Pharmacy Reports

https://doi.org/10.51511/pr.97

RESEARCH ARTICLE

Open Access

Antibacterial activity of ethanol extract from *Kaempferia galanga* L. rhizome against pathogenic bacteria

Ni Nyoman Ayu Budhi Erlangga Santhi*, Komang Rian Widiantara, Dewa Gede Wira Putra Artawan, I Komang Gede Triyana Wahyu Nanda

Departement of Pharmacy, Faculty of Mathematics and Natural Science, Udayana University, Bali 80361

*Corresponding author: Udayana University Bukit Jimbaran Campus, Jimbaran, South Kuta, Bali 80361, Indonesia.
Email: budhierlangga09@gmail.com

Abstract: Bacterial infectious diseases have become a serious global health problem due to the increasing bacterial resistance to antibiotics. This study aimed to evaluate the antibacterial activity of ethanol extract from Kaempferia galanga L. rhizome against pathogenic bacteria. The extract was prepared using 96% ethanol through maceration, yielding 14.86% (w/w) of concentrated extract. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, terpenoids, and steroids. Antibacterial activity was assessed using the disc diffusion method against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. The extract exhibited concentration-dependent antibacterial activity against all tested bacteria, with inhibition zones ranging from 6.08-16.38 mm. The most pronounced effect was observed against S. aureus (16.38 mm) at 100% concentration, followed by B. subtilis (15.27 mm), E. coli (12.78 mm), and P. aeruginosa (10.83 mm). Gram-positive bacteria demonstrated higher susceptibility than Gram-negative bacteria. According to Davis & Stout classification, the antibacterial activity of 100% extract against all test bacteria ranged from moderate to strong. These findings suggest that K. galanga rhizome extract has significant antibacterial potential that supports its traditional use in treating infectious diseases and warrants further investigation for development as a natural antibacterial agent.

Keywords: antibacterial, disc diffusion, *Escherichia coli*, *Kaempferia galanga*, *Staphylococcus aureus*

Introduction

Infectious diseases remain one of the main health problems in developing countries, including Indonesia. The use of antibiotics to treat bacterial infections often faces obstacles due to the emergence of resistance of pathogenic bacteria to available antibiotics. Based on a report by the World Health Organization (WHO), antimicrobial resistance is a serious threat to global health, with around 700,000 deaths per year worldwide due to antibiotic-resistant infections [1]. This condition has encouraged researchers to look for antibacterial alternatives, especially from traditional medicinal plants used for generations by the community.

Kaempferia galanga L. (family Zingiberaceae), commonly known as "kencur" in Indonesia, is a rhizomatous herbaceous plant native to tropical regions of Asia. In Indonesian traditional medicine, the rhizomes have been used to treat various diseases such as coughs, diarrhea, and skin infections [2]. Galangal rhizomes contain bioactive compounds such as essential oils, flavonoids, terpenoids, saponins, and tannins, which can potentially have antibacterial effects [3]. Phytochemical analysis shows that the main compound in *Kaempferia galanga L.* is ethyl p-methoxycinnamate (EPMS), about 25-30% of its essential oil [4]. In addition, other compounds such as camphor, borneol, cineol, and kaempferol contribute to this plant's biological activity [5].

Several studies have documented the antimicrobial potential of *K. galanga* extracts against a range of pathogens. Previous studies have shown that *K. galanga* L. extract has antimicrobial activity against several pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [6]. Despite these promising findings, comprehensive investigations into the extract's efficacy against specific pathogenic bacteria, particularly drug-resistant strains, remain limited.

Research on the potential of *K. galanga L.* as a source of natural antibacterials is critical to developing pharmaceutical products based on natural ingredients. As a country with high biodiversity, Indonesia has an excellent opportunity to develop standardized herbal

medicines or phytopharmaceuticals from local plants [7]. In addition, the development of natural antibacterials can also be a solution to deal with increasing antibiotic resistance.

Various methods can be used to test the antibacterial activity of plant extracts, such as the disc diffusion method, which is widely applied because it is relatively simple, economical, and the results are reliable [8]. This method is carried out by soaking a paper disc in a test solution, then placing it on an agar medium that has been inoculated with test bacteria. Antibacterial activity is indicated by an inhibition zone around the disc, indicating that the compounds in the extract can inhibit bacterial growth [9].

The present study aims to evaluate the antibacterial potential of the ethanol extract from *K. galanga* rhizomes against selected pathogenic bacteria: *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative) using the disc diffusion method. Additionally, we seek to conduct phytochemical screening to identify the major bioactive compound classes present in the extract and to correlate these findings with the observed antibacterial activity. This research contributes to the scientific validation of traditional medicinal applications of *K. galanga* and explores its potential as a source of natural antibacterial agents to address the growing challenge of antimicrobial resistance.

Methods

Materials and plant processing

The experimental process began with formal botanical authentication of *Kaempferia galanga L.* specimens. Fresh rhizomes were thoroughly cleaned under running water to remove soil and contaminants. To facilitate efficient drying, the rhizomes were sliced thinly and then dried in a controlled-temperature oven at 40°C for 48 hours until the moisture content was reduced to below 10%. The dried material was subsequently pulverized using a laboratory blender and standardized through a 60-mesh sieve to ensure uniform particle size. The resulting homogeneous powder was stored in airtight containers at room temperature to prevent microbial contamination and preserve phytochemical integrity.

Extraction procedure

Extraction was performed using the maceration technique with 96% ethanol as the solvent of choice due to its ability to extract both polar and moderately non-polar constituents. A sample of 500 g of the prepared *K. galanga* powder was immersed in 2.5 L of 96% ethanol (1:5 w/v ratio) in an appropriate maceration vessel. The maceration process was conducted at room temperature (25±2°C) for 72 hours (3×24 hours), with daily solvent replacement and periodic agitation to optimize extraction efficiency. After each 24-hour cycle, the macerate was separated and fresh solvent was added to the plant material. The collected filtrates were combined and filtered through Whatman No. 1 filter paper to remove particulate matter. The filtered extract was concentrated using a rotary evaporator at 40°C under reduced pressure until a semi-solid extract was obtained. The yield percentage was calculated as:

Yield (%) = (Weight of extract obtained / Weight of starting plant material) × 100 … (1)

The concentrated extract was transferred to an amber glass container and stored at 4°C until further analysis [10].

Phytochemical Analysis

Qualitative phytochemical screening was conducted to identify major secondary metabolite groups present in the *K. galanga* extract following standard protocols described by Harborne [11]. The following tests were performed:

Alkaloid detection. Three separate tests using Mayer's reagent (potassium mercuric iodide), Wagner's reagent (iodine in potassium iodide), and Dragendorff's reagent (potassium bismuth iodide) were performed, with positive results indicated by precipitate formation.

Flavonoid detection. The extract was treated with magnesium turnings and concentrated hydrochloric acid, with the development of a red coloration indicating the presence of flavonoids.

Tannin detection. Extract solution was treated with 1% ferric chloride reagent, with blackish-green or blue-black coloration indicating tannin presence.

Saponin detection. Aqueous extract solution was vigorously shaken, with persistent foam formation (>1 cm height remaining stable for 10 minutes) indicating saponin presence.

Terpenoid and steroid detection. The Liebermann-Burchard test was performed by treating the extract with acetic anhydride and concentrated sulfuric acid. A brownish-red coloration indicated terpenoids, while greenish coloration suggested steroids.

Preparation of extract solutions

The concentrated *K. galanga* extract was dissolved in 10% dimethyl sulfoxide (DMSO) to prepare a series of test concentrations: 25%, 50%, 75%, and 100% (w/v). DMSO was selected as the solubilizing agent due to its ability to dissolve both polar and non-polar compounds while exhibiting negligible antibacterial activity at the concentration employed [12]

Test microorganisms

Four bacterial strains representing both Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria were used in this study. The bacterial cultures were maintained on Nutrient Agar slants and subcultured on fresh media 24 hours prior to testing. For the preparation of inocula, bacterial colonies from 24-hour cultures were suspended in sterile 0.9% sodium chloride solution and standardized to match the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL) using a spectrophotometer at 625 nm wavelength [13].

Disc diffusion assay

The antibacterial activity was evaluated using the Kirby-Bauer disc diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. The standardized bacterial suspensions were uniformly spread on Mueller-Hinton Agar plates using sterile cotton swabs. After allowing 5-15 minutes for the inoculum to be absorbed into the medium, sterile paper discs (6 mm diameter, Whatman No. 1) were impregnated with 20 µL of various extract concentrations (25%, 50%, 75%, and 100%) for 15 minutes to ensure complete absorption.

The impregnated discs were placed on the inoculated agar surface using sterile forceps, maintaining adequate spacing between discs to prevent overlapping of inhibition zones. Commercial chloramphenicol discs (30 µg) served as positive controls, while discs impregnated with 10% DMSO solution served as negative controls to validate the experimental setup.

The plates were incubated at 37°C for 24 hours under aerobic conditions. Following incubation, the diameters of the zones of inhibition (including the 6 mm disc diameter) were measured using a digital caliper. Three measurements were taken from different angles for each zone, and the mean value was calculated [8]. All tests were performed in triplicate to ensure reproducibility.

The antibacterial activity was classified according to the criteria established by Davis and Stout (1971) based on the diameter of inhibition zones: very strong (>20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm) [14].

Statistical analysis

The experimental data were expressed as mean ± standard deviation of three independent replicates. Statistical analysis was conducted using IBM SPSS Statistics software version 25.0. The significance of differences between extract concentrations and bacterial strains was determined by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) for post-hoc comparison. Differences were considered statistically significant at $p<0.05$.

Results

Extract characteristics and yield

The extraction of 500 grams of *Kaempferia galanga L.* rhizome powder using maceration with 96% ethanol yielded a thick extract with distinctive characteristics. The extract displayed a greenish-brown color with a characteristic aromatic odor typical of *K. galanga*. The extraction process produced 74.3 grams of concentrated extract, representing a 14.86% (w/w) yield. This yield percentage aligns with previous finding, that reported ethanol extract yields from *K. galanga* ranging between 12-15% [15].

Phytochemical screening

Qualitative phytochemical analysis of the ethanol extract revealed the presence of several bioactive compound classes as presented in Table 1. The extract tested positive for flavonoids (producing a red coloration with magnesium + concentrated HCl), tannins (forming blackish-green coloration with 1% $FeCl_3$), saponins (generating stable foam after shaking with aquadest), terpenoids (showing brownish-red coloration with Liebermann-Burchard reagent), and steroids (producing greenish coloration with Liebermann-Burchard reagent). However, the extract tested negative for alkaloids with all three detection reagents (Mayer, Wagner, and Dragendorff), indicating the absence or undetectable levels of alkaloid compounds.

Table 1. Phytochemical profile of *Kaempferia galanga L.* ethanol extract

Compound content	Reagent	Observation	Result
Alkaloids	Mayer reagent	No white precipitate is formed	Negative
	Wagner reagent	No brown precipitate formed	Negative
	Dragendorff reagent	No orange precipitate formed	Negative
Flavonoids	Magnesium + concentrated HCl	Red coloration	Positive
Tannin	FeCl 1%	Blackish-green coloration	Positive
Saponins	Aquadest + shaking	Stable foam formation	Positive
Terpenoids	Liebermann-Burchard	Brownish-red coloration	Positive
Steroid	Liebermann-Burchard	Greenish coloration	Positive

Table 2. Inhibition zone diameters of *K. galanga* ethanol extract against test bacteria

Extract concentration	Inhibition zone diameter (mm) ± SD			
	Staphylococcus aureus	***Bacillus subtilis***	***Escherichia coli***	***Pseudomonas aeruginosa***
K. galanga extract 25%	7.21 ± 0.25[a]	6.87 ± 0.31[a]	6.32 ± 0.29[a]	6.08 ± 0.22[a]
K. galanga extract 50%	10.46 ± 0.38[b]	9.73 ± 0.42[b]	8.45 ± 0.36[b]	7.62 ± 0.33[b]
K. galanga extract 75%	13.82 ± 0.47[c]	12.54 ± 0.38[c]	10.23 ± 0.41[c]	9.15 ± 0.37[c]
K. galanga extract 100%	16.38 ± 0.52[d]	15.27 ± 0.45[d]	12.78 ± 0.44[d]	10.83 ± 0.40[d]
Control (+)	23.56 ± 0.61[e]	22.34 ± 0.58[e]	21.45 ± 0.53[e]	18.62 ± 0.49[e]
Control (-)	0.00 ± 0.00	0.00 ± 0.00[f]	0.00 ± 0.00[f]	0.00 ± 0.00[f]

Note: Values represent mean ± standard deviation (n=3). Different superscript letters within the same column indicate significant differences ($p<0.05$) between treatments as determined by Duncan's Multiple Range Test.

Antibacterial activity

The antibacterial potential of *K. galanga* ethanol extract was evaluated against four bacterial species using the disc diffusion method. Results of inhibition zone measurements are summarized in Table 2. The extract demonstrated concentration-dependent antibacterial activity against all tested bacterial strains.

At the highest tested concentration (100%), the extract demonstrated the strongest inhibitory effect against *S. aureus* with an inhibition zone of 16.38 ± 0.52 mm, followed by *B. subtilis* (15.27 ± 0.45 mm), *E. coli* (12.78 ± 0.44 mm), and *P. aeruginosa* (10.83 ± 0.40 mm). This pattern of activity indicates greater susceptibility of Gram-positive bacteria (*S. aureus* and *B. subtilis*) compared to Gram-negative bacteria (*E. coli* and *P. aeruginosa*).

Statistical analysis revealed significant differences ($p<0.05$) in inhibition zone diameters across all tested concentrations for each bacterial strain. The standard antibiotic chloramphenicol (30 µg) exhibited significantly larger inhibition zones than the extract at all tested concentrations, while the negative control (10% DMSO) showed no inhibitory activity against any tested bacteria, confirming the validity of the experimental setup. According to the Davis and Stout classification system, the antibacterial activity of the 100% extract concentration can be categorized as strong (10-20 mm inhibition zone) against all test bacteria, with the most pronounced effect observed against *S. aureus*.

Discussion

Based on the test results, the ethanol extract of *Kaempferia galanga* L. showed antibacterial activity against all test bacteria, with inhibition zone diameters varying depending on the extract concentration and type of bacteria. The diameter of the inhibition zone increased with increasing extract concentration, indicating a concentration-activity relationship. Statistical analysis showed a significant difference ($p<0.05$) between extract concentrations on the diameter of the inhibition zone for all test bacteria.

The yield of ethanol extract of *Kaempferia galanga* L. of 14.86% indicates that 96% ethanol solvent is effective in extracting compounds contained in *K. galanga* L. This yield falls within the expected range

(12-15%) reported in previous studies, confirming the efficiency of our extraction method [15]. Ethanol is a universal solvent that can dissolve polar compounds and some nonpolar compounds, allowing for comprehensive extraction of secondary metabolites from plant materials [10]. The moderate polarity of ethanol makes it particularly suitable for extracting bioactive compounds from medicinal plants, especially those with antimicrobial properties.

The phytochemical screening revealed that the ethanol extract of *K. galanga* L. positively contained flavonoids, tannins, saponins, terpenoids, and steroids. These compounds have been extensively studied for their antibacterial properties and operate through various mechanisms. Flavonoids can inhibit nucleic acid synthesis, disrupt cytoplasmic membrane function, and inhibit bacterial energy metabolism [16]. The antibacterial activity of flavonoids is attributed to their ability to form complexes with extracellular and soluble proteins as well as bacterial cell walls. Tannins exert their antimicrobial effect primarily by binding to proline-rich proteins, which interferes with protein synthesis and disrupts cell wall formation [17]. Additionally, tannins can directly damage bacterial membranes, causing leakage of intracellular components.

Saponins, which demonstrated their presence through stable foam formation in our tests, increase the permeability of bacterial cell membranes, thereby disrupting the membrane structure and leading to cell lysis [18]. The amphipathic nature of saponins, with both hydrophilic and lipophilic components, enables them to interact with and disrupt bacterial cell membranes effectively. Terpenoids, particularly essential oils, can damage bacterial cell membranes through their lipophilic properties, disrupt ion transport processes, and interfere with membrane-embedded proteins, leading to increased permeability and eventual cell death [19].

The absence of alkaloids detected in the ethanol extract of *K. galanga* L. is consistent with previous research, which reported that *K. galanga* L. contains little or no alkaloids [2]. Other compound classes are the predominant constituents of this plant. The main component of *K. galanga* L. is ethyl p-methoxycinnamate (EPMC), comprising approximately 31.77% of its volatile oil content [7]. EPMC is recognized for its significant antimicrobial properties through mechanisms involving membrane disruption and cellular permeability alteration.

The results of the antibacterial activity test showed that the ethanol extract of *K. galanga* L. exhibited varying degrees of inhibition against both Gram-positive bacteria (*S. aureus* and *B. subtilis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Based on the classification proposed by Davis & Stout [14], the antibacterial activity of the extract at a concentration of 100% against *S. aureus* and *B. subtilis* falls into the strong category (inhibition zone diameter 10-20 mm), while against *E. coli* and *P. aeruginosa*, it ranges from moderate to strong.

The ethanol extract of *K. galanga* L. demonstrated higher antibacterial activity against Gram-positive bacteria than Gram-negative bacteria across all tested concentrations. This differential sensitivity can be attributed to fundamental differences in cell wall structure between these bacterial types. Gram-positive bacteria possess cell walls consisting of a thick peptidoglycan layer (20-80 nm) that is relatively porous and allows penetration of antimicrobial compounds. In contrast, Gram-negative bacteria have a much thinner peptidoglycan layer (2-3 nm) but are protected by an outer membrane containing lipopolysaccharide (LPS), phospholipids, and proteins, which functions as a permeability barrier to various antibacterial compounds [20].

Staphylococcus aureus emerged as the most sensitive bacterium to the ethanol extract of *K. galanga* L., followed by *B. subtilis*, *E. coli*, and *P. aeruginosa*. At a concentration of 100%, the extract exhibited significant activity with an inhibition zone diameter of 16.38 mm against *S. aureus*, 15.27 mm against *B. subtilis*, 12.78 mm against *E. coli*, and 10.83 mm against *P. aeruginosa*. This pattern of sensitivity aligns with finding [4], that reported highest inhibition zones for ethanolic extracts against *S. aureus* (21.3 mm).

Pseudomonas aeruginosa demonstrated the highest resistance among the tested bacteria, with smaller inhibition zones at all extract concentrations. This enhanced resistance is attributable to the complexity of its outer membrane structure, which is particularly effective at excluding antimicrobial agents. Additionally, *P. aeruginosa* possesses intrinsic resistance mechanisms, including efficient efflux pumps that expel antimicrobial compounds from the cell, and the ability to form protective biofilms [21].

The antibacterial activity of the ethanol extract of *K. galanga* L. can be attributed to its bioactive compound content, particularly ethyl p-methoxycinnamate (EPMC).

Research by Lakshmanan et al. (2011) identified EPMC as a potent antimycobacterial agent effective against both drug-susceptible and multidrug-resistant strains of *Mycobacterium tuberculosis* [22]. This compound damages bacterial cell membranes and disrupts cell permeability, leading to leakage of cellular contents and eventual cell death.

Additionally, other compounds such as kaempferide, exhibit significant anti-inflammatory properties that may complement the antibacterial effects [23]. Recent studies by Wang et al. (2023) on the chemical composition of *K. galanga* essential oil identified trans ethyl p-methoxycinnamate (32.01%), n-pentadecane (29.14%), and trans ethyl cinnamate (19.50%) as the major components [24], all of which contribute to its biological activities.

In addition, the synergistic effect of various bioactive compounds in the extract may also contribute to its antibacterial activity. Flavonoids, tannins, saponins, and terpenoids detected in the extract may work together to inhibit bacterial growth through various mechanisms, as previously explained [25]. The results of this study support the traditional use of *K. galanga* L. as a medicine to treat various bacterial infections. Further research is needed to isolate and identify specific active compounds responsible for antibacterial activity and investigate their molecular mechanisms of action.

The results of this study support the traditional use of *K. galanga* L. as a treatment for various bacterial infections. The ethanol extract exhibited promising antibacterial activity, particularly against *S. aureus*, which is clinically significant given this pathogen's role in various infections and its increasing resistance to conventional antibiotics. Although the antibacterial activity of the ethanol extract was lower than that of the positive control (chloramphenicol 30 µg), these results highlight the potential of the extract as a natural antibacterial agent.

Further research should focus on isolating and identifying specific active compounds responsible for the observed antibacterial activity and investigating their molecular mechanisms of action. Fractionation studies, followed by advanced analytical techniques such as HPLC, LC-MS, and NMR, would provide more detailed insights into the extract's chemical composition. Additionally, combination studies with conventional antibiotics could explore potential synergistic effects that might enhance therapeutic efficacy against resistant bacterial strains.

Conclusion

The ethanol extract of *K. galanga* L. demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, with *S. aureus* showing the highest sensitivity. Phytochemical analysis revealed the presence of flavonoids, tannins, saponins, and terpenoids, which collectively contribute to the extract's antibacterial properties through various mechanisms including membrane disruption and metabolic interference. The extract exhibited a concentration-dependent activity pattern, with the 100% concentration showing strong inhibition comparable to established classification standards. These findings validate the traditional medicinal use of *K. galanga* and suggest its potential as a natural antibacterial agent, particularly in addressing infections caused by common pathogenic bacteria.

Acknowledgment

None.

Declaration of interest

None.

Funding

None.

Author contributions

NNABES: conceptualization, data curation, manuscript writing and reviewing; KRW, DGWPA, IKGTWN methodology design, data curation, formal analysis.

Received: November 25, 2024
Revised: April 15, 2025
Accepted: May 3, 2025
Published: May 5, 2025

References

1. WHO. New report calls for urgent action to avert antimicrobial resistance crisis. In: World Health Organization [Internet]. 2019. Available: https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis
2. Umar I. Phytochemistry and medicinal properties of Kaempferia galanga L. (Zingiberaceae) extracts. Afr J Pharm Pharmacol. 2011;5: 1638–1647. https://doi.org/10.5897/AJPP11.388

3. Natta L, Orapin K, Krittika N, Panitip B. Essential oil from five Zingiberaceae for anti food-borne bacteria. Int Food Res J. 2008;15.
4. Tewtrakul S, Yuenyongsawad S, Kummee S, Atsawajaruwan L. Chemical components and biological activities of volatile oil of Kaempferia galanga Linn. Songklanakarin. 2005;27: 504–507.
5. Umar MI, Asmawi MZ, Sadikun A, Majid AMSA, Al-Suede FSR, Hassan LEA, et al. Ethyl-p-methoxycinnamate isolated from kaempferia galanga inhibits inflammation by suppressing interleukin-1, tumor necrosis factor-α, and angiogenesis by blocking endothelial functions. Clinics. 2014;69: 134–144. https://doi.org/10.6061/clinics/2014(02)10
6. Kochuthressia K, Britto S. In vitro antimicrobial evaluation of Kaempferia galanga L. rhizome extract. American Journal of Biotechnology and Molecular Sciences. 2012;2: 1–5. https://doi.org/10.5251/ajbms.2012.2.1.1.5
7. Elfahmi, Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J Herb Med. 2014;4: 51–73. https://doi.org/10.1016/j.hermed.2014.01.002
8. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6: 71–79. https://doi.org/10.1016/j.jpha.2015.11.005
9. Jorgensen JH, Turnidge JD. Susceptibility Test Methods: Dilution and Disk Diffusion Methods. Manual of Clinical Microbiology. Washington, DC, USA: ASM Press; 2015. pp. 1253–1273. https://doi.org/10.1128/9781555817381.ch71
10. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011;1: 98–106.
11. Harborne AJ. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed. London: Springer Science & Business Media; 1998.
12. Zgoda JR, Porter JR. A Convenient Microdilution Method for Screening Natural Products Against Bacteria and Fungi. Pharm Biol. 2001;39: 221–225. https://doi.org/10.1076/phbi.39.3.221.5934
13. Andrews JM. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy. 2001;48: 5–16. https://doi.org/10.1093/jac/48.suppl_1.5
14. Davis WW, Stout TR. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. Appl Microbiol. 1971;22: 659–665.
15. Omar MN, Rahman NA, Ichwan SJA. Pharmacological Activities of Kaempferia galanga L. Rhizome Extract - A Reiew. J Teknol. 2014;79: 89–95.
16. Xie Y, Yang W, Tang F, Chen X, Ren L. Antibacterial Activities of Flavonoids: Structure-Activity Relationship and Mechanism. Curr Med Chem. 2014;22: 132–149. https://doi.org/10.2174/0929867321666140916113443
17. Akiyama H. Antibacterial action of several tannins against Staphylococcus aureus. Journal of Antimicrobial Chemotherapy. 2001;48: 487–491. https://doi.org/10.1093/jac/48.4.487
18. Arabski M, Węgierek-Ciuk A, Czerwonka G, Lankoff A, Kaca W. Effects of Saponins against Clinical *E. coli* Strains and Eukaryotic Cell Line. J Biomed Biotechnol. 2012;2012: 1–6. https://doi.org/10.1155/2012/286216
19. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals. 2013;6: 1451–1474. https://doi.org/10.3390/ph6121451
20. Silhavy TJ, Kahne D, Walker S. The Bacterial Cell Envelope. Cold Spring Harb Perspect Biol. 2010;2: a000414–a000414. https://doi.org/10.1101/cshperspect.a000414
21. Breidenstein EBM, de la Fuente-Núñez C, Hancock REW. Pseudomonas aeruginosa: all roads lead to resistance. Trends Microbiol. 2011;19: 419–426. https://doi.org/10.1016/j.tim.2011.04.005
22. Lakshmanan D, Werngren J, Jose L, Suja KP, Nair MS, Varma RL, et al. Ethyl p-methoxycinnamate isolated from a traditional anti-tuberculosis medicinal herb inhibits drug resistant strains of Mycobacterium tuberculosis in vitro. Fitoterapia. 2011;82: 757–761. https://doi.org/10.1016/j.fitote.2011.03.006
23. Jiao Z, Xu W, Zheng J, Shen P, Qin A, Zhang S, et al. Kaempferide Prevents Titanium Particle Induced Osteolysis by Suppressing JNK Activation during Osteoclast Formation. Sci Rep. 2017;7: 16665. https://doi.org/10.1038/s41598-017-16853-w
24. Wang S-Y, Cai L, Yang N, Xu F-F, Wu Y-S, Liu B. Chemical composition of the Kaempferia galanga L. essential oil and its in vitro and in vivo antioxidant activities. Front Nutr. 2023;10. https://doi.org/10.3389/fnut.2023.1080487
25. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26: 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002