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Endophytic Bacteria Producing Alkaloid as Antibacterial and Antioxidant Agent from *Hibiscus tiliaceus* Leaves

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Abstract

It is known that endophytes can produce the same or similar secondary metabolites as their host plants. Medicinal plants have been proven to be a source of microorganisms that can make certain metabolites. Our previous research succeeded in isolating the endophytic bacteria *Staphylococcus warneri* strain 25 from *waru* leaves (*Hibiscus tiliaceus*), which had antioxidant and antibacterial activity and contained saponins and alkaloids. In this study, alkaloids were extracted from the endophytic bacteria found in *waru* leaves. Subsequently, their bioactivity was assessed for antibacterial properties against *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* using the well diffusion method. Additionally, their antioxidant activity was evaluated through the DPPH method, with gallic acid serving as the standard. A cyclic amine alkaloid compound was isolated, exhibiting a Minimum Inhibitory Concentration (MIC) of 23 ppm against *E. coli*, *S. typhi*, and *S. aureus* bacteria, accompanied by inhibitory zones measuring 7 mm, 8 mm, and 6 mm, respectively. Additionally, the compound had very strong antioxidant activity, with an IC_{50} value of 50.11 ppm.

1. Introduction

The presence of endophytic bacteria in plant tissues means plants automatically use them to protect host plants from pathogenic microorganisms. Endophytic bacteria and fungi are currently categorized as promising resources of novel natural products with applications in medicine, agriculture, and the plant industry [1]. Endophytic bacteria are considered to be an effective agent of bioactivity [2]. Endophytic microorganisms serve as significant sources for discovering bioactive compounds. Their symbiotic association with plants enables them to produce a diverse array of bioactive molecules [3]. Endophytic bacteria extracted from traditional medicinal plants are known to synthesize compounds with antibacterial, antifungal, and antiseptic properties.

Moreover, secondary metabolites derived from endophytic bacterial cultures have demonstrated cytotoxic, antimicrobial, antiviral, and anticancer activities in previous studies [4]. The growing interest in endophytic bacteria emphasizes their utilization due to their ability to enhance plant defense responses through the formation of secondary metabolites [5]. Endophytic bacteria can produce new secondary metabolites with significant bioactivity potential [6]. Utilizing endophytic bacteria to produce bioactive compounds tailored to their hosts offers several advantages. These include a faster life cycle, enabling rapid production, scalability for large-scale production without extensive land requirements, and the ability to obtain new bioactive compound components by manipulating various environmental conditions [7].

Secondary metabolites play a crucial role in plants overcoming diverse environmental stresses. The metabolite-based approach can be used to characterize metabolic alterations, encompassing compounds such as alkaloids, phenolics, terpenoid glycosides, and flavonoids [5]. According to Rahayu *et al.* [8], Hibiscus leaves contain alkaloids, flavonoids, saponins, and tannins. However, the presence of this compound in hibiscus plants varies; some may contain it in higher or lower quantities. This discrepancy arises because the formation of secondary metabolites is influenced by factors such as climate, plant genetics, humidity, temperature, geography, and soil

mineral and nutrient content. Presently, extensive research has been conducted on the bioactivity of extracts from the *waru* (*Hibiscus tiliaceus*) plant, including its leaf extract, which has shown promising antibacterial and antioxidant properties [9].

Previous research revealed that endophytic bacteria isolated from Hibiscus tiliaceus leaves exhibited a cocci form, predominantly comprising Gram-positive bacteria closely resembling Staphylococcus warneri strain AW 25. These endophytic bacteria synthesized alkaloid and saponin secondary metabolites, displaying inhibitory effects against the growth of *Staphylococcus aureus* and *Salmonella typhi* while also demonstrating antioxidant properties. Optimal outcomes were observed after a 22- hour incubation period, coinciding with the initial decline phase in the growth of endophytic bacteria [10]. Building upon previous findings, it becomes necessary to isolate alkaloid compounds synthesized by bacteria extracted from *waru* leaves. This research aims to isolate and characterize alkaloids from the *waru* plant while also acquiring data on their antibacterial and antioxidant activities.

2. Experimental

2.1. Tools and Materials

The tools used were a set of laboratory glassware, a set of laminar airflow, a set of analytical balances, Tryptic Soy Broth (Merck), DI water, Dragendorff's reagent (Merck), Mayer's reagent (Merck), HCl (Merck), NH_4OH (Merck), ethyl acetate (Merck), pH paper, DPPH (Sigma), Ciprofloxacin, GCMS-QP2010S (Shimadzu), FTIR (PerkinElmer Spectrum IR 10.6.1).

2.2. Production of Secondary Metabolites

Endophytic bacterial isolates were inoculated in liquid media and incubated in an incubator shaker for 3 hours at room temperature as a starter, then re- inoculated with 2 mL in 200 mL of liquid media and incubated in an incubator shaker at 125 rpm for 22 hours at room temperature [10]. The culture obtained was centrifuged for 9 minutes at a speed of 6000 rpm. The supernatant obtained was subjected to liquid-liquid extraction to obtain the alkaloid extract. Subsequently, both pre- and post-isolation alkaloids underwent phytochemical screening tests to confirm the presence of alkaloids.

2.3. Alkaloid Extraction

After centrifugation, 50 mL of the secondary metabolites were added with 2 M HCl until the pH reached 3. This acidic solution was then subjected to liquid-liquid extraction with 50 mL of ethyl acetate until two distinct layers formed: an ethyl acetate layer and a water layer. To the aqueous layer containing alkaloid salts, NH_4OH was added to adjust the pH to 8-9, followed by another extraction with 50 mL of ethyl acetate. The ethyl acetate layer obtained in this final step was evaporated, yielding a concentrated alkaloid extract [11].

2.4. Alkaloid Screening

A volume of 2 mL of supernatant was mixed with 10 drops of 2 N HCl. Subsequently, the resulting solution from both the production stage (2.2) and the extraction stage (2.3) was divided into two different reaction tubes. The first tube was dripped with 3 drops of Mayer's reagent, while the second tube was dripped with 3 drops of Dragendorff's reagent. A positive test was evidenced by the formation of a cloudy white precipitate in the first tube and a brick-red precipitate in the second tube [12].

2.5. Characterization Using GC-MS

The sample was prepared by dissolving it in ethyl acetate, chosen for its ease of solubility and volatility. In GC-MS analysis, a volume of 1 µL of the isolated alkaloid compound extract was injected into the GC-MS system equipped with an Agilent HP-5MS UI column ((5%-Phenyl)-methylpolysiloxane) measuring 30 meters in length. Helium served as the carrier gas, guiding the separation process within the RT 1 MS Restech column connected to an MS detector (Mass Spectrometer). The obtained mass spectrum data was compared with the data in the Wiley and NIST libraries contained in the GC-MS software to ascertain the composition of the alkaloid sample.

Chemical components were identified using GCMS-QP2010S (Shimadzu) with the following operational conditions: a column temperature of 60°C, an injection temperature of 290°C, and employing split injection mode with a pressure of 12.3 kPa and a total flow rate of 21.8 mL/min. The column flow was set at 2.00 mL/min, corresponding to a linear velocity of 51.6 cm/sec. A purge flow rate of 3.0 mL/min and a split ratio of 8.4 were utilized. The ion source temperature was maintained at 300°C, while the interface temperature was set to 250°C.

2.6. Characterization Using FTIR

Five mg of the alkaloid isolate was identified for its functional groups using FTIR, where infrared light was emitted in the direction of the interferogram. The light enters the sample compartment, then passes through the mirror to the detector for final measurement, and is passed to the recorder to be translated into a spectrum.

2.7. Antibacterial Activity Test Using Well Diffusion Method

The antibacterial activity test was conducted by inoculating 20 µL of pathogenic bacterial culture using the spread method over the entire surface of an agar plate. Prior to inoculation, the number of bacterial colonies was standardized to 0.5 McFarland units by mixing 9.95 mL of 1% H_2SO_4 and 0.05 mL of 1% BaCl solution, resulting in a turbidity equivalent to 0.5 McFarland units. The inoculated agar plates were then incubated until the absorbance reached 0.08-0.1 at a wavelength of 600 nm. The agar plate (containing agar nutrient media) was aseptically perforated using a sterile cork borer. Antibacterial agent samples of 40 µL of various concentrations (21, 23, 25, 27, and 30 ppm) were added to the wells. Additionally, 40 µL of Ciprofloxacin 5 ppm was used as a positive control.

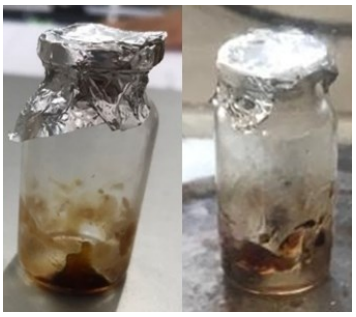


Figure 1. Alkaloid extract

Subsequently, agar plates were incubated for 24 hours at room temperature. During this incubation period, the samples diffused within the agar, inhibiting the growth of the test bacteria. The resulting clear zones were measured using a caliper or ruler [13]. At this stage, Minimum Inhibitory Concentration (MIC) data was acquired for each test bacteria. MIC data was determined by cultivating the test bacteria at varying concentrations of the sample until a point where the sample no longer inhibited the growth of the test bacteria.

2.8. Antioxidant Test

A 1000 ppm stock solution was prepared by dissolving a 25 mg alkaloid sample in 25 mL of ethanol. Sample solutions were made from this stock solution into different concentrations, from 10 to 60 ppm, with intervals of 10 ppm. Subsequently, 1 mL of the metabolite from each concentration variation was added with 3 mL of 0.1 mM DPPH, homogenized, and left for 30 minutes. The absorbance of the solution was measured using UV-Vis at a maximum wavelength of 517 nm. The absorbance value was used to calculate the IC_{50} value using Equation (1).

$$\%\ inhibition = \frac{A_{\ control} - A_{test\ sample}}{A_{\ control}} \times 100\% \qquad (1)$$

The calculation results were inserted into the regression equation with sample concentration on the x- axis and % inhibition value on the y-axis. The IC_{50} value was calculated when the inhibition value was 50% using the equation y = ax + b. Gallic acid served as the positive control in this antioxidant test.

3. Results and Discussion

3.1. Secondary Metabolite Production

The objective of producing secondary metabolites from endophytic bacterial isolates is to acquire compounds anticipated to possess antibacterial activity. Based on the growth curve data of endophytic bacteria obtained in the research [10], the production of secondary metabolites is conducted during the final phase of bacterial growth, specifically at the 22nd hour.

During the production of secondary metabolites, the endophytic bacterial culture undergoes centrifugation to separate the residue from the supernatant. The supernatant, acquired thereafter, contains metabolite compounds derived from endophytic bacteria, whereas the residue constitutes the outcome of the cell lysis process of endophytic bacteria. Subsequently, the obtained supernatant was subjected to phytochemical screening, followed by the isolation of alkaloids.

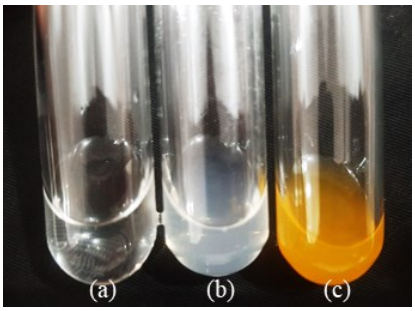


Figure 2. Alkaloid screening results with (a) control, (b) Mayer's reagent, and (c) Dragendorff's reagent

3.2. Alkaloid Isolation

The objective of isolating alkaloid compounds is to extract alkaloids from the secondary metabolites produced by endophytic bacteria. The isolation of alkaloids from the secondary metabolites of endophytic bacteria involves a liquid-liquid extraction method, which relies on the differences in component distribution between two immiscible liquid phases. In this study, the separation was conducted using two solvents that do not mix: water and ethyl acetate. The process of isolating secondary metabolite alkaloids from endophytic bacteria began with a salting-out reaction using HCl until the solution reached a pH of 3, facilitating the formation of alkaloid salts. Subsequently, the solution was extracted using ethyl acetate, resulting in the formation of two layers: a lower water layer and an upper ethyl acetate layer. The alkaloid salts formed dissolve in the water layer, while non-alkaloid compounds such as flavonoids, tannins, lipids, and proteins dissolve in the ethyl acetate layer. The alkaloid salts dissolved in the water layer were subsequently converted back to their free, insoluble form by adding NH_4OH until the pH reached 9.

The alkaline solution was then subjected to extraction using ethyl acetate to isolate the alkaloids in their free form from the salt. The free alkaloids present in the ethyl acetate layer were concentrated using a rotary evaporator to obtain an alkaloid extract. This evaporation method serves to concentrate the solution by evaporating the solvent to separate dissolved substances with boiling points higher than that of the solvent. This evaporation aimed to decrease the solution volume and lower the water activity before proceeding to the next stage [14]. The evaporation process was conducted at a temperature of 77°C to vaporize the ethyl acetate solvent [15]. The concentration of the ethyl acetate layer yielded 3.15 grams of brownish solid alkaloid extract from 25 liters of secondary metabolites (Figure 1). Subsequently, the isolated alkaloid extract was tested for qualitative alkaloid and characterized using FTIR and GCMS.

3.3. Alkaloid Screening

Alkaloid screening aims to verify the presence of qualitative alkaloid compounds in the extract obtained from alkaloid isolation. Alkaloid screening was performed using Mayer and Dragendorff reagents. The obtained results indicated a positive test, marked by the formation of a cloudy white precipitate upon the addition of Mayer's reagent and the formation of a brick red precipitate upon the addition of Dragendorff's reagent.

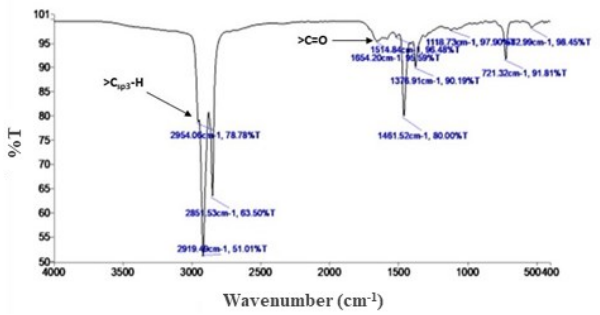


Figure 3. FTIR spectrum of the sample

Based on Figure 2, the positive reaction observed in the Dragendorff reagent test for alkaloids indicates the formation of an orange precipitate at the solution's bottom. This occurrence validates the presence of nitrogen, which forms coordinate covalent bonds with the potassium ion (K^+) in potassium tetraiodobismuthate, a metal ion. Consequently, a potassium-alkaloid complex precipitate is formed, resulting in the appearance of a brick-red or orange-coloured precipitate, indicative of a positive test for alkaloid [16, 17]. In Mayer's reagent, a positive test is signified by the formation of a white precipitate [16]. This is due to the presence of nitrogen atoms in alkaloids, which possess lone pairs of electrons, enabling them to form coordinate covalent bonds with metal ions [18]. It is anticipated that the nitrogen in the alkaloid undergoes a reaction with the K^+ metal ion derived from potassium tetraiodomercurate(II), resulting in the formation of a potassium-alkaloid complex that precipitates out.

The positive identification of alkaloids in the isolated thick extract aligns with findings from Sarjono *et al.* [10], suggesting that endophytic bacteria from *waru* leaves have the capability to produce alkaloids as secondary metabolites. Research by Brader *et al.* [19] further supports this notion, indicating that metabolites are produced through interactions between endophytic microbes and plants, occurring through bilateral synthesis and induction from endophytic microbes. This interaction between endophytes and their host plants can induce the synthesis of various secondary metabolite compounds. Hibiscus leaves, for instance, are known to contain alkaloids, amino acids, carbohydrates, organic acids, fatty acids, saponins, sesquiterpenes, steroids, and triterpenes. Characterization was conducted using FTIR and GCMS to confirm the presence and functional groups of the isolated alkaloids.

3.4. Analysis Using FTR

The functional group analysis test contained in the sample was carried out using FTIR spectrophotometry, and produced the spectrum in Figure 3. The appearance of a spectrum at a wavenumber of 2954 cm^{-1} indicates the presence of saturated hydrocarbon chains (vibration >Csp^3-H) within the sample components. Conversely, the carbonyl group is detected at a wavenumber of 1654 cm^{-1}. NH bending vibrations typically occur within the range of 1640-1514 cm^{-1}; however, NH vibrations are not observable in the IR spectrum. This absence of NH vibrations is attributed to the proposed alkaloid structure lacking an aromatic ring but containing a tertiary amine group and a carbonyl group, which does not exhibit prominent NH group absorption. Additionally, there is no evident absorption for >Csp^2-H vibrations.

The proposed compound represents only one type of alkaloid present in the crude extract, with many unidentified compounds remaining (as per GC-MS data). This discrepancy between FTIR data and the proposed alkaloid content derived from GC-MS analysis arises because FTIR analysis utilizes crude extract samples rather than pure compounds. Considering the proposed structure, the alkaloid includes a tertiary amine group and a carbonyl group, leading to the absence of noticeable NH group absorption.

3.5. Alkaloid Analysis Using GC-MS

GC-MS analysis is employed to separate compounds and offers highly sensitive detection capabilities, along with providing specific structural details derived from alkaloid extracts of endophytic bacteria [20]. Additionally, it can identify bioactive compounds with antibiotic properties [8].

The operational principle of GC-MS involves the separation of components within a mixture through gas chromatography, followed by the creation of a precise mass spectrum for each component. The outcomes of a mass spectrometry examination are referred to as spectra, while the separation results obtained through gas chromatography are represented as chromatograms.

Gas chromatography (GC) is utilized to separate components within a chemical mixture present in a sample. In this process, the components to be separated are transported by a gas through a column. Upon injection, the mixture is partitioned between the carrier gas and the stationary phase. The stationary phase selectively retains components based on their distribution coefficients, resulting in the formation of multiple bands within the carrier gas. These bands of components exit the column alongside the carrier gas flow and are recorded over time by the detector, yielding a chromatogram.

Mass spectrometry, on the other hand, determines molecular weight by identifying the ratio of mass to charge. It functions as a detector to analyze the molecular structure of samples. Figure 4 illustrates the chromatogram obtained from the analysis conducted using GC-MS. Based on the chromatogram depicted in Figure 4 and corroborated by the spectrograms in Figures 5a, b, and c, it is evident that the extract comprises alkaloid compounds. Specifically, the alkaloid compound is observed at retention times of 27.105 minutes (compound 1), 29.135 minutes (compound 2), and 29.570 minutes (compound 3).

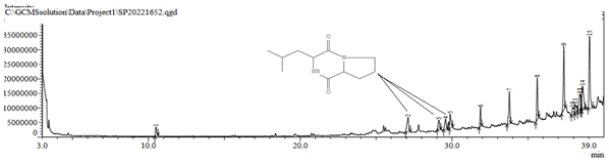
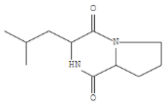
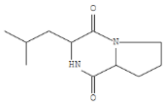
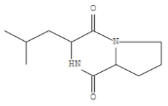


Figure 4. GC-MS chromatogram

Table 1. Analysis results using GCMS

Peak	TR (minute)	Area (%)	Name	Structure
2	27.106	5.73	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	
3	29.136	3.04	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	
4	29.572	3.78	1,4-diaza-2,5-dioxo-3-isobutyl bicyclo[4.3.0]nonane	

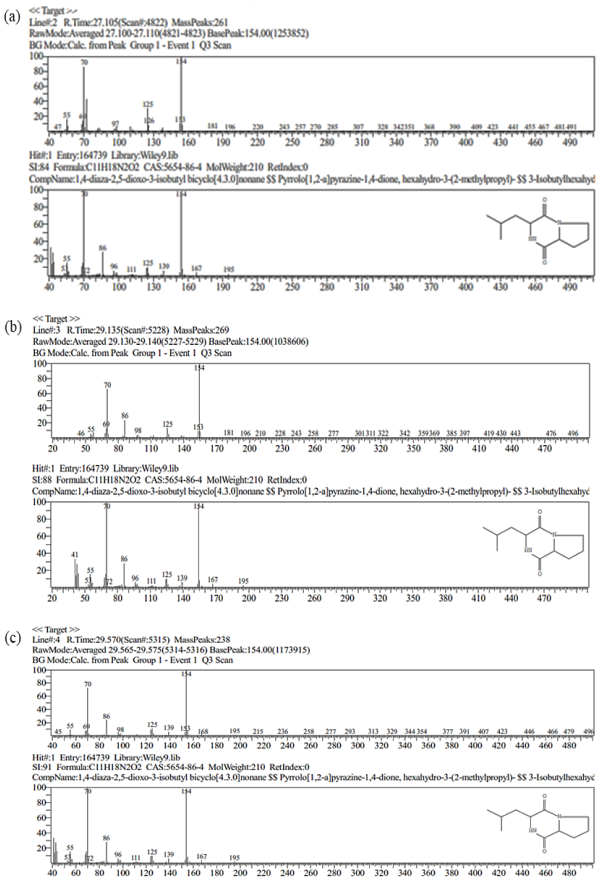


Figure 5. Spectrogram of (a) compound 1 (tR 27.105 min), (b) compound 2 (tR 29.135 min), and (c) compound 3 (tR 29.570 min)

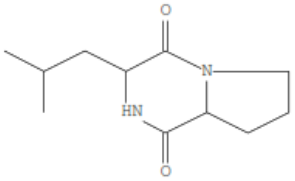


Figure 6. The proposed alkaloid structure

Based on Table 1, the three proposed alkaloid compounds are identified within peak 2, designated as compound 1, with an area of 5.73%. Peak 3, labeled as compound 2, exhibits an area of 3.04%, while peak 4, referred to as compound 3, displays an area of 3.78%. It is important to note that this GC-MS data reflects the analysis of impure compounds, yielding a total alkaloid content of approximately 12.55%.

The proposed structure for all three compounds is depicted in Figure 6. Although these alkaloid compounds share the same structure, they are diastereomers of each other, resulting in their appearance at distinct retention times. Based on the findings from FTIR and GCMS analyses, it is proposed that the alkaloid synthesized by the endophytic bacteria of *waru* leaves constitutes a cyclic amine with the molecular structure illustrated in Figure 6.

3.6. Antibacterial Activity

The antibacterial test was conducted employing the well diffusion method. This method enables the assessment of the inhibitory effect of secondary metabolites, specifically alkaloids, on the growth of pathogenic bacteria, including *S. typhi* and *E. coli* as Gram-negative bacteria and *S. aureus* as Gram-positive bacteria. Ciprofloxacin served as the positive control in this study due to its broad-spectrum antibiotic properties, allowing it to effectively inhibit the growth of both Gram-positive and Gram-negative bacteria. The alkaloids obtained were dissolved into various concentrations, specifically 21, 23, 25, 27, and 30 ppm. The absorbance of the test bacteria was measured at 5×10^8 CFU/mL, which is equivalent to the 0.5 McFarland standard. This step was undertaken to standardize the bacterial count. The results of the antibacterial test are depicted in Figures 7 and 8.

Figure 7 illustrates one of the final results of the antibacterial test, while Figure 8 presents the comprehensive outcome of alkaloid inhibition zones against the test bacteria. Notably, the zone of inhibition observed in Gram-negative bacteria is larger compared to Gram-positive bacteria, as depicted in both figures. This discrepancy arises from variances in the cell wall structure between the two bacterial types. Gram-negative bacteria possess cell walls composed of lipopolysaccharides, which are endotoxins and exhibit greater pathogenicity compared to Gram-positive bacteria [21]. Alkaloids exert antibacterial activity by disrupting the peptidoglycan layer within bacterial cell walls. This interference impedes the formation of cell wall layers, ultimately leading to bacterial cell death [22].

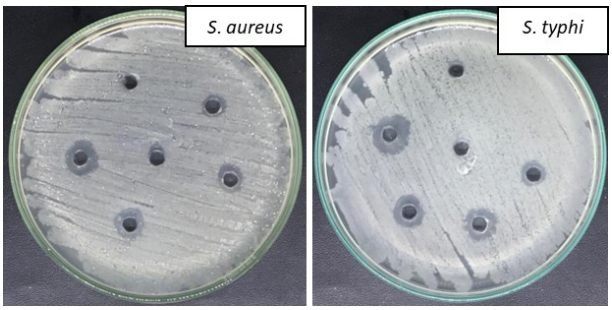


Figure 7. Appearance of antibacterial activity results against *S. aureus* (Gram-positive bacteria) and *S. typhi* (Gram-negative bacteria)

The peptidoglycan layer of Gram-negative bacteria is thinner compared to that of Gram-positive bacteria. Gram-negative bacterial cell walls contain relatively more lipids, resulting in a thinner peptidoglycan structure compared to Gram-positive bacteria such as *S. aureus*. Gram-positive bacteria have cell walls with thick and robust peptidoglycan layers but fewer lipids. Consequently, alkaloids find it easier to damage the cell walls of Gram-negative bacteria like *S. typhi* and *E. coli* compared to *S. aureus* due to the structural differences in their cell walls [23, 24]. This is proven by the existence of a larger zone of inhibition in *S. typhi* and *E. coli* bacteria than the zone of inhibition in *S. aureus* bacteria.

The outer structure of the peptidoglycan in Gram-positive bacteria comprises layers of lipoproteins, phospholipids, and polymers [25]. Chitemerere and Mukanganyama [26] argue that the greater inhibition observed in Gram-negative bacteria stems from their cell walls, which consist of only 10% peptidoglycan and 11- 22% lipids, whereas Gram-positive bacteria possess a thicker cell wall structure composed of over 50% peptidoglycan, polysaccharides (such as teichoic acid), and 1-4% lipids. Interestingly, the MIC obtained for all bacteria was consistent at a concentration of 23 ppm.

From the results, it is proposed that cyclic amine alkaloid compounds exhibit antibacterial properties. Alkaloids, being basic and semi-polar compounds, feature a fundamental structure containing a nitrogen atom capable of interacting with amino acids in bacterial cell walls and DNA [27]. The inhibition mechanism between cyclic amines and bacterial cells, resulting in the formation of an inhibitory zone, involves the amine group (-NH) in cyclic amines forming hydrogen bonds with the oxygen atom (-O) present in the cell walls of Gram-positive bacteria (peptidoglycan) and Gram-negative bacteria (lipopolysaccharide). This interaction leads to incomplete formation of the cell wall layer due to structural changes. Additionally, hydrogen bonds form with nucleotides, the building blocks of bacterial DNA, thereby impeding the synthesis of amino acid compounds through transcription and translation processes and altering the composition of amino acids in the DNA chain [28, 29]. These actions culminate in bacterial lysis and subsequent cell death.

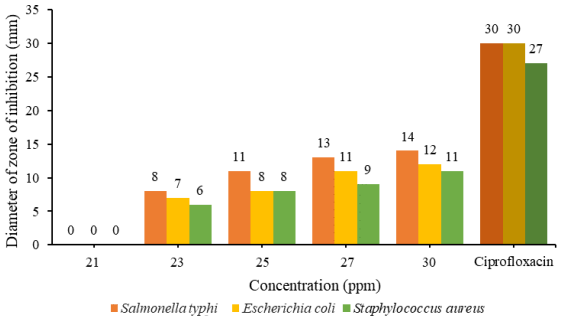


Figure 8. Data on the antibacterial activity of alkaloid extracts

This reaction alters the structure and arrangement of amino acids and disrupts the genetic integrity of DNA strands, ultimately leading to damage to the cell wall and bacterial DNA, resulting in cell lysis and bacterial death. Alkaloids thus function as antibacterial agents by interfering with the components constituting the peptidoglycan in bacterial cells, preventing the complete formation of the cell wall layer and ultimately causing bacterial cell death [30].

3.7. Antioxidant Activity

Antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. DPPH functions as a free radical and an oxidizing agent, reacting with antioxidant compounds present in the sample [31]. When antioxidant activity is present, DPPH undergoes decolorization or a reduction in color intensity, transitioning from purple to yellow when measured at a wavelength of 517 nm. This method relies on the interaction between free electrons in free radicals and hydrogen atoms from antioxidants. Consequently, the reduction of free radicals by antioxidants leads to a shift from radical compounds to non-radical compounds, evident by the color change of the solution from purple to yellow [32, 33].

As depicted in Figure 9, a higher absorbance corresponds to a greater percentage of inhibition. This relationship arises from the significant suppression of DPPH by antioxidant compounds present in the sample. The extent of antioxidant activity exhibited by secondary metabolites can be quantified through the determination of the IC_{50} value. An IC_{50} value of less than 50 ppm classifies a compound as a very strong antioxidant, while values ranging from 50 to 100 ppm indicate strong antioxidant activity. Compounds with IC_{50} values between 100 and 150 ppm are categorized as having medium antioxidant activity, whereas values between 151 and 200 ppm signify weak antioxidant activity.

The IC_{50} value of the secondary metabolites obtained was found to be 50.11 ppm, indicating that the compounds derived from *waru* leaves exhibit exceptionally strong antioxidant activity. This potency can be attributed to the presence of alkaloids, the sole compounds detected in the sample, known for their antioxidative properties, allowing for optimal reduction of DPPH. The efficacy of alkaloid compounds in reducing DPPH is elucidated by their cyclic amine structure, as depicted in Figure 9.

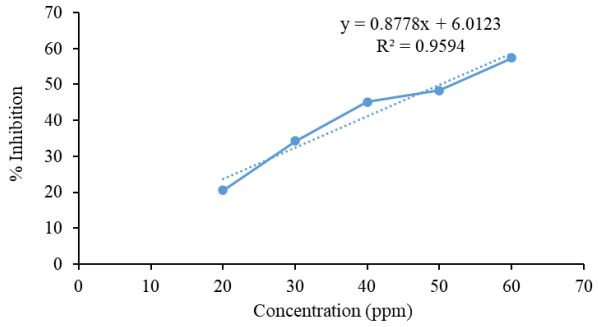


Figure 9. Antioxidant activity curve of alkaloid extract

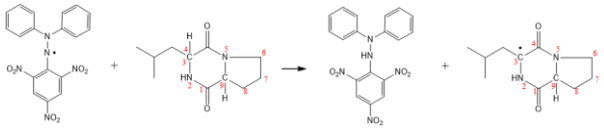


Figure 10. Reaction between DPPH and cyclic amine

The presence of H-alpha at C number 3 and C number 9 in cyclic amines confers acidic properties, attributed to the positive charge on the carbon atom adjacent to the carbonyl group. This charge distribution weakens the bond between the carbon atom and the alpha hydrogen, rendering the compound acidic [34]. Consequently, the release of H-alpha facilitates the reduction of DPPH free radicals, converting them into non-radical compounds. This property defines the antioxidant capacity of a compound.

In the current study, gallic acid served as a positive control due to its established role as a natural antioxidant. Its structure features multiple phenolic groups capable of effectively reducing DPPH free radicals. Gallic acid demonstrated an IC_{50} value of 27 ppm in this study. In prior research, secondary metabolites from endophytic bacteria *Staphylococcus warneri* strain AW 25 exhibited an IC_{50} of 400.34 ppm. These findings suggest that the alkaloid extract exhibits superior antioxidant activity compared to the crude extract. Consequently, the extraction process employed in this study effectively enhances antioxidant activity.

4. Conclusion

Based on the findings from FTIR and GCMS analyses, alkaloids were successfully isolated from endophytic bacteria sourced from hibiscus leaves. These alkaloids are identified as cyclic amines, constituting approximately 12.55% of the extract. Notably, the cyclic amine compound exhibits potent antioxidative properties, as demonstrated by its ability to inhibit DPPH radicals with an IC_{50} value of 50.11 ppm, categorizing it as a strong antioxidant.

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