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Comparison of Antibacterial Activity of White and Black Kencur Rhizome Juices Against Shigella sp

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Abstract

Kaempferia galanga L. (commonly known as white kencur) is a medicinal plant from the Zingiberaceae family, widely distributed in tropical and subtropical regions. In developing countries such as Indonesia, the use of herbal remedies has gained popularity due to their affordability and abundance. Kencur rhizomes are known to contain various bioactive compounds, including essential oils, saponins, flavonoids, and polyphenols, which are reported to have antibacterial properties. This study aimed to evaluate and compare the antibacterial activity of white kencur (*Kaempferia galanga*) and black kencur (*Kaempferia parviflora*) rhizome juices against *Shigella* sp. using the diffusion method. A quasi-experimental research design was employed. The study population consisted of white and black kencur rhizomes, which were processed into 60% concentration juices. A total of 32 samples were prepared, comprising 16 replicates for each kencur type. The antibacterial activity was assessed by measuring the inhibition zones formed around the samples using the agar diffusion method. The results demonstrated that white kencur juice exhibited antibacterial activity, with an average inhibition zone diameter of 7.5 mm. In contrast, black kencur juice showed no inhibition zone, indicating a lack of antibacterial effect against *Shigella* sp.

Keywords: *Kaempferia galanga*, *Kaempferia parviflora*, *Shigella* sp., antibacterial activity.

Introduction

Kencur (*Kaempferia galanga* L.) is an herbaceous plant belonging to the Zingiberaceae family, widely recognized for its medicinal properties across various communities. Native to tropical Asia—including China, Indonesia, Thailand, Taiwan, Malaysia, and India—kencur has long been utilized as a traditional remedy, especially in Ayurvedic medicine. The rhizome of kencur is the primary source of its bioactive compounds and has been traditionally employed in the treatment of conditions such as diarrhea, migraines, inflammation, malaria, bronchitis, skin diseases, helminthiasis, cough, asthma, wounds, rheumatism, fever, ulcers, and hemorrhoids.[1]

Both white kencur and black kencur (*Kaempferia parviflora*) contain compounds known to inhibit bacterial cell wall synthesis. These compounds exhibit bactericidal effects by disrupting the synthesis of bacterial cell wall enzymes. Additionally, they can inhibit bacterial protein synthesis, either by exerting bactericidal or bacteriostatic effects, without affecting normal host cells. Some components also demonstrate bacteriostatic properties by altering cell membrane permeability.[2]

Prolonged use of synthetic antibiotics is associated with several adverse effects, including digestive disorders, allergic reactions, headaches, kidney damage, and the development of antibiotic resistance [3]. In contrast, plant-derived herbal medicines generally pose fewer risks and side effects, although they often require extended and consistent use to be effective.[4]

The emergence of antibiotic-resistant bacteria, such as *Shigella* spp., which have shown resistance to fluoroquinolones and ciprofloxacin[5], underscores the urgent need for alternative antimicrobial agents. In this context, herbal medicine is gaining renewed attention due to its relatively safer profile compared to synthetic antibiotics.[6]

The rhizomes of *Kaempferia galanga* contain several active antibacterial compounds, including flavonoids, essential oils, saponins, and polyphenols [7]. Each of these compounds has distinct mechanisms of action against bacterial cells. Flavonoids denature bacterial proteins and irreversibly damage cell membranes[8]. Saponins reduce the surface tension of bacterial cell membranes, causing them to become permeable and ultimately lyse.[9]

White and black *kencur* (*Kaempferia galanga* and *Kaempferia rotunda*) have long been utilized in traditional medicine, but recent scientific investigations have begun to elucidate their distinct phytochemical compositions and corresponding medicinal properties. *Kaempferia galanga* is rich in bioactive compounds such as ethyl p-methoxycinnamate, ethyl cinnamate, kaempferol, and kaempferide, which contribute to its antimicrobial, antioxidant, and anti-inflammatory activities[10] . In contrast, *Kaempferia rotunda* contains significant amounts of benzyl benzoate, bornyl acetate, and zingiberene, compounds known for their antimicrobial and antioxidant properties[11] . Both species

have demonstrated efficacy against various pathogens, including Gram-negative bacteria such as *Shigella flexneri*[12]. Given the rising concern of antibiotic resistance in *Shigella* spp., exploring the differential antibacterial effects of these two *kencur* species is of scientific and clinical interest

A study by Susanti (2018)[13] demonstrated the antibacterial potential of kencur rhizome extract against *Escherichia coli*. The results showed increasing inhibition zone diameters with higher concentrations: 30% extract yielded a 27 mm zone, 45% yielded 30 mm, 60% yielded 32 mm, and 75% yielded 34 mm.

Based on this background, the present study aims to investigate the inhibitory effects of white and black kencur rhizome extracts (Kaempferia galanga and Kaempferia parviflora, respectively) against *Shigella* spp. using the diffusion method.

Materials and methods

This study employed a descriptive-analytical method with a quasi-experimental design. A quasi-experimental design includes a control group but lacks full randomization, which limits its ability to completely control for external variables that may influence the results.

Population and Sample

The research population consisted of white kencur rhizomes (*Kaempferia galanga*) and black kencur rhizomes (*Kaempferia parviflora*), which were sourced from the Sungai Durian Village, Kubu Raya Regency.

The study used white and black kencur rhizome juices at a 60% concentration as the test samples. The inclusion criteria for the samples were as follows fresh rhizomes, free from rot and physical defects. The samples were divided into two treatment groups (white and black kencur juice), with 16 replications per group, totaling 32 experimental units.

Antibacterial Testing Method

The antibacterial activity was assessed using the disc diffusion method. Filter paper discs were impregnated with the respective kencur juice extracts and placed on Mueller-Hinton Agar (MHA) plates that had been inoculated with *Shigella* sp. The diameter of the inhibition zone surrounding each disc indicated the antibacterial efficacy of the extract, reflecting bacterial sensitivity[14].

Sample Preparation

A total of 200 grams each of white *kencur* (*Kaempferia galanga*) and black *kencur* (*Kaempferia parviflora*) rhizomes were collected from Sungai Durian Village. The rhizomes were thoroughly washed with distilled water to remove any dirt or contaminants. After cleaning, each type of rhizome was grated, squeezed, and filtered using sterile gauze. This traditional cold extraction method is considered efficient for preserving heat-sensitive bioactive compounds such as essential oils and flavonoids, while also yielding a relatively high volume of extract with minimal degradation. The resulting filtrate (juice) was collected in a sterile beaker and covered with sterile gauze to maintain hygiene. This extract was designated as the 100% stock solution, from which a 60% concentration was subsequently prepared for use in the antibacterial test.

Preparation of McFarland Turbidity Standard

Adapted from Kurniawan et al 2015[15],the 0.5 McFarland turbidity standard was prepared by mixing 0.5 mL of 1.175% barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) with 9.5 mL of 1% sulfuric acid (H_2SO_4) in a clean test tube. The solution was mixed thoroughly until homogeneous. This standard was stored in a tightly sealed test tube to prevent evaporation and was kept in a dark environment at room temperature. It remained stable for up to six months and was shaken before each use.

Preparation of *Shigella* sp. Bacterial Suspension

Following the method described by Kuswiyanto (2016), pure colonies of *Shigella* sp. were collected from culture media using a sterile inoculating loop. The colonies were then transferred into a test tube containing 5 mL of sterile 0.9% NaCl solution and mixed until a homogeneous bacterial suspension was formed. The turbidity of the bacterial suspension was adjusted by visually comparing it to the McFarland 0.5 standard to ensure uniform bacterial concentration.

Preparation of Antibacterial Discs

According to the guidelines from the Ministry of Health of the Republic of Indonesia (2014), 32 sterile blank paper discs (6 mm in diameter) were prepared. Using sterile forceps, the discs were immersed in 60% juice extracts of either white kencur (Kaempferia galanga) or black kencur (Kaempferia parviflora) for 5–10 minutes. After soaking, the discs were briefly air-dried to remove excess liquid and then carefully placed onto the surface of Mueller-Hinton Agar (MHA) plates that had been inoculated with Shigella sp. The placement followed a standard distance of approximately 2 cm between the disc and the edge of the plate and 3 cm between individual discs.

Preparation of Negative Control

A negative control was prepared by dipping a sterile cotton swab into the bacterial suspension, pressing and rotating it along the inner wall of the test tube to remove excess liquid. The swab was then used to evenly spread the suspension across the surface of a Mueller-Hinton Agar plate. A sterile 6 mm disc soaked in 0.9% NaCl solution was placed on the inoculated agar plate to serve as the negative control disc. Plates were incubated at 37°C for 24 hours.

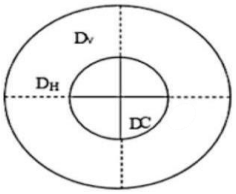
Inoculation of *Shigella* sp. on MHA Plates

A sterile cotton swab was dipped into the prepared Shigella sp. bacterial suspension. After pressing the swab along the side of the test tube to remove excess fluid, the bacteria were evenly spread across the surface of Mueller-Hinton Agar plates. The plates were then allowed to rest for 10 minutes to allow absorption of the inoculum. Discs previously soaked in either white or black kencur juice were allowed to drain for 5 minutes, then placed on the inoculated MHA surface. The discs were gently pressed using sterile forceps to ensure full contact with the agar surface. Finally, the plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zones was measured to assess antibacterial activity.

Antimicrobial activity was assessed by measuring the size of the inhibition zone formed around each well. The inhibition zones were recorded in millimeters (mm) by measuring both the vertical diameter (DV) and horizontal diameter (DH) using a standard ruler

Legend:
Dv: diameter vertical
Dh: diameter Horizontal

$$\frac{DV+DH}{2} = \ldots m$$



Statistical Analysis

Data from this study were analyzed using univariate analysis to describe key variables, including the maximum, minimum, and mean values of the inhibition zones. A comparative test was also conducted to determine whether there were statistically significant differences in the antibacterial effects between white and black *kencur* extracts. All measurements were processed using computerized tools to ensure accuracy and consistency.

Results

Table 1: Clear Zone Results of White Galangal Rhizome Juice and Water Gangal Rhizome Juice Against the Growth of Bacteria Shigella sp

Replication	Inhibition zone (mm)	
	White Kencur	**Black Kencur**
1	7 mm	6 mm (no inhibition)
2	7 mm	6 mm (no inhibition)
3	8 mm	6 mm (no inhibition)
4	7 mm	6 mm (no inhibition)
5	7 mm	6 mm (no inhibition)
6	8 mm	6 mm (no inhibition)
7	8 mm	6 mm (no inhibition)
8	8 mm	6 mm (no inhibition)
9	8 mm	6 mm (no inhibition)
10	7 mm	6 mm (no inhibition)
11	7 mm	6 mm (no inhibition)
12	8 mm	6 mm (no inhibition)
13	7 mm	6 mm (no inhibition)
14	8 mm	6 mm (no inhibition)
15	8 mm	6 mm (no inhibition)
16	7 mm	6 mm (no inhibition)
Rata-Rata	7,5 mm	6 mm (no inhibition)

Table 1 presents the results of observations on the antibacterial activity of white and black kencur (Kaempferia spp.) rhizome extracts against the growth of *Shigella* sp. The findings reveal that the white kencur extract exhibited an average inhibition zone of 7.5 mm. This inhibition zone size indicates a relatively strong antibacterial effect, with the highest observed value reaching 8 mm and the lowest at 7 mm, suggesting a consistent and stable response across the tested samples.

In contrast, the black kencur extract showed a significantly smaller average inhibition zone of 6 mm, with all samples exhibiting the same value. There was no observable variation or notable increase in inhibition zone size, indicating that the black kencur extract did not demonstrate substantial antibacterial activity against *Shigella* sp. under the tested conditions.

Table 2: Descriptive analysis results

	N	**Min**	**Max**	**Mean**	**Std. Deviasi**
White Kencur	16	7	8	7.5	0.51640
Black Kencur	16	6	6	6	0

Table 2 presents the descriptive statistical analysis of the inhibition zone measurements, including the number of samples, minimum and maximum values, mean, and standard deviation. The data show that 16 samples were tested for each type of *kencur* (white and black). For white *kencur*, the inhibition zone ranged from a minimum of 7 mm to a maximum of 8 mm. The mean inhibition zone was 7.5 mm, with a standard deviation of 0.51640, indicating relatively low variability and a stable distribution around the mean value.

In contrast, for black *kencur*, all samples consistently produced an inhibition zone of 6 mm, resulting in a standard deviation of 0. This indicates complete uniformity across all trials and no observable variation in antibacterial activity.

Table 3: Independent T-Test Results Comparing Inhibition Zones of White and Black Kencur Extracts

Variable	**White Kencur**	**Black Kencur**
Sample size (n)	16	16
Mean (mm)	7.5	6.0
Standard deviation	0.5	0.0
t-value	11.62	–
p-value	1.24×10^{-12}	–
Statistical decision	Significant ($p < 0.05$)	–
Interpretation	Greater antibacterial effect	–

Table 3 shows that antibacterial activity of white and black *kencur* (*Kaempferia galanga*) rhizome juice, extracted through a manual squeezing technique to obtain the liquid component. Sixteen samples of white *kencur* juice were tested, yielding inhibition zones ranging from 7 mm to 8 mm, with a mean of 7.5 mm. In contrast, the black *kencur* rhizome produced inhibition zones of 6 mm, equivalent to the disc diameter, suggesting the absence of antibacterial activity under the experimental conditions.

Discussion

These findings differ markedly from those reported by Putra (2025)[16], who observed a mean inhibition zone of 10.88 mm for *Kaempferia parviflora* extract against *Escherichia coli*, which is categorized as moderate antibacterial activity. The divergence in antibacterial efficacy between studies may be primarily attributed to differences in extraction methodology. Putra employed solvent-based extraction, a technique well-documented for its ability to efficiently isolate secondary metabolites. In contrast, the current study utilized mechanical squeezing, a comparatively less efficient method for extracting polar and non-polar phytochemicals. While both studies applied the same concentration (60%), the extraction technique critically influences the yield and composition of bioactive constituents, particularly flavonoids, saponins, alkaloids,

and tannins—compounds known for their antimicrobial properties.

Black kencur (*Kaempferia parviflora*) may exhibit lower antibacterial efficacy compared to white kencur (*Kaempferia galanga*) due to differences in their phytochemical compositions. While *K. parviflora* is rich in methoxyflavones known for their antioxidant and anti-inflammatory properties, these compounds may not possess strong direct antibacterial activity. In contrast, *K. galanga* contains a higher concentration of ethyl p-methoxycinnamate (EPMC), a compound that has demonstrated significant antimicrobial effects against various pathogens, including *Escherichia coli* and *Staphylococcus aureus*[17]. This disparity in active constituents likely contributes to the observed differences in antibacterial potency between the two species.

Extraction efficacy remains a pivotal factor in phytochemical and pharmacological investigations. Mechanical squeezing may not sufficiently rupture plant cellular matrices, potentially leading to suboptimal recovery of active compounds. The absence of phytochemical screening in this study further limits the ability to confirm the presence and concentration of antibacterial constituents, precluding a deeper mechanistic understanding of the observed effects.

Methodological constraints also likely contributed to the reduced antibacterial performance. Although 16 replications were performed, the extract used across replicates originated from a single batch of mechanically processed juice. Inadequate homogenization may have led to sedimentation and uneven distribution of active compounds, thereby affecting the reproducibility and consistency of inhibition zones. Moreover, the soaking process was not proportionally scaled to accommodate the number of replications, which, combined with sample limitations, may have negatively impacted extraction yield and bioactive concentration.

Despite these limitations, prior studies have substantiated the antibacterial potential of *K. galanga* rhizomes. The species is known to contain bioactive phytochemicals such as flavonoids, essential oils, saponins, and polyphenols[7]. These compounds function through distinct antibacterial mechanisms. Flavonoids can bind to bacterial enzymes, denature proteins, and disrupt membrane integrity while saponins alter membrane surface tension and permeability, ultimately inducing bacterial cell lysis.[9]

Furthermore, the concentration of phenolic compounds and the antioxidant activity of *Kaempferia* species have been reported to vary in response to environmental conditions, such as light exposure and soil composition[18]. These environmental influences could significantly impact the phytochemical profile and, consequently, the antibacterial efficacy of the plant material.

Conclusion

White kencur (Kaempferia galanga) juice at 60% concentration exhibited weak antibacterial activity against *Shigella sp.*, with a mean inhibition zone of 7.5 mm, while black kencur (Kaempferia parviflora) showed no detectable antibacterial effect under the tested conditions

Conflict of interest

Authors state no conflict of interest

Reference style

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