F3 (9%), with inhibition zones of 12.9 mm against S. aureus and 12.7 mm against E. coli. The inhibition zone increased as the concentration of betel leaf ethanol extract increased from 5% to 9%.

g. Best Formulation

Based on the overall evaluation, F3 containing 9% betel leaf ethanol extract demonstrated the greatest antibacterial activity among the tested formulations. F3 produced inhibition zones of 12.9 mm against S. aureus and 12.7 mm against E. coli, while maintaining homogeneous and stable physical characteristics during 12 weeks of storage and producing no observable skin irritation. Therefore, F3 (9%) was selected as the best formulation in this study. The results of the parameter evaluation can be seen in the table 2

***Table 2**. Evaluation of stock parameters*

Evaluation parameter	F0 (Blank)	F1 (5%)	F2 (7%)	F3 (9%)
Color	Yellow	Greenish-brown	Greenish-brown/brown	Brown
Odor	Odorless	Betel leaf odor	Betel leaf odor	Betel leaf odor
Ph	9.0	8.0	9.5	8.8
Foam height (mL)	78	76	70	72
Stability (12 weeks)	Stable	Stable	Stable	Stable
Skin irritation	None	None	None	None
S. aureus inhibition zone (mm)	–	7.0	9.0	12.9
E. coli inhibition zone (mm)	–	7.0	9.5	12.7

4. CONCLUSIONS

The variation in the concentration of betel leaf (*Piper betle* L.) ethanol extract affected the antibacterial activity of the hand sanitizer preparations against *Staphylococcus aureus* and *Escherichia coli*. The phytochemical screening showed that the betel leaf ethanol extract contained saponins, flavonoids, alkaloids, tannins, phenols, steroids, and essential oils, which may contribute to its antibacterial activity.

All formulations (F0, F1, F2, and F3) maintained their physical form and stability during 12 weeks of storage. The pH values were 9.0 for F0, 8.0 for F1, 9.5 for F2, and 8.8 for F3, while the foam heights were 78, 76, 70, and 72 mL, respectively. No observable allergic reactions or skin irritation occurred following use of the formulations.

The antibacterial activity increased with increasing betel leaf ethanol extract concentration. F1 (5%) produced inhibition zones of 7.0 mm against both *S. aureus* and *E. coli*, while F2 (7%) produced inhibition zones of 9.0 mm and 9.5 mm, respectively. F3 (9%) produced the largest inhibition zones, measuring 12.9 mm against *S. aureus* and 12.7 mm against *E. coli*. Therefore, F3 (9%) was identified as the best formulation based on its antibacterial activity, physical stability, and absence of observable skin irritation.

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