JPPIPA 11(9) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

The Antibacterial Activities of *Piper betle* and *Allium cepa* Extracts Against *Staphylococcus aureus*

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Received: March 11, 2025
Revised: August 17, 2025
Accepted: September 25, 2025
Published: September 30, 2025

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DOI: 10.29303/jppipa.v11i9.10850

Abstract: Indonesia rich in biodiversity, both animals and plants. This is known as ethnobotany. Local people learn to use natural herbs to meet their daily needs, and some commonly used herbs are *Piper betle* (betel leaf) and *Allium cepa* (red onion). They often use *P. betle* or *A. cepa* to cure various skin infections, one of which is furuncles. This study aimed to explore ethanol extracts of *P. betle, A. cepa,* and their combination against *Staphylococcus aureus,* the main bacterial cause of furuncles, using petri dish. The inhibition zones in petri dishes were measured using a caliper to evaluate the antibacterial efficacy. Each sample was tested with 3 replicates and kanamycin was used as positive control. The *P. betle* extract exhibited the strongest inhibitory effect with an inhibition zone of 17.70 ± 0.30 mm, followed by *P. betle* and *A. cepa* with ratio 1:1 produced an inhibition zone of 15.63 ± 0.58 mm. The ethanol extract of *A. cepa* showed the lowest inhibition zone only 14.38 ± 0.35 mm and this inhibition zone was similar to that of kanamycin as positive control.

Keywords: *Allium cepa*; Antibacterial; *Piper betle*; *Staphylococcus*

Introduction

Indonesia ranks second after Brazil in terms of terrestrial biodiversity (National Geographic Indonesia, 2019). This abundant biodiversity is utilized by local communities and passed down from generation to generation through customs, traditions, and ancestral recipes (Fallo et al., 2022). Plants are widely used to meet various needs, from food to traditional medicine. The use of traditional medicine has been known to the Indonesian people long before the use of synthetic drugs (Nurkomaria et al., 2023).

Spices that are often used as traditional medicine by Indonesian are betel leaves (*Piper betle*) and shallots (*Allium cepa*). The ethanol extract of *P. betle* mainly contains phenolic compounds and saponins (Sonphakdi et al., 2024). Flavonoids and tannins are phenolics compounds (Alara et al., 2021). Plants produce flavonoid in response to microbial infections. Flavonoids are powerful antimicrobial agents with various mechanisms of action as antibacterials (Imran et al., 2025). Tannins are well known both as astringent and antibacterial agents (Kováč et al., 2022). Saponins have antibacterial and antifungal activity by destroying the cell membrane of microbes (Mehta, 2020; Ervianingsih et al., 2025).

Biswas et al. (2022) reported that *P. betle* exhibits anti-inflammatory, antioxidant, analgesic, antifungal, and antibacterial activities. Nayaka et al. (2021) reported that *P. betle* showed fungistatic and fungicidal activity, as well as bacteriostatic and bactericidal activity depending on its ratio. Antifungal agents are compounds or drugs that work by inhibiting or eradicating the growth of fungi while antibacterial agents are refer to compounds or drugs that capable of inhibiting (fungistatic or bacteriostatic) or eradicating

How to Cite:
Setiawan, V., Triono, N., Agustina, A. K., Moira, L. A., Biutifasari, V., & Budipramana, K. (2025). The Antibacterial Activities of Piper betle and Allium cepa Extracts Against Staphylococcus aureus. *Jurnal Penelitian Pendidikan IPA*, *11*(9), 97–102. https://doi.org/10.29303/jppipa.v11i9.10850

(fungicidal or bactericidal) the growth of molds or bacteria (Haryani et al., 2025; Dey et al., 2025).

In India, *P. betle*, known as "green gold," is famous for its medicinal properties related to its nutritional value and is used as a mouthwash after meals (Chowdhury et al., 2020; Kirve, 2024). The utility of *P. betle* as mouthwash perhaps due to phenolic compounds since phenolic compounds have activities as preservatives, disinfectant, and antiseptic (Brooks et al., 2013). In Srilanka, fresh juice of *P. betle* leaves is frequently used to treat skin rashes, wounds, and cuts as topical (Arambewela et al., 2010). Local people in Kapanewon Berbah, Yogyakarta, Indonesia, reported the traditional use of *P. betle* and *A. cepa* to treat this infection. They clean the infected area around the furuncle with boiling water containing *P. betle*, followed by the topical application of *A. cepa*. This method has been practiced from generation to generation among the local population.

A. cepa contains allicin, alliin, diallyl sulfide, and various phenolic compounds, and is widely recognized as an antimicrobial and antiparasitic agent (Chakraborty et al., 2022). In Turkey, *A. cepa* is in list of ethnobotany used to treat furuncle (Goc et al., 2021). Meanwhile, in Italy, *A. cepa* used as topical to cure abscess and furuncle (Quave, 2008). These reports indicate that both *P. betle* and *A. cepa* are traditional plants frequently used for their antimicrobial activity.

Skin is one of the largest parts of human body that is exposed to the environment. As a barrier, skin is vulnerable to microorganisms that can cause infection (Grice et al., 2011; Aman et al., 2020). Symptoms of skin infections caused by bacteria or fungi include pain, swelling, and redness around the infected area (Neman, 2022). One type of skin infection is a furuncle, which can lead to the formation of an abscess. This infection develops due to bacterial infection that attacks hair follicles and progresses into an abscess containing pus and dead tissue. The primary cause of this condition is *Staphylococcus aureus*, a bacterium commonly found on human skin, particularly in hairy areas such as the neck, armpits, or thighs (Ibler et al., 2014; Estrada-Chávez, 2024). As mentioned before, *P. betle* and *A. cepa* are frequently used traditionally for their antimicrobial activity. Therefore, this study aims to explore the potential synergistic effects of these two plants against *S. aureus.*

Method

Plant Materials

This study used various materials to support the antibacterial testing and the formulation of the developed preparation. The leaves of *P. betle* and bulb of *A. cepa* were obtained from a local market in Surabaya, Indonesia. *S. aureus* (ATCC 6538) was used as the test bacterium, while kanamycin was used as a positive control to compare the antibacterial effectiveness of the plant extracts. Nutrient agar (HiMedia, India) was used as a bacterial growth medium. Additional materials supporting this study included sterile distilled water (Ikapharmindo, Indonesia) as solvent, 96% ethanol (technical grade) for extraction, filter paper (Whatman # 41, Maidstone England) for filtration.

Equipments

An incubator (Binder BD115, Germany), autoclave (All-American, USA), rotary evaporator (Buchi R-200, Switzerland), oven (Memmert, Germany), analytical balance (Ohaus, USA), micropipette (Acura-Socorex, Switzerland), UV-Visible spectrophotometer (Jasco, Japan), and glassware (Iwaki, Japan).

Extraction

The leaves of *P. betle* and *A. cepa* bulb were ground into powder and sieved using 100 mesh. Next, each powder was weighed according table 1.

Table 1. Ratio of *P. betle* and *A. cepa* for Extraction

P. betle leaf	*A. cepa* bulb (g)	Ratio	Dimeter inhibition (mm)
0 g	50 g	0:1	14.38±0.38
50 g	0 g	1:0	17.70±0.30
25 g	25 g	1:1	15.63±0.58
50 g	25 g	2:1	13.53±0.27
25 g	50 g	1:2	14.33±0.38
Kanamycin			14.40±0.41

Five hundred milligrams of each powder or mixture was extracted with 300 ml of 96% alcohol for 24 hours. The residues were re-extracted for 4 days. The filtrate extracts were evaporated using a rotary evaporator, yielding 20 ml of concentrated extract.

Antibacterial Evaluation of Plant Extracts against Staphylococcus aureus

The *S. aureus* bacterial inoculum was prepared by measuring the transmittance using a spectrophotometer set at ʎ of 580 nm, until a transmittance of 25% was reached. Both the 30 ml base layer and 20 ml seed layer were sterilized at 121°C for 30 minutes. The base layer was transferred to a petri dish and allowed to cool and solidify. Ten microliters of inoculum were mixed with seed layer then poured onto solid base layer and let it solidify. Subsequently, wells were created in the agar using a ring, with the number of wells depending on the number of samples to be tested. Each extract was prepared by diluting it with sterile distilled water at a 1:1 ratio. Each well contain 50 µL sample of diluted extract with 3 replications. As a negative control, 100 µL of sterile water was added to one of the wells, and as a

positive control, 50 µL of 100 ppm kanamycin was added to another well. The petri dish was then incubated at 32.5°C for 24 hours. Measure the inhibition zones using caliper to evaluate the antibacterial effectiveness of the tested extracts sample.

Result and Discussion

The issue of bacterial resistance is currently one of the main concerns in the field of health, so the search for new antibacterial compounds continues to be encouraged (Guglielmi et al., 2020). Natural sources such as medicinal plants are gaining increasing attention due to their great potential in inhibiting the growth of pathogenic bacteria. Plant extracts can be effective against bacteria, but the results can vary significantly depending on the efficiency of the extraction. Various factors such as solvent type, temperature, extraction time, and extraction method play a significant role (Nawaz et al., 2020). Ethanol is classified as a polar solvent, making it dominant in extracting polar compounds such as phenolic compounds, flavonoids, saponins, and alkaloids, which can act as antibacterial agents (Putri et al., 2024). In this study, we investigated the antibacterial effects of ethanol extracts from *P. betle* or *A. cepa*, as well as a combination of both, against *S. aureus*, a gram-positive bacterium commonly found on mucous membranes and skin (Taylor & Unakal, 2025).

This study showed that all ethanol extracts from *P. betle* or *A. cepa* or a combination of both were active against *S. aureus* because they had a similar or even higher inhibition diameter than the inhibition diameter of kanamycin as a positive control (14.40 mm). The ethanol extract of *P. betle* yielded the highest inhibition diameter of 17.70 mm, followed by the combination of *P. betle* and *A. cepa* (ratio 1:1) with an inhibition diameter of 15.63 mm. The ethanol extract of *A. cepa* alone only produced an inhibition diameter of 14.38 mm, similar to the combination of *P. betle* and *A. cepa* in a 1:2 ratio, which was 14.33 mm. The mixture of *P. betle* ethanol extract and *A. cepa* in a 1:1 ratio also provides the best combination compared to other ratios such as 1:2 or 2:1. According to the classification by Davis et al. (1971), inhibition zones below 5 mm are classified as weak, 5–10 mm as moderate, 11–19 mm as strong, and above 20 mm as very strong (Davis & Stout, 1971). Based on this standard, all the extracts analyzed fall into the strong category.

Valle et al. (2015) studied ethanol extracts from 12 plants traditionally grown in the Philippines, including *Zingiber officinalis, Tinospora rumphii, Phyllanthus niruri, Moringa oleifera, Mitrephora lanotan, Vitex negundo, Psidium guajava, Curcuma longa, Centella asiatica, Cassia alata*, and *P. betle* against methicillin-resistant *Staphylococcus aureus* (MRSA). They reported that only ethanol extracts from *P. betle* showed the best antibacterial activity as an inhibitor of MRSA. Phumat et al. (2017) studied ethanol extracts from *Sesbania grandiflora, Phyllanthus emblica, Momordica charantia, Andrographis paniculta,* and *P. betle* against two strains of oral pathogenic *Streptococcus* and two strains of *Candida*. *P. betle* showed the highest antibacterial activity among the other plants and against all bacteria tested. Another study conducted by Teanpaisan et al. (2017) also investigated 12 traditional Thai plants commonly used as antibacterial and antibiofilm agents. Twelve traditional Thai plants, including *Zingiber officinale, Ocimum sanctum, O. basilicum, O. africanum, Mentha cordifolia, Piper sarmentosum, Curcuma zedoaria, C. longa, P. nigrum, P. chaba,* and *P. betle*, were tested against *Fusobacterium nucleatum, Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans, Lactobacillus salivarius,* and *L. fermentum*, which commonly infect the mouth. The antibacterial test of *P. betle* ethanol extract showed the best inhibition against all tested bacteria. They also revealed that the main compound found in the ethanol extract is 4-chromanol, which acts as a dual inhibitor as an antibiofilm and antibacterial agent.

Sagar et al. (2020) tested red onion skin *A. cepa* against *Streptococcus agalactiae, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus cereus*, and *S. aureus*. This result is consistent with Anh et al. (2023), who reported the successful of ethanol extract of *A. ascalonicum* skin against *S. aureus,* as well as by Octaviani et al. (2019) on *Trichophyton mentagrophytes, Escherichia coli, Salmonella thypi, Staphylococcus epidermidis,* and *S. aureus.* On the other hand Anindita et al. (2023) reported the use of onion skin from *A. ascalonicum* but it did not significantly inhibit the growth of *S. aureus*. Our findings revealed that the addition *P. betle* to *A. cepa* in a ratio of 1:1 could increase the inhibition diameter of *A. cepa* itself from 14.38 mm to 15.63 mm. However, a higher ratio of *P. betle* to *A. cepa* at a 2:1 ratio resulted in a decrease in the inhibition diameter to 14.33 mm, which was the lowest inhibition value among all ratios. A higher ratio of *A. cepa* to *P. betle* (2:1) resulted in an inhibition diameter of 14.33 mm, similar to the inhibition diameter of *A. cepa* (14.38 mm) and kanamycin (14.40 mm). Based on the similarity of the inhibition diameter between the ethanol extract *A. cepa* 14.38 mm and kanamycin 14.40 mm, it is assumed that the ethanol extract *A. cepa* as natural herb has similarity potency to synthetic kanamycin.

Conclusion

The ethanol extract of *P. betle* showed highest antibacterial activity against *S. aureus* 17.70 mm. The best combination ethanol extract of *P. betle* and *A. cepa* in a 1:1 ratio was 15.63 mm. Although the ethanol extract of *A.*

cepa had the lowest antibacterial zone 14.33 mm, this antibacterial zone similar to antibacterial zone of kanamycin as positive control 14.40 mm.

Acknowledgements
Christian High School of Petra 2, Surabaya, Indonesia for providing the laboratory facilities.

Author Contributions
Conceptualization K. B, V. S, N. T., A. K. A. Investigation L. A. M., K. B., V. S., V. B., N. T., A. K. A. Drafting K.B, V.S, L.A.M, V. S. Review and editing K.B, V.S

Funding
No external funding.

Conflicts of Interest
Authors declare there are no conflicts of interest.

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