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Research Article
Investigation of Antibacterial and Antifungal Activities of *Etlingera balikpapanensis* Extracts against *Staphylococcus aureus* and *Candida albicans*

[1]Hasyrul Hamzah, [2]Sylvia Utami Tunjung Pratiwi, [1]Dwi lestari, [1]Diah Ayu Azizah, [1]Nur Atika Astriani, [3]Arsiullah Jabbar and [4]Ahmad Marzuki

[1]Faculty of Pharmacy, Muhammadiyah University East Kalimantan, Samarinda, 75124, Indonesia
[2]Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, 55281, Indonesia
[3]Faculty of Pharmacy, Halu Oleo University, Kendari, 93232, Indonesia
[4]Faculty of Laboratory Technology, Health Polytechnic Ministry of Health, Maluku, 97233, Indonesia

Abstract
Background and Objective: Antimicrobial resistance has created a demand for alternative therapeutic agents derived from natural products. *Balikpapan ginger* (*Etlingera balikpapanensis* A.D. Poulsen) is an underexplored species with potential medicinal value. This study aimed to evaluate the antibacterial and antifungal activity of its stem extract against *Staphylococcus aureus* and *Candida albicans*. **Materials and Methods:** The extract was obtained through maceration using 96% ethanol. Antimicrobial activity was tested using the disc diffusion method at concentrations of 0.5, 1 and 2%. The diameters of inhibition zones were measured and results were analyzed statistically to assess dose-dependent effects. **Results:** The extract exhibited significant antibacterial activity against *S. aureus* and notable antifungal activity against *C. albicans*. Inhibition zones increased proportionally with extract concentration, with the highest activity observed at 2%. The antimicrobial effect is attributed to the presence of flavonoids and tannins in the extract. **Conclusion:** *Balikpapan ginger* stem extract demonstrates promising antibacterial and antifungal potential, supporting its application as a natural antimicrobial agent in health-related fields. Further studies are recommended to isolate active compounds and evaluate *in vivo* efficacy.

Key words: *Balikpapan ginger*, *Etlingera balikpapanensis*, antimicrobial activity, *Staphylococcus aureus*, *Candida albicans*, natural therapeutics

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Corresponding Author: Hasyrul Hamzah, Faculty of Pharmacy, Muhammadiyah University East Kalimantan, Samarinda, Indonesia
Tel: +62 852-4007-8947

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious diseases remain one of the leading causes of morbidity and mortality worldwide, particularly those associated with microbial resistance and chronic infections. The National Institutes of Health estimates that nearly 65-80% of human infections are related to biofilm formation, which makes them more persistent and difficult to treat[1]. Biofilm-associated infections are especially problematic in hospital environments, where they contribute to prolonged hospitalization, high treatment costs and significant mortality rates. In Indonesia and other developing countries, the burden of biofilm-related infections continues to rise in parallel with the increased use of medical devices and the growing prevalence of immunocompromised patients[2].

Among the microorganisms responsible for biofilm-associated diseases, *Staphylococcus aureus* and *Candida albicans* are of particular concern. The *S. aureus* is a major bacterial pathogen, frequently involved in bloodstream infections, pneumonia, endocarditis and wound infections. Its ability to form biofilms on medical implants such as catheters and prosthetic devices enables the pathogen to evade host immune responses and survive antimicrobial treatment. Moreover, the emergence of Methicillin-Resistant *S. aureus* (MRSA) has made infections more difficult to control, often resulting in severe complications and increased mortality[2].

On the other hand, *Candida albicans* is the most common fungal pathogen in humans, responsible for a wide range of infections from superficial mucosal candidiasis to life-threatening bloodstream infections. The *C. albicans* is also a strong biofilm former, particularly on medical devices and mucosal surfaces, where biofilm communities display resistance levels up to 1,000 times higher than planktonic cells. When *S. aureus* and *C. albicans* coexist, they often develop synergistic polymicrobial biofilms. These mixed biofilms enhance microbial survival, promote antibiotic and antifungal tolerance and increase virulence, leading to infections that are extremely challenging to eradicate. Such infections can result in chronic inflammation, sepsis and even death, representing a critical issue in both community and hospital settings[3].

Given these challenges, there is an urgent need to discover novel antimicrobial agents that can target biofilm-forming pathogens effectively. Natural products, especially those derived from medicinal plants, are an important source of bioactive compounds with antimicrobial and antibiofilm potential. The genus *Etlingera*, belonging to the Zingiberaceae family, has long been recognized in traditional medicine for its pharmacological properties, including antimicrobial, antioxidant and anti-inflammatory activities[4].

Of particular interest is *Etlingera balikpapanensis*, a plant species endemic to Kalimantan, Indonesia. Despite its limited scientific exploration, preliminary phytochemical analysis of *Etlingera* species suggest the presence of diverse secondary metabolites such as flavonoids, terpenoids and phenolic compounds, which are known to exhibit antimicrobial activity. Investigating the antibacterial and antifungal potential of *E. balikpapanensis* extracts against biofilm-forming pathogens like *S. aureus* and *C. albicans* could provide valuable insights into its therapeutic potential. Moreover, this research highlights the importance of conserving Indonesia's unique biodiversity while addressing the urgent global need for new strategies to combat biofilm-associated infections[5].

The study of *Etlingera balikpapanensis* extracts against these clinically relevant pathogens is therefore timely and important. Such investigations can bridge the gap between traditional knowledge and modern medicine, while also contributing to biodiversity-based drug discovery. Ultimately, the exploration of this plant's antimicrobial activity may support the development of natural therapeutic agents and provide scientific validation for the medicinal value of a unique species native to Indonesia.

Over the past few decades, plants from the ginger family have been the subject of intensive scientific research. A variety of bioactive compounds, such as tannins, flavonoids, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides and alkaloids, have been successfully isolated from different parts of these plants and studied for their pharmacological benefits[5]. This study evaluates the antibacterial and antifungal activity of *Balikpapan ginger* (*Etlingera balikpapanensis*) stem extract against *Staphylococcus aureus* and *Candida albicans* using the disc diffusion method.

MATERIALS AND METHODS

Equipment and material: The equipment used includes: Erlenmeyer flask (Iwaki, Japan), test tube (Iwaki), horn spoon, spatula, gloves, mask (KF 94), laminar airflow (LAF), water bath (Faithful), rotary vacuum evaporator (Büchi, Switzerland), digital scale (Ohaus, USA), hotplate (Maspion), micropipette (Dlab, China), autoclave (All American, USA), incubator, Bunsen burner and disc paper. The materials used include: *Balikpapan ginger* stem extract, 96% ethanol, alcohol (OneMed), Fluconazole, Chloramphenicol, PDA media (OXOID), NA media (HIMEDIA), sterile distilled water, pure culture of *Staphylococcus aureus* ATCC 6538 and pure culture of *Candida albicans* ATCC 10231.

Sample preparation: The *Balikpapan ginger* stem (*Etlingera balikpapanensis* A.D. Poulsen) sample was obtained from Batu Ampar District, East Kutai Regency, East Kalimantan Province in July, 2025.

Plant identification: The determination of the *Balikpapan ginger* plant was conducted at the Mulawarman Herbarium, Tropical Forest Biodiversity Ecology and Conservation Laboratory, Faculty of Forestry, Mulawarman University. Based on the identification results with letter number 362/UN17.4.08/LL/2024, it was confirmed that the plant used is indeed the *Balikpapan ginger* (*Etlingera balikpapanensis* A.D. Poulsen).

Preparation of simplisia: The young and old stem parts (which are mixed), as well as their branches, are cleaned using running water to remove dirt. After that, these parts are dried and placed in plastic bags to keep the material fresh. The dried samples can be turned into powder by cutting them into small pieces to facilitate grinding[6].

Extraction of *Balikpapan ginger* stems: The stem extract of *Etlingera balikpapanensis* (*Balikpapan ginger*) was obtained through maceration. The powdered sample was immersed in 96% ethanol (semi-polar solvent) for 3×24 hrs, protected from light with black cloth. The mixture was stirred periodically and the filtrate was separated by filtration. Remaceration was conducted for 2×24 hrs to maximize compound recovery, followed by filtration. The combined filtrates were evaporated using a rotary evaporator at 50°C and subsequently concentrated in a water bath at 50°C to remove residual solvent. The resulting thick extract was weighed and the percentage yield was calculated as the ratio of extract weight to raw material weight.

Identification details of secondary metabolites:

- **Alkaloid:** The alkaloid test was conducted qualitatively by preparing the extract in a test tube, then adding a few drops of HCl and two drops of Dragendorff's reagent. The presence of an orange precipitate indicates that the sample contains alkaloids. Meanwhile, if a reddish-brown precipitate forms, the test results confirm that the sample is positive for aslkaloids[7]
- **Flavonoid:** The flavonoid content test was conducted qualitatively using a sufficient amount of concentrated *Balikpapan ginger* stem extract, which was then transferred into a test tube. Next, hot distilled water was added, followed by a few drops of HCl and a small amount of Mg powder. If the sample turns yellowish-brown, it indicates the presence of flavonoids[8]
- **Tannin:** The tannin test was performed qualitatively. A certain amount of *Balikpapan ginger* stem extract was placed in a test tube, followed by the addition of 95% ethanol and a few drops of FeCl solution[9]
- **Saponin:** The test for detecting saponins was carried out by preparing a sufficient amount of extract in a test tube, then adding distilled water, shaking the mixture vigorously and letting it sit for 15 min. Afterward, a few drops of concentrated HCl were added. If the foam remains after the addition of HCl, it can be concluded that the sample contains saponins[10]
- **Steroid:** Steroids were tested qualitatively by preparing *Balikpapan ginger* stem extract in a test tube. Then, 95% ethanol, chloroform, anhydrous acetic acid and a small amount of H_2SO_4 were added. After being gently mixed, the solution was left to stand for a few minutes. A color change to blue or green indicates the presence of steroids in the sample[11]
- **Terpenoid:** The terpenoid test was conducted by taking 1 ml of the plant sample and adding the Liebermann-Burchard reagent, which consists of 2 mL of chloroform, 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. After the mixture was gently shaken and left to stand for a few minutes, the color change was observed. The test result is considered positive if a red or purple color appears, along with a brown ring[12]

Bacterial strain: *Staphylococcus aureus* (ATCC 25923) was refined into BHI medium and brooded at 37 for 24 hrs. Bacterial thickness was inspected utilizing a spectrophotometer and acclimated to McFarland 0.1 norm ($0.5\text{-}1.5\times10^8$ CFU/mL)[2].

***Candida albicans* test fungus preparation:** The isolate tested in this study was *Candida albicans*. After being inoculated from SDA media into 15 mL of YPD media, a number of fungal colonies were left to incubate for an entire night at room temperature with constant shaking. After which they were PBS-washed and centrifuged for 15 min at 3000 rpm. The pellet obtained was resuspended with RPMI media and a suspension of C. *albicans* $1\text{x}10^8$ CFU/mL was prepared in 5 mL of RPMI media (equalized to McFarland standard 0.5) A total of 1 mL was taken from this suspension and added to 9 mL of RPMI medium to obtain a suspension of *C. albicans* colonies $1\text{x}10^7$ CFU/mL as a stock solution. A working solution was made with a dilution of 1:10 to obtain a suspension of *C. albicans* 1×10^6 CFU/mL[6].

Antibacterial activity testing: The antibacterial test was conducted using the disk diffusion method. Several advantages of this approach include its low cost, ease of implementation, time efficiency and the simplicity of the equipment used. Before use, all equipment was sterilized using an oven and an autoclave.

The testing process involved placing paper disks impregnated with the test compound at concentrations of 0.5, 1 and 2% onto agar media. Chloramphenicol was used as the positive control due to its antibacterial properties, while 96% ethanol was used as the negative control. The samples were then incubated at 37°C for 24 hrs. The bacteria-free zone formed around the paper disks was then measured using a calliper to determine the diameter of the inhibition zone[13].

Antifungal activity testing: The test is performed using the disk diffusion method with agar media. Paper discs containing the test materials at concentrations of 0.5, 1 and 2% are placed on the media. The diameter of the inhibition zone is measured using a caliper. Fluconazole is used as a positive control and 96% ethanol as a negative control. Incubation is carried out at 37°C for 24 hrs. All equipment is sterilized before use. This method is simple, quick and cost-effective. The diameter of the transparent zone that forms around the paper discs is measured using a caliper/ruler[14].

RESULT AND DISCUSSION

Extraction: The maceration and remaceration processes produced a thick ethanolic extract of *Balikpapan ginger* stem. The yield percentage obtained from the extraction is presented in Table 1. The obtained yield reflects the efficiency of the maceration technique in extracting bioactive compounds from the raw material.

Maceration was selected as the extraction method because it avoids heating, thereby preventing the degradation of thermolabile compounds and maintaining the stability of secondary metabolites. The use of 96% ethanol as a solvent was appropriate due to its semi-polar nature, which enables the extraction of a wide spectrum of compounds ranging from polar to non-polar. In addition, protecting the sample from light during the maceration process helped minimize the degradation of photosensitive compounds, while applying a moderate temperature (50°C) during evaporation and solvent removal ensured that active constituents were not lost.

The relatively high yield indicates that the process successfully extracted a significant portion of the secondary metabolites, which may contribute to the antimicrobial potential of *Balikpapan ginger*. These findings support the suitability of maceration with ethanol as an efficient extraction approach for preserving and concentrating the bioactive constituents of the plant[15-18].

Based on the table above, the weight of the raw material obtained was 257 g and the resulting thick extract weighed 26.38 g, with a yield of 10.2% of the raw material weight.

Data Identification: Based on the identification results of secondary metabolite groups in the *Balikpapan ginger* stem extract, it was found that several secondary metabolite groups were detected, including alkaloids, flavonoids, tannins, saponins and terpenoids. The results of the secondary metabolite tests are shown in Table 2.

The results of secondary metabolite testing on the extract of Balikpapan ginger stem, extracted using the maceration method, showed positive results for alkaloids, flavonoids, tannins, saponins and terpenoids. The extract of *Balikpapan ginger* stem was found to be negative for steroids.

The secondary metabolite analysis of the *Balikpapan ginger* stem extract indicated the presence of alkaloids, flavonoids, tannins, saponins and terpenoids. The extract contains alkaloids, which were identified by a color change when tested with Mayer's, Borchardt's and Dragendorff's reagents. The positive reaction for alkaloids occurs because alkaloids, with nitrogen atoms containing free electron pairs, can replace iodide ions in these reagents[19]. The results were as follows: A white precipitate with Mayer's reagent, a brown precipitate with Bouchardat's reagent and a reddish-brown precipitate with Dragendorff's reagent, indicating the presence of alkaloids in the Balikpapan ginger stem.

Table 1: Extraction yield calculation results

	Extract yield	
Simplisia weight (g)	Concentrated extract (g)	Yield (%)
257	26.38	10.2

Table 2: Data Identification of secondary metabolite compound groups in *Balikpapan ginger* stem

	Secondary metabolite test					
Sample	Alkaloid	Flavonoid	Tannin	Saponin	Steroid	Terpenoid
Extract of *Balikpapan ginger* stem	+	+	+	+	-	+

+: Secondary metabolites identified and -: Secondary metabolites not identified

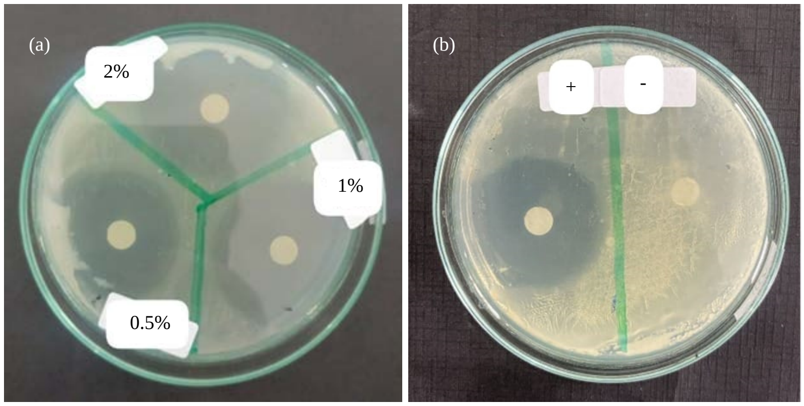

Fig. 1(a-b): Antibacterial activity of *Etlingera balikpapanensis* stem extract against *Staphylococcus aureus*, (a) Antibacterial activity of the extract at concentrations of 0.5, 1 and 2% and (b) Antibacterial activity with positive control (Chloramphenicol) and negative control (1% ethanol)

Flavonoid testing showed a positive result if a yellow, reddish or orange color appeared after adding Magnesium (Mg) and concentrated Hydrochloric Acid (HCl). This color change indicates the formation of a flavylium salt, which is characteristic of flavonoid compounds[20].

Tannin testing on the *Balikpapan ginger* stem extract showed a yellow color after adding 1% $FeCl_3$ reagent. This color formation occurred because tannin compounds interacted with Fe^{3+} ions, forming a complex between tannins and $FeCl_3$, with oxygen atoms in tannins coordinating with the central Fe^3 atom as a ligand[18].

Saponin testing showed a positive result with the formation of foam ranging from 1 to 10 cm, which did not dissipate after adding 1 drop of 2N HCl. This foam formation occurred because saponins have both hydrophobic and hydrophilic groups that form foam when shake. The addition of 2N HCl increases polarity, strengthens hydrophilic group bonds and stabilizes the foam. The micelle structure formed, with polar groups facing outward and nonpolar groups facing inward, also supports foam formation[18].

In steroid testing, the *Balikpapan ginger* stem extract did not contain steroid compounds, as indicated by the absence of a blue or blue-green color change after adding the reagent[10]. On the other hand, the terpenoid test showed a positive result due to a color change to red or purple, as well as the formation of a brown ring in the solution after adding Lieberman-Burchard reagent[18].

Overall, the secondary metabolite testing results of the *Balikpapan ginger* stem extract, extracted using the maceration method, indicated the presence of alkaloids, flavonoids, tannins, saponins and terpenoids, while steroid compounds were not detected in the extract.

Assessment of antibacterial potential: The antibacterial activity testing of *Balikpapan ginger* stem extract was conducted using three concentration variations: 0.5, 1 and 2% against *Staphylococcus aureus*. The results of the antibacterial activity test of *Balikpapan ginger* stem extract can be seen in Fig. 1a-b.

Based on the data obtained from the antibacterial activity test against *Staphylococcus aureus*, it is evident that increasing the concentration of the tested compound results in a larger inhibition zone diameter. At a 0.5% concentration, the inhibition zones were 13.5 mm for *S. aureus*. As the concentration increased to 1 and 2%, the inhibition zones expanded to 16.66 and 18.83 mm for *S. aureus* (Table 3). This indicates that the compound has potential antibacterial activity against both bacterial strains, with effectiveness improving as the concentration increases.

As a positive control, Chloramphenicol exhibited inhibition zones of 19.66 mm for *S. aureus*. This suggests that Chloramphenicol has a strong antibacterial effect against *S. aureus*. The negative control, which did not contain active compounds, showed no inhibition zones, confirming the validity of the results.

Previous studies have shown that chloramphenicol has significant antimicrobial activity against microorganisms. A study published in 2020 found that chloramphenicol exhibited antimicrobial activity against *S. aureus*[21].

These results are in line with a study, which reported that extracts from the *Etlingera* genus exhibited antibacterial activity against various pathogenic bacteria. Additionally, flavonoids present in ginger extract are known to have significant antibacterial properties. According to a study published by MDPI, flavonoids can inhibit the growth of various pathogenic microorganisms, including antibiotic-resistant bacteria[22].

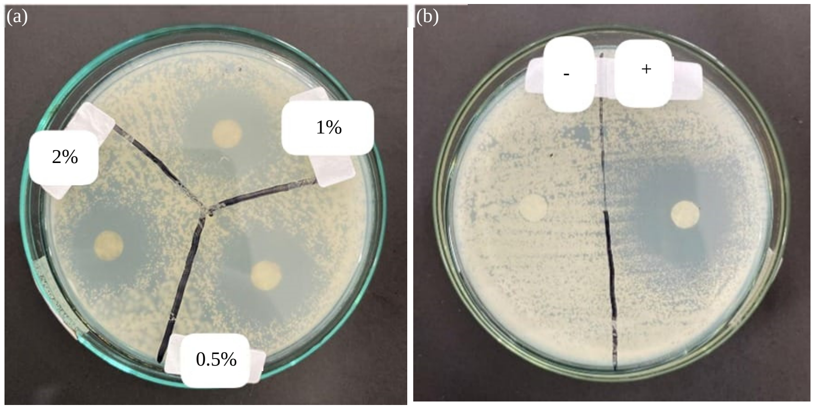

Fig. 2(a-b): Antifungal activity of *Etlingera balikpapanensis* stem extract against *Candida albicans*, (a) Antifungal activity of the extract at concentrations of 0.5, 1 and 2% and (b) Antifungal activity with positive control (Fluconazole) and negative control (1% ethanol)

Table 3: Antibacterial Inhibition zone diameter of *Balikpapan ginger* stem extract

Treatment	Average inhibition zone diameter (mm)
0.5%	13.50
1%	16.66
2%	18.83
P	19.66
N	0.00

P: Positive control (*Chloramphenicol*) and N: Negative control (*S. aureus*)

Table 4: Antifungal inhibition zone diameter of *Balikpapan ginger* stem extract

Treatment	Average inhibition zone diameter (mm)
0.5%	8.6
1%	11.0
2%	12.5
P	18.8
N	0.0

P: Positive control (Fluconazole) and N: Negative control (*C. albicans*)

The antibacterial activity of flavonoids involves interactions with bacterial cell membranes, disrupting their integrity and inhibiting bacterial metabolic processes. Furthermore, flavonoids can inhibit biofilm formation and multidrug-resistance pumps in bacteria, contributing to their effectiveness as antibacterial agents[23].

Overall, the extract of *Balikpapan ginger* stem demonstrates potential as an effective natural antibacterial agent against *Staphylococcus aureus*, with activity increasing as the extract concentration rises. Further research is needed to identify the active components in the extract and to better understand its mechanisms of action.

Antifungal activity: The antifungal activity testing of Balikpapan ginger stem extract was conducted using three concentration variations: 0.5, 1 and 2% against *Candida albicans*. The results of the antibacterial activity test of *Balikpapan ginger* stem extract can be seen in Fig. 2a-b.

Based on the data obtained from the antifungal activity test against *Candida albicans*, it is evident that as the concentration of the tested compound increases, the inhibition zone diameter also increases. At a 0.5% concentration, the inhibition zone was 8.6 mm, indicating antifungal activity, though still relatively small. As the concentration increased to 1 and 2%, the inhibition zones grew to 11 and 12.5 mm, respectively (Table 4). This suggests that the compound has potential to inhibit the growth of *Candida albicans* with increasing concentration.

As a positive control, Fluconazole showed more significant results with an inhibition zone diameter of 18.8 mm. This indicates that Fluconazole is more effective in inhibiting the growth of *Candida albicans* compared to the tested compounds at the given concentrations. The negative control, which did not contain active compounds, showed no inhibition zone, confirming that the results are valid as there was no influence from external factors.

Although the compound tested showed potential in inhibiting the growth of *Candida albicans*, its effectiveness is still lower than that of Fluconazole. This indicates that the compound has moderate antifungal activity and increasing the concentration may enhance its effectiveness. However, to achieve effectiveness comparable to Fluconazole, further research is needed to determine the optimal concentration and the underlying mechanism of action of the compound[24].

CONCLUSION

This study shows that *Balikpapan ginger* stem extract (*Etlingera balikpapanensis*) has antibacterial effects against *Streptococcus mutans* and *Staphylococcus aureus* and antifungal effects against *Candida albicans*. The antimicrobial activity increases with extract concentration, with the best results at 2%. These effects are attributed to bioactive compounds like flavonoids, tannins, alkaloids, saponins and terpenoids, which disrupt microbial cell membranes and inhibit key enzymes. The findings support the potential of *Balikpapan ginger* stem extract for oral health, but further research is needed to assess its long-term effectiveness and clinical applications..

SIGNIFICANCE STATEMENT

This study discovered the antimicrobial potential of *Balikpapan ginger* (*Etlingera balikpapanensis*) stem extract, which can be beneficial for developing alternative therapeutic agents against bacterial and fungal infections. The findings highlight that the extract exhibits dose-dependent inhibitory effects against *Staphylococcus aureus* and *Candida albicans*, suggesting its role as a natural source of bioactive compounds such as flavonoids and tannins. This research contributes to the search for plant-based antimicrobials, addressing the urgent challenge of antimicrobial resistance. Importantly, this study will help researchers to uncover the critical areas of phytochemical exploration and pharmacological validation that many researchers were not able to explore. Thus a new theory on the integration of underutilized Zingiberaceae species into antimicrobial drug discovery may be arrived at.

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