



Research Article

Evaluation of the antimicrobial activity of ethanol extract from galangal rhizome (*Kaempferia galanga* L.) in the Purbalingga accession

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ABSTRACT

Galangal (*Kaempferia galanga* L.) is a medicinal plant known for its antimicrobial properties. Its rhizome contains bioactive compounds such as alkaloids, flavonoids, tannins, phenols, quinones, steroids, and triterpenoids, which have been reported to inhibit the growth of *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans*. This study aimed to evaluate the antimicrobial activity of the ethanol extract of galangal rhizome from the Purbalingga accession. The antimicrobial activity was assessed using the disc diffusion method to determine the inhibition zone diameter (IZD), while the minimum inhibitory concentration (MIC) and minimum killing concentration (MKC) were determined using solid agar dilution. Extract concentrations of 10%, 30%, 50%, 70%, and 90% were tested for IZD. Clindamycin was used as a positive control for *S. epidermidis* and *P. acnes*, while nystatin was used for *C. albicans*; 10% DMSO served as the negative control. MIC values were evaluated at concentrations of 1.25%, 2.5%, 5%, and 10%. The ethanol extract inhibited the growth of *S. epidermidis* at a concentration of 10%, with an IZD of 8.38 mm. The inhibition zones for *P. acnes* and *C. albicans* were 8.42 mm and 9.24 mm, respectively. The MIC was 10% for *S. epidermidis* and 2.5% for both *P. acnes* and *C. albicans*. The MKC for *P. acnes* and *C. albicans* was determined to be 5%. The ethanol extract of *K. galanga* rhizome from the Purbalingga accession exhibits antimicrobial potential against *S. epidermidis*, *P. acnes*, and *C. albicans*, suggesting its potential use in antimicrobial applications.

Keywords: antimicrobial activity, ethanolic extract, *Kaempferia galanga* L., Purbalingga accession

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INTRODUCTION

Infectious diseases are prevalent in Indonesia, where the tropical climate, high humidity, heat, and poor hygiene contribute to an increasing incidence of infections, particularly those affecting the skin. These infections are caused by pathogenic or opportunistic microorganisms, including bacteria and fungi such as *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans* (Fitriani et al. 2023). *S. epidermidis* is an opportunistic bacterium that can cause acne, skin infections, and body or oral odor, particularly in individuals with weakened immune systems (Rafika et al. 2020). Meanwhile, *P. acnes* contributes to acne formation by triggering inflammation in the sebaceous glands, commonly affecting the face, neck, chest, and back (Asrianti et al. 2024). Additionally, *C. albicans* is responsible for candidiasis, which affects the skin, mouth, and genitals. It becomes pathogenic when the host's immune system is compromised (Rahayu et al. 2019).

Infectious diseases are commonly treated with antimicrobial agents. However, inappropriate antimicrobial use such as unnecessary prescriptions, unregulated public use, incorrect dosages, and improper treatment durations can contribute to the growing problem of antimicrobial resistance (Sweileh, 2021). Antimicrobial resistance occurs when bacteria or fungi are no longer inhibited or killed by standard doses of antibiotics or antifungals, including at their minimal inhibitory concentrations (Humaida, 2014). To address this issue, alternative therapies using medicinal plants are being explored as potential sources of new antimicrobial agents. Medicinal plants offer several advantages, including affordability, accessibility, and relatively lower side effects (Chaugule & Barve, 2023). Herbal medicines have played a significant role in health promotion, disease prevention, and treatment, and many have been developed into modern pharmaceuticals. As a result, the global use of medicinal plants for therapeutic purposes continues to rise (Romadhona & Permatasari, 2024).

Galangal (*Kaempferia galanga* L.), a member of the Zingiberaceae family, is a widely cultivated medicinal plant, primarily valued for its rhizome. Traditionally, its rhizome has been used to treat skin diseases, wounds, and rheumatism (Khairullah et al. 2021). Studies have shown that galangal rhizomes contain secondary metabolites such as flavonoids, tannins, saponins, and essential oils, which exhibit antimicrobial properties (Annisa et al. 2016). Galangal rhizomes also contain the active compound ethyl-paramethoxy cinnamate (EPMC) at concentrations of 2.5–4.0% (Preetha et al. 2016), which has been reported to inhibit the growth of *Mycobacterium tuberculosis* and *Candida albicans* (Omar et al. 2014). Additionally, Elya et al. (2016) found that galangal rhizome extract inhibited *Staphylococcus aureus* and *Staphylococcus epidermidis* at concentrations of 1.2–2.4%, while *Propionibacterium acnes* was inhibited at concentrations as low as 0.6%. Furthermore, Primawati & Jannah (2019) reported that methanol extracts of galangal rhizomes effectively inhibited *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus*.

Research on the antimicrobial potential of Purbalingga accession galangal rhizomes remains limited. The Purbalingga accession contains 1.4–2.8% essential oil and 27.9–74.8% ethyl-paramethoxy cinnamate (EPMC) (Subaryanti et al. 2021), highlighting its potential for further antimicrobial research, particularly against *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans*. This study aims to provide scientific data on the antimicrobial potential of ethanol extracts from Purbalingga accession galangal rhizomes.

METHODS

The test material was galangal rhizome (*Kaempferia galanga* L.), obtained from galangal farmers in Bedagas Village, Pengadegan District, Purbalingga Regency, Central Java, Indonesia, at an altitude of 100 meters above sea level (m asl). The rhizomes were dark brown, pithy, fresh, free from rot, and had a distinctive aromatic odor. They were collected from 12-month-old plants. The tested microorganisms included *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans*, obtained from the Microbiology Laboratory collection of the Pharmacy Study Program, Faculty of Pharmacy, National Institute of Science and Technology, Jakarta.

Ethanol Extract of Galangal Rhizomes

The extract was prepared using the maceration method, which involves soaking galangal rhizome powder in 96% ethanol. a total of 500 g of rhizome powder was placed in a maceration vessel and mixed with 5000 mL of 96% ethanol (1:10 ratio). The mixture was covered with aluminum foil and left to macerate for 24 hours with occasional stirring. The filtering process was repeated at least twice or until the solvent became clear. The filtrate was then evaporated using a vacuum rotary evaporator at $\pm 50^{\circ}\text{C}$ until a concentrated extract was obtained. The extract was stored in a tightly sealed container and protected from light.

Phytochemical Screening

Alkaloids Identification. Alkaloid identification was performed on the ethanol extract (0.5 g), which was placed in a test tube. Next, 1 mL of 2 N HCl and 9 mL of distilled water were added. The mixture was heated in a water bath for 2 minutes, cooled, and then filtered using filter paper. The resulting filtrate was divided into three portions for further testing. Mayer's Test: In the first tube, two drops of Mayer's reagent were added. A positive result was indicated by the formation of a white to yellowish precipitate. Bouchardat's Test: In the second tube, Bouchardat's reagent was added. A brown precipitate indicated a positive result. Dragendorff's Test: In the third tube, Dragendorff's reagent was added. The formation of a brick-red precipitate confirmed the presence of alkaloids (Kalaiselvi et al. 2016).

Flavonoids Identification. Flavonoid identification was performed on 1 g of extract, which was placed in a test tube. Next, 10 mL of distilled water was added, and the mixture was heated for 5 minutes. After cooling, it was filtered using filter paper. A 5 mL portion of the filtrate was taken, and 0.1 g of magnesium (Mg), 2 mL of amyl alcohol, and 1 mL of concentrated HCl were added. The mixture was shaken and allowed to separate. The presence of flavonoids was confirmed by the appearance of a red, orange, or yellow color in the amyl alcohol layer (Kalaiselvi et al. 2016).

Tannins Identification. Tannin identification was performed on 0.5 g of the extract, which was placed in a test tube. Next, 10 mL of distilled water was added, and the mixture was heated in a water bath. After cooling, it was

filtered using filter paper. The filtrate was then treated with 1–3 drops of 1% FeCl₃ solution. The presence of tannins was confirmed by the appearance of a bluish-black or green color (Kalaiselvi et al. 2016).

Saponins Identification. Saponin identification was performed on 0.5 g of the extract, which was placed in a test tube. Next, 10 mL of hot distilled water was added. After cooling, the mixture was shaken vigorously for 10 seconds. The presence of saponins was confirmed if a stable foam persisted even after the addition of 1 drop of 2 N HCl (Kalaiselvi et al. 2016).

Phenolics Identification. Phenol identification was performed on 0.5 g of the extract, which was placed in a test tube. Next, 10 mL of distilled water was added, and the mixture was heated in a water bath. After cooling, it was filtered using filter paper. The filtrate was then treated with 1–3 drops of 5% FeCl₃ solution. The presence of phenols was confirmed by the appearance of a green or blue-black color (Kalaiselvi et al. 2016).

Quinones Identification. Quinone identification was performed on 0.5 g of the extract, which was placed in a test tube. Next, 10 mL of distilled water was added, and the mixture was heated in a water bath. After cooling, it was filtered using filter paper. Then, 1–3 drops of 1 N NaOH were added to the filtrate. The presence of quinones was confirmed by the appearance of a red color (Kalaiselvi et al. 2016).

Steroids and Triterpenoids Identification. Steroid and triterpenoid identification was performed on 1 g of the extract, which was placed in a test tube. Then, 20 mL of ether was added to a tightly closed container, and the mixture was filtered. The filtrate was transferred to a porcelain cup and evaporated. The dried residue was then treated with Liebermann-Burchard reagent. The presence of steroids was indicated by a blue or blue-green color, while the presence of triterpenoids was indicated by a red, pink, or purple color (Kalaiselvi et al. 2016).

Antimicrobial Activity Assay

Microbial Culture Media. Nutrient Agar (NA) was used as the growth medium for *Staphylococcus epidermidis* and *Propionibacterium acnes*, while Potato Dextrose Agar (PDA) was used for *Candida albicans*. The respective powdered media were dissolved in distilled water, heated until completely dissolved, and sterilized in an autoclave at 121°C for 15 minutes.

Microbial Stocks and Preparation of Inoculum. Microbial rejuvenation was performed by streaking *Staphylococcus epidermidis* and *Propionibacterium acnes* onto Nutrient Agar (NA) and *Candida albicans* onto Potato Dextrose Agar (PDA). The plates were incubated at 37°C for 24 hours to reactivate microbial metabolism and obtain pure cultures. Microbial suspensions were prepared by transferring a 24-hour-old colony into 5 mL of 0.9% physiological saline (NaCl) and vortexed to homogenize. The turbidity was adjusted to match the 0.3 McFarland standard ($\sim 9.0 \times 10^8$ CFU/mL). Dilution Process: *S. epidermidis* and *P. acnes* were diluted to 10^7 CFU/mL by transferring 1 mL of a 10^8 CFU/mL suspension into 9 mL of 0.9% NaCl. *C. albicans* was diluted to 10^6 CFU/mL through a two-step process: 1 mL of a 10^8 CFU/mL suspension was transferred into 9 mL of 0.9% NaCl. 1 mL of this 10^7 CFU/mL suspension was transferred into another tube containing 9 mL of 0.9% NaCl. (Gayathiri et al. 2018).

Determination of Inhibition Zone Diameter (IZD), Minimum Inhibitory Concentration (MIC), and Minimum Killing Concentration (MKC). The antimicrobial activity of galangal rhizome extract was evaluated using the disc diffusion method. Sterile 6 mm paper discs were used for testing. Approximately 15 mL of sterile Nutrient Agar (NA) medium was poured into petri dishes and allowed to solidify. Then, 0.1 mL of bacterial suspension was evenly spread over the surface using a sterile spreader rod and allowed to dry. Paper discs containing 20 μ L of extract at different concentrations were placed onto the medium. Negative control: A disc soaked in 20 μ L of 10% DMSO. Positive control: Clindamycin disc for bacterial strains and nystatin disc for fungal strains. Incubation Conditions: Bacteria: 37°C for 24 hours. Fungi: 37°C for 48 hours. After incubation, antimicrobial activity was assessed by measuring the inhibition zone diameter (IZD) (clear zone around the disc). The experiment was performed in triplicate (George & Hima, 2019).

Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MKC) Determination. The minimum inhibitory concentration (MIC) was determined using the solid agar dilution method. The tested extract concentrations, based on the lowest inhibition zone diameter (IZD) results, were 1.25%, 2.5%, 5%, and 10%. For MIC testing, 0.1 mL of each extract concentration was mixed with 0.1 mL of bacterial suspension in a petri dish. Then, 15 mL of molten Nutrient Agar (NA) medium (45–50°C) was added and mixed until homogeneous. The plates were incubated at 37°C for 24 hours for bacteria and 37°C for 48 hours for fungi. The minimum killing concentration (MKC) was defined as the lowest extract concentration that completely inhibited microbial growth, with no visible colonies observed (Koeth & Miller, 2023).

RESULTS AND DISCUSSION

Ethanol Extract of Galangal Rhizome

Maceration of galangal rhizomes yielded 6.23% extract. A yield below 10% suggests a low concentration of secondary metabolites (Senduk et al., 2020). Yield is influenced by various factors, including the drying process, which reduces water content, and sieving, which can lead to particle retention in the filter (Nurdyansyah et al., 2019).

The extract was then concentrated using a vacuum rotary evaporator to remove the solvent, producing 31.16 g of a thick, brownish-yellow extract. This extract was subsequently used for antimicrobial testing and phytochemical screening.

Phytochemical Screening

Phytochemical screening identifies the chemical compounds present in plants (Agustina & Handayani, 2017). Phytochemicals protect plants from herbivores and environmental stressors such as drought, extreme temperatures, heavy metals, and salinity (Angin et al., 2019). Additionally, certain phytochemicals serve as antioxidants and medicinal raw materials for humans. The phytochemical screening results for galangal rhizome powder and extract are presented in Table 1.

Table 1. Phytochemical screening results of galangal rhizome powder and ethanol extract

No.	Compounds	Reagents	Observation	Results	
				Powder	Extract
1.	Alkaloids	Mayer	White Presipitate	+	+
		Dragendorff	Orange Presipitate	+	+
		Bourchardat	Brown Presipitate	+	+
2.	Flavonoids	NaOH	Orange Layer	+	+
3.	Tannins	FeCl ₃ 5%	Balckish Green	+	+
4.	Phenols	FeCl ₃ 5%	Black/Blackish Blue	+	+
5.	Saponins	Aquadest	Stable Foam	+	+
6.	Kuinons	NaOH 1 N	Yellowish Red	+	+
7.	Steroids	Lieberman-Burchard	Blue/Blue Green	—	+
8.	Triterpenoids	Lieberman-Burchard	Red/Red Pink	—	+

Notes: (+) Indicates the presence of the compound; (–) Indicates the absence of the compound

The phytochemical screening results in Table 1 indicate that galangal rhizome contains alkaloids, flavonoids, tannins, phenols, saponins, quinones, steroids, and triterpenoids. However, in powdered form, steroids and triterpenoids were absent. This finding aligns with Rajendra et al. (2011), who reported that methanol extract of galangal rhizomes contains steroids, triterpenoids, alkaloids, flavonoids, carbohydrates, resins, and proteins. Similarly, Dash et al. (2014) found that methanol extract contains carbohydrates, tannins, flavonoids, proteins, steroids, alkaloids, and resins. Additionally, Wang et al. (2021) reported the presence of terpenoids, phenols, flavonoids, fatty acids, and esters in galangal rhizomes.

Antimicrobial Activity Assay

The inhibition zone diameter (IZD) test results for the ethanol extract of galangal rhizome against *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans* are presented in Table 2.

Table 2. Inhibition Zone Diameter (IZD) of ethanol extract of galangal rhizome against *S. epidermidis*, *P. acnes*, and *C. albicans*

Extract Concentration (%)	IZD Value (mm)		
	<i>S. epidermidis</i>	<i>P. acnes</i>	<i>C. albicans</i>
10	8,38 ± 0,44	8,42 ± 0,62	9,24 ± 0,47
30	10,44 ± 0,87	11,12 ± 0,52	10,76 ± 0,40
50	9,42 ± 0,61	11,43 ± 0,86	11,38 ± 0,92
70	8,98 ± 0,80	10,99 ± 0,70	13,50 ± 0,93
90	0 ± 0	10,78 ± 0,94	13,76 ± 0,92
Control +	27,71 ± 0,67	35,78 ± 0,83	24,82 ± 0,87
Control —	0 ± 0	0 ± 0	0 ± 0

Note: Control (+): Clindamycin (*S. epidermidis* and *P. acnes*), Nystatin (*C. albicans*). Control (–): 10% DMSO

Antimicrobials are substances that function to inhibit the growth and eliminate microbes (bacteria or fungi) responsible for infections (Magani et al. 2020). Four key factors influence the success of antimicrobial testing: extract concentration, chemical composition, diffusion ability, and microbial type (Lestari et al. 2016). The antimicrobial test results in Table 2 indicate that the ethanol extract of galangal rhizome exhibits inhibitory activity against bacterial growth, as evidenced by the formation of a clear zone around the paper disc. *Staphylococcus epidermidis* demonstrated the following inhibition zone diameters (IZD) at different extract concentrations; 10% =

8.38 mm (medium inhibition), 30% = 10.44 mm (strong inhibition), 50% = 9.42 mm (medium inhibition), 70% = 8.98 mm (medium inhibition), 90% = 0 mm (no inhibition zone observed) (Indryani et al. 2020).

The highest inhibitory effect of the ethanol extract of galangal rhizome against *Staphylococcus epidermidis* was observed at a concentration of 30%. For *Propionibacterium acnes*, the inhibition zone diameters (IZD) at different extract concentrations were as follows; 10% = 8.42 mm (medium inhibition), 30% = 11.12 mm (strong inhibition), 50% = 11.43 mm (strong inhibition), 70% = 10.99 mm (strong inhibition), 90% = 10.78 mm (strong inhibition). The highest inhibitory effect against *P. acnes* was observed at 50% concentration. A decline in inhibition was noted for *S. epidermidis* at 50%, 70%, and 90%, as well as for *P. acnes* at 70% and 90%. This decrease in antimicrobial activity at higher concentrations may be attributed to factors such as reduced diffusion efficiency or compound instability. *S. epidermidis* is known to exhibit resistance or reduced sensitivity to certain antimicrobials, indicating the need for more potent antimicrobial agents to effectively inhibit its growth (Dewi et al. 2012).

This phenomenon occurs due to a decrease in the diffusion process at higher concentrations. As the concentration increases, the diffusion of the substance from the disc to the surface of the medium becomes more limited, reducing its ability to inhibit bacterial growth. The porosity of agar is influenced by its concentration and gel structure, which affect molecular diffusion. Typically, 1.5% agar (standard microbiological agar), pore size ranges between 50–200 nm. Higher agar concentrations ($\geq 3\%$); pore size decreases to below 50 nm, restricting molecular diffusion. Several factors influence diffusion; molecular weight; larger molecules (>1000 Da) diffuse more slowly. Hydrophilicity/hydrophobicity; hydrophilic molecules diffuse more efficiently than hydrophobic ones. Agar composition and gel strength: increased cross-linking reduces pore size, limiting diffusion. At high concentrations, antimicrobial substances may not diffuse effectively due to their size, charge, and solubility. As a result, higher concentrations do not always correlate with larger inhibition zones (Hossain, 2024).

The inhibition zone of the ethanol extract of galangal rhizomes against *S. epidermidis* and *P. acnes* is smaller than that of the positive control, clindamycin. Clindamycin exhibits an inhibition zone of 27.71 mm against *S. epidermidis* and 35.78 mm against *P. acnes*. This difference occurs because clindamycin is an antibiotic derived from lincomycin, which has bacteriostatic activity against Gram-positive aerobic and anaerobic bacteria (Gunawan & Gan, 2016). As a bacteriostatic agent, clindamycin inhibits microbial growth without directly killing the bacteria (Susanto et al. 2022).

Based on the secondary metabolites present in the powder and extract of galangal rhizomes, the antibacterial activity is suspected to be associated with these compounds. These metabolites can interfere with bacterial metabolism, leading to growth inhibition or cell death. Various secondary metabolites in galangal rhizomes exhibit antibacterial activity, as evidenced by the formation of clear inhibition zones through different mechanisms of action. Flavonoids inhibit nucleic acid synthesis by disrupting transcription and replication (Noor, 2017). Saponins act by damaging the plasma membrane, which is semipermeable and regulates the transport of metabolites in and out of the cell. Any disruption or structural damage to the plasma membrane can impair its function as a selective barrier (Julianto, 2019). Steroids damage the semipermeable plasma membrane, disrupting the transport of metabolites into and out of the cell. Tannins inhibit cell wall synthesis by damaging the peptidoglycan layer, which is essential for bacterial cell wall integrity. Triterpenoids interfere with the transport of essential ions into the cell and bind to lipids and carbohydrates, leading to disruptions in cell wall permeability (Samputri et al. 2020).

The ethanol extract of galangal rhizomes also exhibits inhibitory activity against *C. albicans*, as indicated by the formation of a clear zone around the paper disc after 48 hours of incubation at 37°C. At a concentration of 10%, the inhibition zone diameter (IZD) is 9.24 mm (medium category). Higher concentrations result in stronger inhibition, with IZDs of: 30%: 10.76 mm (strong category), 50%: 11.38 mm (strong category), 70%: 13.50 mm (strong category), 90%: 13.76 mm (strong category). The highest concentration of galangal rhizome ethanol extract (90%) exhibits the strongest inhibitory effect against *C. albicans*. The variation in inhibition zone size at different concentrations is influenced by several factors, including inoculum size. Microorganisms are introduced into a medium while still viable and in the exponential growth phase. A larger inoculum tends to result in a smaller inhibition zone (Khusuma et al. 2019). Incubation time also plays a crucial role, as it ensures that fungi are maintained at optimal temperature and duration to monitor their growth and development.

The extract concentration also influences the inhibition zone size. Higher concentrations facilitate faster diffusion, resulting in greater antifungal activity and a wider inhibition zone (Pasaribu et al. 2018). The inhibition zone of the ethanol extract of galangal rhizomes against *C. albicans* was smaller than that of the positive control, nystatin. This is because nystatin has potent antimycotic activity against yeast-like fungi such as *C. albicans* (Sousa et al. 2023). The antifungal activity of galangal rhizome extract is likely attributed to its secondary metabolites, which disrupt fungal cell metabolism, leading to growth inhibition or cell death (Wang et al. 2021).

Various secondary metabolites in galangal rhizomes exhibit antifungal activity, as indicated by the formation of clear inhibition zones. These compounds act through different mechanisms of action, including: Flavonoids: inhibit nucleic acid synthesis by disrupting transcription and replication (Noor, 2017). Tannins: inhibit cell wall synthesis (Samputri et al., 2020). Triterpenoids: disrupt ion transport into cells and bind to lipids and carbohydrates, leading

to altered cell wall permeability (Mioc et al. 2022). Saponins exhibit high toxicity against fungi. Their antifungal mechanism is associated with interactions between saponins and membrane ergosterol. These compounds act by reducing surface tension in sterol membranes and fungal cell walls, increasing membrane permeability. As a result, intracellular fluid is drawn out of the cell, leading to the loss of essential nutrients, metabolic substances, enzymes, and proteins. This disruption inhibits fungal growth or leads to cell death (Julianto, 2019). Steroids, on the other hand, inhibit fungal growth by targeting the cytoplasmic membrane or interfering with the development and proliferation of fungal spores (Samputri et al. 2020).

The antimicrobial activity test of the ethanol extract of galangal rhizome from the Purbalingga accession demonstrated its ability to inhibit the growth of *S. epidermidis*, *P. acnes*, and *C. albicans*. This activity is likely influenced by the presence of phytochemical compounds, including alkaloids, flavonoids, tannins, phenols, saponins, quinones, steroids, and triterpenoids. Therefore, the ethanol extract of galangal rhizome from the Purbalingga accession has the potential to be developed as a natural alternative treatment for skin diseases such as acne, skin infections, and candidiasis. After the inhibition zone diameter (IZD) test confirmed the inhibitory capacity of the ethanol extract of galangal rhizome, the next step was to determine the minimum inhibitory concentration (MIC) to identify the lowest extract concentration that still inhibits microbial growth. MIC testing was conducted by progressively reducing the extract concentration obtained from the IZD test until reaching the lowest concentration that still showed an inhibition zone. In this study, the lowest effective concentration observed was 10%, so the MIC test was performed using concentrations of 10%, 5%, 2.5%, and 1.25%. Concentration distribution was carried out by dilution using 10% DMSO solvent. Dilution aims to get a lower concentration so that it can be found which concentration is the best treatment (Table 3).

Table 3. Minimum Inhibitory Concentration test results of galangal rhizome extract

Extract Concentration (%)	Microbial Growth		
	<i>Staphylococcus epidermidis</i>	<i>Propionibacterium acnes</i>	<i>Candida albicans</i>
1,25	+	+	+
2,5	+	+	+
5	+	—	—
10	+	—	—
Control +	+	+	+
Control —	—	—	—

Note: (+) = Bacterial growth occurs; (—) = No bacterial growth occurs; Control + = Clindamycin for bacteria and Nystatin for fungi; Control — = Nutrient Agar (NA) medium for *S. epidermidis* and *P. acnes* and Potato Dextrose Agar (PDA) for *C. albicans*

Table 3 indicates that the most effective treatment for *S. epidermidis* occurs at a concentration of 1.25%, as bacterial growth is still observed at concentrations of 10%, 5%, 2.5%, and 1.25%. For *P. acnes*, the minimum inhibitory concentration (MIC) is 2.5%, as bacterial growth is present at this concentration but absent at 5%. Similarly, for the fungus *C. albicans*, the MIC is 2.5%, where growth is still observed, whereas at 5%, no fungal growth is detected. These MIC results are attributed to the secondary metabolites present in galangal rhizome extract, which exhibit antimicrobial properties. These bioactive compounds interfere with microbial metabolism, ultimately inhibiting their growth. After conducting the MIC test and confirming that the ethanol extract of galangal rhizome can inhibit the growth of *P. acnes* and *C. albicans*, the next step is to determine the Minimum Kill Concentration (MKC). MKC refers to the lowest concentration capable of completely eliminating microbial growth, as indicated by the absence of visible colonies in the growth medium. This confirms that the test microbes have been effectively killed by the ethanol extract of galangal rhizome. The purpose of the MKC test is to identify the minimum concentration required to achieve microbial eradication. The results of the MKC test for *P. acnes* and *C. albicans* are presented in Table 4.

Table 4. Minimum Kill Concentration test results of galangal rhizome extract

Extract Concentration (%)	Microbial Growth	
	<i>Propionibacterium acnes</i>	<i>Candida albicans</i>
2,5%	+	+
5%	—	—
Control +	+	+
Control —	—	—

Note: (+) = Bacterial growth observed, (—) = No bacterial growth observed, Control (+) = Positive control, (Clindamycin for bacteria, Nystatin for fungi), Control (–) = Negative control (Nutrient Agar (NA) for *Propionibacterium acnes*, Potato Dextrose Agar (PDA) for *Candida albicans*)

Based on Table 4, no microbial growth was observed at a concentration of 5%. This is attributed to the secondary metabolites in the galangal rhizome extract, which exhibit antimicrobial properties by disrupting microbial metabolism, ultimately leading to cell death. Additionally, microbial sensitivity to the ethanol extract of galangal rhizome varies, as some microbes develop resistance as a natural survival mechanism. The MKC is influenced not only by microbial type but also by the concentration of the extract, which determines the effectiveness of its active compounds in eliminating test microbes (Al Fajrie & Nurhayani, 2023).

CONCLUSION

The ethanol extract of galangal rhizome has the potential to inhibit the growth of *Staphylococcus epidermidis*, *Propionibacterium acnes*, and *Candida albicans*. At a 10% concentration, the inhibition zone diameters (IZD) were 8.38 mm for *S. epidermidis*, 8.42 mm for *P. acnes*, and 9.24 mm for *C. albicans*. The minimum inhibitory concentration (MIC) was 10% for *S. epidermidis* and 2.5% for *P. acnes* and *C. albicans*. The minimum kill concentration (MKC) for *P. acnes* and *C. albicans* was 5%.

CONFLICTS OF INTEREST

The author declares that the data presented in this manuscript do not pose any conflict of interest with any parties. If a conflict is discovered at a later date, the author assumes full responsibility. This manuscript is based on original research and has neither been published nor submitted to other journals.

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