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Phytochemical Examination, GC-MS Analysis, and Antibacterial Activity of Methanol Extract of Dadap Leaves (*Erythrina variegata* L.) Against *Staphylococcus aureus* and *Escherichia coli* Bacteria using Disc Diffusion Method

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ABSTRACT. This study examines the antibacterial activity of a methanol extract from the leaves of Dadap (*Erythrina variegata* L.) against *Staphylococcus aureus* and *Escherichia coli*. Bacterial infections are a major health issue in Indonesia, so the development of plant-based natural medicines is being pursued. The Dadap plant has the potential to treat bacterial infections. This study aims to identify the types of secondary metabolites in the methanol extract of Dadap leaves, analyze the active compounds using Gas Chromatography-Mass Spectroscopy (GC-MS), and evaluate their antibacterial activity. Extraction was performed by maceration with methanol, and antibacterial testing was conducted by disk diffusion on Mueller-Hinton Agar with extract concentrations of 5, 10, 20, and 40% (w/v). The extracts were diluted in sterile distilled water. The results exhibited antibacterial activity against *S. aureus* and *E. coli,* with inhibition zones of 14 mm (strong) and 8.8 mm (moderate) at a 40% concentration, respectively. These activities might be linked to the active compounds found in the Dadap leaves extract using phytochemical and GC-MS analysis. The phytochemical analysis showed that the *E. variegata* L. leaf extract contains alkaloids, steroids, terpenoids, saponins, flavonoids, phenols, and tannins. Furthermore, the GC-MS chromatogram identified 11 compounds, including 7 with antibacterial activity, such as neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, hexadecanoic acid, methyl ester, n-hexadecanoic acid, phytol, methyl stearate, and stigmasterol. Overall, the crude extract of Dadap leaves showed a potential candidate for development as an antibiotic.

INTRODUCTION

The Dadap plant (*Erythrina variegata* L.), a member of the Fabaceae family, holds significant potential as a medicinal plant for treating a variety of ailments. Traditionally, it has been used to alleviate conditions such as stress, fever, coughs, wounds, and bacterial infections (Chu *et al.*, 2019; Eltayeb and Mousnad, 2020). In addition to these traditional uses, the Dadap plant exhibits various bioactivities, including antioxidant (Abdulrahman *et al*., 2022), anthelmintic (Roring *et al*., 2019), antidiabetic (Sinulingga *et al*., 2020), antimycotic (Hardani *et al*., 2020), anti-inflammatory (Hasim *et al*., 2019), anti-diarrheal (Suhaimi and Kartikasari, 2020), antipyretic (Kusuma and Anggraini, 2022), and antibacterial properties. The plant's scientific name, *E. variegata* L., is sometimes used synonymously with *Erythrina indica* Lam. (Kumari, P. and Kumari, C., 2017). Dadap plants can be classified as follows.

Kingdom : Plantae
Divisi : Spermatophyta
Class : Dicotyledoneae
Ordo : Fabales
Family : Fabaceae
Genus : *Erythrina*
Species : *E. variegata* L.

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The leaves of *E. variegata* L. have three ovate leaves (3-foliolate), with a rhombus shape and blunt leaf tips. The young part of the stem bark has fine vertical stripes of green, grey, light brown, or whitish. The stem also has small spines (Kumari, P. and Kumari, C., 2017). The morphology of the *E. variegata* L. plant is shown in Figure 1.

Figure 1. Dadap (*E. variegata* L.) plant morphology.

The Dadap plant has the ability to act as an antibacterial, inhibiting bacterial growth and killing bacteria. This ability stems from its secondary metabolite content. Secondary metabolites are compounds produced by plants that possess bioactive properties and can be used as ingredients in medicine (Rohama and Zainuddin, 2021). Antibacterial activity can be obtained from plant extracts that contain secondary metabolites such as alkaloids, saponins, Terpenoids, flavonoids, phenols, and tannins (Syafriana *et al*., 2024). Hemmalakshmi *et al*. (2016) also reported that phytochemical screening of the leaves, flowers, and bark of *E. variegata* L. reveals the presence of alkaloids, flavonoids, saponins, terpenoids, steroids, and tannins. The bioactivity of the Dadap plant as an antibacterial can be tested by observing the effect of the Dadap extract on the growth of test bacteria. Antibacterial testing is generally carried out using two methods: the dilution method and the diffusion method. The dilution method involves observing low concentrations that can inhibit the growth of bacteria or other microorganisms in liquid or solid media containing antibacterial substances (Najiya, 2022).

Meanwhile, the diffusion method is used to determine antibacterial activity by measuring the diameter of the clear zone formed around the disc or well. This clear zone is formed by the solubility and diffusion of the material being tested, which inhibits the growth of microorganisms (Geofani *et al*., 2022). In this study, antibacterial testing was performed using the agar diffusion method (Kirby-Bauer) because it is simple, easy to apply, and effective for determining antibacterial activity (Fransisca *et al*., 2020). Additionally, disc diffusion testing is faster, relatively inexpensive, easy to perform, and does not require specialized skills (Intan *et al*., 2021).

The identification of the methanolic extract of *E. variegata* L. leaves was conducted using Gas Chromatography-Mass Spectroscopy (GC-MS), which is preferred over Liquid Chromatography-Mass Spectroscopy (LC-MS) for the analysis of methanolic plant extracts because it is more effective at analyzing volatile compounds such as Terpenoids and esters. GC-MS offers superior separation capability for volatile compounds and demonstrates higher sensitivity in detecting light organic compounds. Furthermore, GC-MS is more economical and efficient compared to LC-MS, which requires more expensive equipment and more complex operational conditions. Therefore, GC-MS is more suitable for methanolic extracts containing volatile compounds (Melati, 2021). Given this, research on the antibacterial activity of *E. variegata* L. leaves extract against *S. aureus* and *E. coli* bacteria is crucial. The findings from this study are expected to serve as a valuable reference for the development of natural antibacterials, particularly those derived from Dadap leaves.

RESEARCH METHODS

The tools used in this study include a rotary evaporator, erlenmeyer flask, filter paper, test tubes and racks, autoclave, petri dish, laminar air flow, tube needle, incubator, caliper, measuring cup, beaker glass, dropper pipette, micropipettes, spirit burners, spray wire mesh, scales, measuring pipettes, calipers, tweezers, aluminum foil, object glass, glass funnel, and rod stirrer. The material used is Dadap (*E. variegata* L.) leaves sourced from Ketambe Village, Southeast Aceh Regency. Other materials include *S. aureus* and *E. coli* bacteria, distilled water, methanol (CH_3OH), ammonia (NH_3), chloroform ($CHCl_3$), 2N sulfuric acid (H_2SO_4), Mayer, Wagner, and Dragendorff reagent, sodium hydroxide (NaOH), ammonium hydroxide (NH_4OH), n-hexane, lieberman bouchard reagent, ferric chloride ($FeCl_3$), hydrochloric acid (HCl), magnesium powder, sodium hydroxide (NaOH), Nutrient Agar

(NA) media, Nutrient Broth (NB) media, 0.9% sodium chloride (NaCl) solution, Mueller Hinton Agar (MHA), and ciprofloxacin.

Extraction

The Dadap leaves used in this study are dried leaves. The leaves are allowed to dry for one week, avoiding direct sunlight, and then crushed to obtain a dry powder sample. A total of 500 g of powdered *E. variegata* L. leaves is combined with 500 mL of methanol for the extraction process using the maceration method. The maceration process lasts for 48 hours. Afterward, the extract is concentrated using a rotary evaporator (Halimatussakdiah *et al*., 2018). Once concentrated, the resulting extract is used for phytochemical analysis and antibacterial activity testing.

Phytochemical Examination

Alkaloid

The methanol extract of *E. variegata* L. leaves was mixed with 1 mL of ammonia, then 10 mL of chloroform was added, and the mixture was filtered. The filtrate was mixed with 10 mL of 2 N Sulfuric Acid (H_2SO_4), shaken, and allowed to separate into two layers. The Sulfuric Acid layer was divided into two test tubes and tested with Mayer, Wagner, and Dragendorff reagents. A white precipitate with Mayer's reagent, a yellow precipitate with Wagner's reagent, and an orange precipitate with Dragendorff's reagent indicated the presence of alkaloids (Situmorang *et al*., 2024).

Flavonoid

Flavonoid compounds were tested using methods that involved adding HCl and magnesium powder, which resulted in a positive reaction indicated by an orange color change. The sample volume used for the flavonoid test was 2 mL (Iskandar, 2020).

Steroids, Terpenoids, and Saponins

The methanol extract of *E. variegata* L. leaves was re-extracted using n-hexane solution. The extract was then tested with Lieberman-Bourchard reagent. A color change to blue or green indicated the presence of steroids, while a reddish-brown color indicated the presence of terpenoids. The saponin test was performed by adding a few drops of distilled water to the extract sample and shaking vigorously. A positive saponin result was indicated by the presence of stable foam remaining for 15 minutes after shaking (Situmorang *et al*., 2024).

Fenol

The methanol extract of *E. variegata* L. leaves was mixed with 5 mL of distilled water, followed by the addition of a few drops of $FeCl_3$ solution. The presence of phenols was confirmed by the formation of a bluish-black color (Situmorang *et al*., 2024).

Tanin

The sample extract was treated with a FeCl3 solution, and the presence of tannins was indicated by a blackish-green color change (Situmorang *et al*., 2024).

GC-MS Analysis

Briefly, GC-MS analysis was performed using a GC-MS instrument controller DESKTOP-EVK9RSD equipped with a detector, a TG-5MS column, and helium gas. The operating conditions were: total flow rate at the separate vent was 50 mL/min; flow rate through the column was 1 mL/min (inlet flow: 1 mL/min). The split and purge flows were 50 mL/min and 5 mL/min, respectively. One microliter of the sample was injected, and the temperature gradient started at 40 °C and reached 300 °C (end) over 52 min (Sari *et al*., 2018).

Antibacterial Assay

Sterilization

The sterilization of equipment, the production of NA and MHA media, and the preparation of bacterial suspensions were conducted following the procedures outlined in the study by Astriani *et al*. (2021).

NB Media

The preparation of NB media was conducted according to the procedures outlined by Pinta *et al*. (2017).

Bacterial Regeneration

Five milliliters of NA media were poured into a Petri dish and left to solidify. Subsequently, bacterial cultures of *S. aureus* and *E. coli* were transferred using a sterile loop and incubated at 37 °C for 24 hours (Astriani *et al*.,

2021). After incubation, seven bacterial colonies were transferred into an Erlenmeyer flask containing NB media for each bacterial type. The cultures were then incubated at room temperature for 24 hours to obtain pure bacterial cultures (Pinta *et al*., 2017).

Assay of Antibacterial Activity of Methanol Extract of E. variegata L. Leaves

The methanol extract of Dadap leaves was prepared at concentrations of 5%, 10%, 20%, and 40% and tested for antibacterial activity using the agar disk diffusion method (Kirby-Bauer). Petri dishes containing 25 mL of MHA media were sterilized, and a bacterial suspension was spread on the surface. Discs impregnated with the extract, ciprofloxacin (as a positive control), and distilled water (as a negative control) were placed on the agar. After 24 hours of incubation at 37 °C, the zones of inhibition were measured to evaluate antibacterial activity, and the results were categorized according to the Davis-Stout criteria (Trisia *et al*., 2018).

RESULTS AND DISCUSSION

The identification results of secondary metabolite compounds from the leaves extract of *E. variegata* L. are presented in Table 1. Based on Table 1, the methanol extract of *E. variegata* L. leaves contains a variety of compounds, including alkaloids, steroids, Terpenoids, saponins, flavonoids, phenols, and tannins. Mohammed *et al*. (2023) also reported that alkaloids, flavonoids, phenols, and tannins were present in the methanol extract of Dadap leaves. Methanol, being a polar solvent, has the unique ability to dissolve a wide range of both polar and nonpolar compounds, due to its small molecular structure and the presence of both hydroxyl (–OH) and methyl (–CH_3) groups. The hydroxyl group facilitates interactions with polar compounds through hydrogen bonding, while the methyl group interacts with nonpolar substances. This makes methanol an effective solvent for extracting various secondary metabolites with different polarities (Marpaung and Romelan, 2019).

Table 1. Phytochemical test results of Dadap leaves extract.

Secondary Metabolites	Methanol Extract
Alkaloids	+
Steroids	+
Terpenoidss	+
Saponins	+
Flavonoids	+
Phenol	+
Tannins	+

The methanol extract of Dadap leaves was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS) to identify its active compounds. This technique combines gas chromatography (GC) for quantitative analysis and mass spectrometry (MS) for molecular structure determination (Melati, 2021). The analysis revealed 26 peaks in the chromatogram in Figure 2, indicating the concentrations of various compounds in the sample (Novilda *et al*., 2022). According to Table 2, 11 of the 7 identified compounds exhibit antibacterial activity, including neophytadiene, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, hexadecanoic acid, methyl ester, n-hexadecanoic acid, phytol, methyl stearate, and stigmasterol.

Antibacterial activity was evaluated to assess the effect of the test sample, with three repetitions (U1-U3). The bioindicators used were Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. Ciprofloxacin served as the positive control, and distilled water as the negative control. The positive control was used to compare inhibition zone diameters, while the negative control ensured that distilled water, used to prepare the agar medium and dilute extracts, did not contain antibacterial substances. Antibacterial activity was tested using the disk diffusion method, and the inhibition zone diameters were measured after 24 hours of incubation with methanol extracts of Dadap leaves against *S. aureus* and *E. coli*. The measurements were taken with a caliper in millimeters (mm) (Atmaja *et al*., 2017).

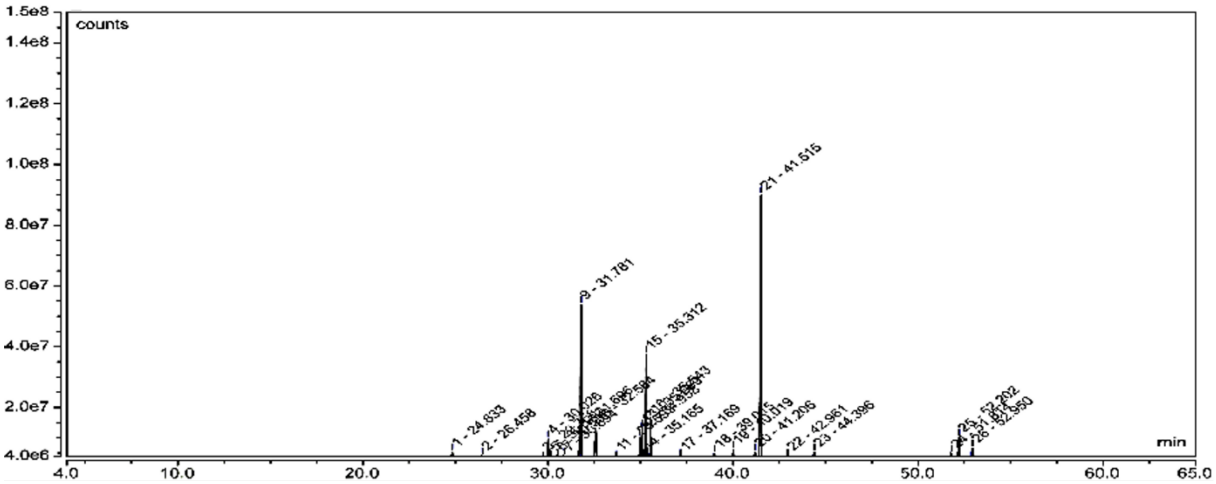


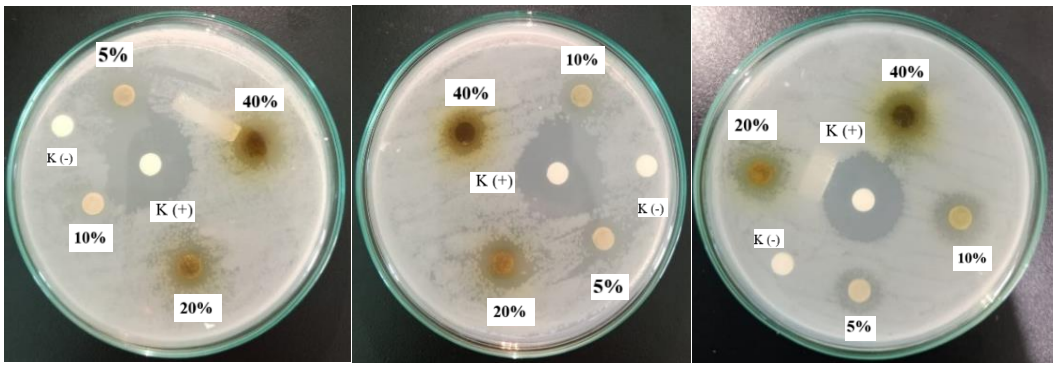
Figure 2. GC chromatogram of the methanol extract of *E. variegata* L. leaves.

Table 2. Volatile compounds identified in the methanol extract of Dadap leaves.

Compound Name	Molecular Formula	Molecular Weight (g/mol)	Retention Time (minute)	Area Percent (%)	Similarity Index (%)	Type of Compounds
7-Oxabicyclo[4.1.0]heptan-3-ol, 6-(3 hydroxy-1-butenyl)-1,5,5-trimethyl-	$C_{13}H_{22}O_3$	226.3120	26.458	1.08	81	Terpenoids
Neophytadiene	$C_{20}H_{38}$	278.5	30.026	2.55	89.7	Terpenoids
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	30.894	0.67	80.5	Isoprenoid
7-Hexadecenoic acid, methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.4	31.696	1.98	87.3	Unsaturated Fatty Acids Steroid Jadi Steroids
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	31.781	17.75	95.3	Saturated Fatty Acids
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	270.5	32.584	5.74	86	Saturated Fatty Acids
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.5	34.958	2.72	89.7	Unsaturated Fatty Acids Steroid Jadi Steroids
Phytol	$C_{20}H_{40}O$	296.5	35.312	1.21	92.2	Isoprenoid
Methyl stearate	$C_{19}H_{38}O_2$	298.5	35.543	4.51	91	Saturated Fatty Acids
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.5	40.019	1.30	83.6	Terpenoids
Stigmasterol	$C_{29}H_{48}O$	412.7	52.202	2.63	86.1	Steroid

In this study, antibacterial activity was tested at four concentrations of the sample: 5, 10, 20, and 40% w/v, with three replicates per concentration. The inhibition zone produced by the methanol extract of *E. variegata* L. leaves against *S. aureus* is shown in Figure 3, and the corresponding diameters of the inhibition zones are listed in Table 3. Based on the data presented in Table 3, there are variations in the diameter of the inhibition zones at different extract concentrations. At a 5% w/v concentration, the methanol extract exhibited antibacterial activity against *S. aureus*, with an average inhibition zone of 7.6 mm, categorized as medium activity. However, at concentrations of 10, 20, and 40% w/v, the inhibition zones increased to 12.8 mm, 13.3 mm, and 14 mm, respectively, placing them in the strong activity category. The increase in the inhibition zone diameter with higher extract concentrations can be attributed to the higher concentration of active compounds, which are more effective

at inhibiting bacterial growth (Nurhayat *et al*., 2020). Alkaloids, flavonoids, tannins, steroids, and terpenoids are key compounds involved in inhibiting bacterial growth. Alkaloids prevent peptidoglycan formation in bacterial cell walls, leading to cell membrane damage. Flavonoids disrupt peptidoglycan biosynthesis via their hydroxyl groups, leading to bacterial cell death. Tannins impair bacterial cell adhesion, interfere with protein transport, and damage cell wall polypeptides, leading to lysis. Steroids and Terpenoids damage the cell membrane by increasing permeability, causing leakage of cell contents (Nurjannah *et al*., 2022).



U1 **U2** **U3**

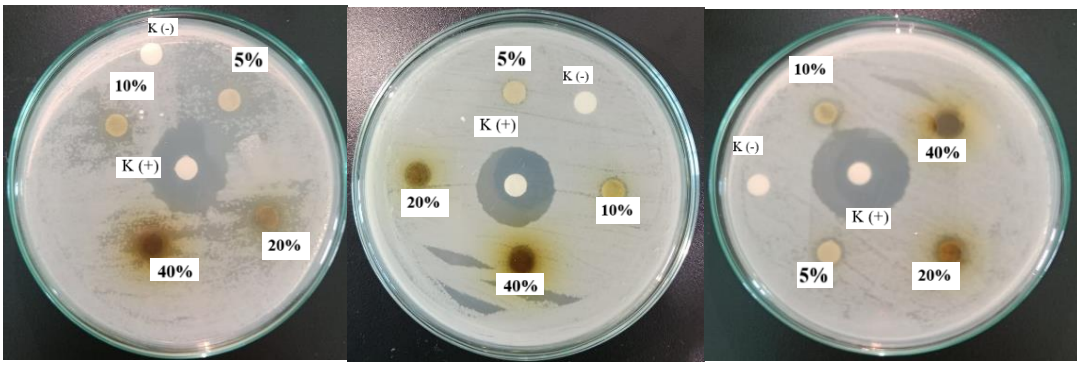
Figure 3. Zone of inhibition of *E. variegata* L. leaves extract against *S. aureus* bacteria.

Table 3. The diameter value of the inhibition zone in *S. aureus* bacteria.

Sample	Sample Concentration (% w/v)	Diameter of the Inhibition Zone (mm)			Average (mm)	Inhibition Zone Category
		U1	U2	U3		
Dadap Leaves Extract	5	12	0	11	7.6	Medium
	10	13	13,5	12	12.8	Strong
	20	13	12	15	13.3	Strong
	40	15	13	14	14	Strong
Positive Control		24	25	24	24.3	Very Strong
Negative Control		0	0	0	0	Not Obstructing

The antibacterial activity of the methanol extract from *E. variegata* leaves was also evaluated against *E. coli* bacteria. The inhibition zones produced by the methanol extract of *E. variegata* leaves against *E. coli* are shown in Figure 4, with the corresponding inhibition zone diameters recorded in Table 4. Based on Table 4, the methanol extract of *E. variegata* L. leaves at concentrations of 5, 10, 20, and 40% w/v produced inhibition zone diameters against *E. coli* bacteria with average measurements of 7.3 mm, 8 mm, 8.3 mm, and 8.8 mm, respectively, which are classified as medium activity. This moderate effect is attributed to the double membrane structure of *E. coli*, which includes both the inner and outer membranes, both of which are permeable, making the bacterium more resistant to antibacterial agents (Ulfah, 2020). The study utilized both positive and negative controls to compare the antibacterial effectiveness of the methanol extract of *E. variegata* L. leaves. Ciprofloxacin, used as the positive control, demonstrated a clear zone diameter of 24 mm against *S. aureus* and 22.6 mm against *E. coli*, both classified as very strong antibacterial activity. Ciprofloxacin was chosen for its broad-spectrum properties, accessibility, and widespread use (Fajrina *et al*., 2021). This antibiotic works by inhibiting the DNA gyrase enzyme, which is involved in bacterial cell division, making it bacteriostatic (Muslim *et al*., 2020).

Table 3 and Figure 4 show that the inhibition zone of ciprofloxacin is significantly larger compared to that of the methanol extract of *E. variegata* L. leaves. This is likely due to the fluorine atom in ciprofloxacin, which disrupts the topoisomerase enzymes II and IV, essential for bacterial DNA replication and separation (Dewangga and Qurrohman, 2019). The negative control used was distilled water, which has no antibacterial activity and does not interfere with the test results for the extract's bacterial inhibition. Distilled water is also used to ensure that the solvent does not influence the antibacterial test outcomes (Welfalini *et al*., 2022). According to Nurjannah *et al*. (2022), distilled water as a negative control does not produce an inhibition zone in antibacterial testing.



U1 **U2** **U3**

Figure 4. Zone of inhibition of *E. variegata* L. leaves extract against *E. coli* bacteria.

Table 4. The Diameter value of the inhibition zone in *S. aureus* bacteria.

Sample	Sample Concentration (% w/v)	Diameter of the Inhibition Zone (mm)			Average (mm)	Inhibition Zone Category
		U1	U2	U3		
Dadap Leaves Extract	5	6	8	8	7.3	Medium
	10	7	9.5	8	8	Medium
	20	7	9	9	8.3	Medium
	40	8	9	9.5	8.8	Medium
Positive Control		21	22	25	22.6	Very Strong
Negative Control		0	0	0	0	Not Obstructing

The antibacterial potency, as indicated by the diameter of the inhibition zone, is categorized into four levels: weak, moderate, strong, and very strong. This zone appears as a clear area surrounding the paper disc. Several factors influence the size of the inhibition zone, including sample concentration (greater concentrations tend to produce larger zones), the thickness of the agar medium (thicker layers typically result in smaller zones), incubation duration (set at 24 hours to support optimal bacterial growth) and incubation temperature (maintained at 37 °C, which is ideal for most bacterial species). Prolonged incubation may increase the likelihood of the formation of resistant mutants or promote the growth of less sensitive microbial populations (Welfalini *et al.*, 2022).

CONCLUSION

The methanol extract demonstrated antibacterial activity, effectively inhibiting the growth of *S. aureus* and *E. coli*. At the highest concentration (40%), the extract produced a strong inhibition zone of 14 mm against *S. aureus* and a moderate inhibition zone of 8.8 mm against *E. coli*. The antibacterial activities might be due to the secondary metabolite in the methanol extract. The phytochemical and GC-MS analysis proved that Dadap leaves are rich in bioactive compounds.

CONFLICT OF INTEREST

The author hereby declares that during the entire process of carrying out the research, data collection, analysis, writing, and preparing the manuscript for this journal, there were no conflicts of interest that could affect the objectivity and integrity of the research results. There are no personal, financial, or professional relationships that could be considered a potential conflict of interest that could influence the conduct of this research or publication.

AUTHOR CONTRIBUTION

HH and UA: Research Design, Statistical Analysis, Interpretation of Results, and Manuscript Revision; YTS: Experiment, Data Collection, Manuscript Writing, and Manuscript Revision. VM; Methodology Development (Antibacterial Testing) and Manuscript Revision.

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