A Study on the Anatomy and Antibacterial Activity of Boesenbergia rotunda Rhizomes Against Staphylococcus aureus

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Boesenbergia rotunda is a plant belonging to the Zingiberaceae family. This plant is known to contain various bioactive compounds with potential as natural antibacterial agents. This study aims to determine the microscopic anatomical characteristics, secondary metabolite content, and antibacterial activity of Boesenbergia rotunda rhizome extract against Staphylococcus aureus. The research methods used included microscopic observation, phytochemical screening, and antibacterial activity testing using the disk diffusion method. Anatomical observations revealed that the Boesenbergia rotunda rhizome consists of parenchyma tissue with starch granules scattered throughout the ground tissue. Phytochemical screening results indicated the presence of flavonoids, alkaloids, terpenoids, saponins, and tannins. Antibacterial activity testing showed that the Boesenbergia rotunda rhizome extract was able to inhibit the growth of Staphylococcus aureus, producing an inhibition zone with a diameter of ±17 mm. This inhibition zone diameter falls into the category of strong inhibition. This strong antibacterial activity is believed to result from the synergistic action of secondary metabolites that work through mechanisms of cell membrane damage, disruption of membrane permeability, and inhibition of bacterial cell wall synthesis. Based on the research results, the rhizome of Boesenbergia rotunda has the potential to be developed as a natural antibacterial source derived from herbal materials.
Keywords: Boesenbergia rotunda, antibacterial, phytochemistry, microscopic anatomy, Staphylococcus aureus

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1. Introduction

Infectious diseases remain a global health concern due to their high incidence rates and the growing challenges in controlling infections caused by antimicrobial resistance. Most infectious diseases are caused by pathogenic bacteria, one of which is *Staphylococcus aureus* (Gherardi, 2023). *Staphylococcus aureus* is a Gram-positive bacterium that can cause various infections in humans, ranging from skin and soft tissue infections, abscesses, and pneumonia to severe conditions such as sepsis (Touaitia et al., 2025).

The rise in antibiotic resistance among *S. aureus* has become a serious problem in the treatment of infections. According to a report by the World Health Organization (WHO), antimicrobial resistance directly causes approximately 1.27 million deaths annually and contributes to nearly 5 million deaths globally due to infections that are difficult to treat (WHO, 2023). One group of bacteria of particular concern is methicillin-resistant *Staphylococcus aureus* (MRSA), which exhibits resistance to various β-lactam antibiotics, thereby limiting treatment options (Murray et al., 2022). Irrational antibiotic use whether through off-label use or inappropriate antibiotic selection is one of the main factors accelerating the development of bacterial resistance (Yuan et al., 2025). These conditions have spurred research into alternative antibacterial sources derived from natural materials that are potentially safer and more sustainable.

One medicinal plant with potential as a source of antibacterial compounds is *Boesenbergia rotunda* (temu kunci), a member of the Zingiberaceae family. The rhizome of this plant has been empirically used in traditional medicine to treat digestive disorders, inflammation, and mild infections (Wang et al., 2025). Several studies have reported that *B. rotunda* rhizomes contain various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, and terpenoids, which are known to possess biological activity, including antimicrobial potential (Silalahi et al., 2017).

Previous research on *B. rotunda* has largely focused on identifying secondary metabolites or testing the antibacterial activity of extracts in isolation. Phytochemical studies provide information on the presence of bioactive compounds in *B. rotunda* rhizomes, but have not yet provided insight into the relationship between the morphological and anatomical characteristics of the crude drug as parameters for raw material authentication. Conversely, studies on antibacterial activity generally only evaluate the extract's ability to inhibit bacterial growth without being accompanied by the characterization of the crude drug, which is essential for the standardization of natural products.

In pharmacognosy, the anatomical identification of the crude drug through microscopic observation plays a crucial role in ensuring the authenticity, identity, and quality of medicinal plant raw materials (Archana et al., 2023). Therefore, an integrative approach combining anatomical characterization, phytochemical screening, and antibacterial activity evaluation is necessary to generate more comprehensive information regarding the potential of B. rotunda rhizomes as candidates for natural-based medicinal raw materials.

The novelty of this study lies in its integrative approach, which combines an anatomical study of the rhizome, a phytochemical profile, and an in vitro evaluation of the antibacterial activity of *Boesenbergia rotunda* rhizome extract against *Staphylococcus aureus* within a single study. This approach is expected not only to provide evidence of the plant's bioactivity potential but also to provide information on the characteristics of the crude drug that supports the authentication and standardization of natural materials in the development of pharmaceutical science.

2. Literature Review and Problem Statement

The development of natural-product-based antibacterial agents continues to be a focus of research in response to the rising incidence of bacterial resistance to conventional antibiotics. One pathogenic bacterium of particular concern is *Staphylococcus aureus*, which is known to cause a wide range of infections, from skin infections to more serious systemic infections. The rise in antibiotic resistance in this bacterium has spurred the exploration of various medicinal plants with potential as sources of new antibacterial compounds (Ahmed et al., 2024; Yuan et al., 2025). Plants from the Zingiberaceae family are known to be rich in secondary metabolites such as flavonoids, alkaloids, terpenoids, and phenolic compounds that contribute to antimicrobial activity. One member of this family that is widely used in traditional medicine is *Boesenbergia rotunda* (temu kunci), which has been reported to possess various biological activities, including antibacterial, anti-inflammatory, antioxidant, and antifungal properties (Sanguansermsri & Sanguansermsri, 2024).

Several previous studies have shown that the rhizome extract of *Boesenbergia rotunda* contains major bioactive compounds such as pinostrobin, panduratin A, flavonoids, and essential oils, which contribute to the plant's antimicrobial activity (Chahyadi et al., 2014; Eng-Chong et al., 2012). In addition to the identification of chemical constituents, the anatomical characterization of crude drug materials is also a crucial aspect of pharmacognosy research, as it can serve as a parameter for the authentication and standardization of herbal medicinal raw materials. The anatomical structure of rhizomes is known to be closely related to the sites of biosynthesis and storage of secondary metabolites that play a role in the

plant's pharmacological activity (Fahn, 1991; Noviana et al., 2022). Therefore, studies linking a plant's anatomical characteristics to its phytochemical composition and biological activity are essential for supporting the development of evidence-based medicinal plants.

Although various studies have reported the antibacterial activity of *Boesenbergia rotunda*, most of them have focused on identifying bioactive compounds or testing biological activities separately. Studies that integrate the microscopic anatomical characterization of the rhizome, phytochemical profiles, and antibacterial activity against *Staphylococcus aureus* remain relatively limited. This lack of information served as the basis for this study. Therefore, this study aims to examine the microscopic anatomical characteristics of the rhizome, identify the content of secondary metabolites, and evaluate the in vitro antibacterial activity of *Boesenbergia rotunda* rhizome extract against *Staphylococcus aureus*. The results of this study are expected to provide scientific information supporting the use of *Boesenbergia rotunda* as a source of natural antibacterial agents while also strengthening the standardization of medicinal plant crude drugs.

3. Method

This study was an experimental study that involved microscopic observation of the rhizome's anatomy, analysis of secondary metabolite content, and evaluation of the antibacterial efficacy of *Boesenbergia rotunda* rhizome extract against *Staphylococcus aureus*. The stages of this study included the preparation of crude drug, microscopic anatomical characterization of the rhizome, sample extraction, phytochemical screening, and testing of antibacterial activity using the in vitro disc diffusion method.

Equipment and Materials

The equipment used in this study included an analytical balance, a blender, a drying oven, a light microscope, an autoclave, an incubator, a laminar air flow hood, micropipettes, Petri dishes, test tubes, measuring cylinders, Erlenmeyer flasks, sterile forceps, and a vernier caliper for measuring the diameter of the inhibition zone.

The materials used included *Boesenbergia rotunda* rhizomes, 96% ethanol (Merck, analytical grade) as an extraction solvent, sterile distilled water, Nutrient Agar (NA) medium, *Staphylococcus aureus* bacterial culture, and sterile disk paper. Reagents used in the phytochemical tests included Mayer's and Wagner's reagents for alkaloids, $FeCl_3$ solution for tannins, magnesium solution and concentrated HCl for flavonoids, as well as other reagents used to identify saponins and terpenoids.

Instruments

The instruments used in this study included a light microscope for microscopic observation of the rhizome's anatomical structure, a bacterial incubator with an operating temperature of 37°C for bacterial incubation, and an autoclave with a sterilization temperature of 121°C at a pressure of 15 psi for sterilizing equipment and media. The diameter of the inhibition zone was measured using a vernier caliper with a precision of 0.01 mm. The observational data were then analyzed descriptively to determine the potential antibacterial activity of the Boesenbergia *rotunda* rhizome extract.

Procedure

a. Preparation of the Crude Drug
   Boesenbergia rotunda rhizomes were washed under running water, cut into pieces, and dried in an oven at ±40°C until a dry crude drug was obtained. The dried crude drug was then ground using a blender to produce a crude drug powder (Haerani et al).
b. Sample Extraction

The *Boesenbergia rotunda* rhizome crude drug powder was extracted using the maceration method with 96% ethanol as the solvent. The crude drug powder was soaked in the solvent for 3 × 24 hours with occasional stirring. The filtrate from the maceration process was then filtered and concentrated using an evaporator until a concentrated extract was obtained (Haerani et al).

c. Anatomical Examination of the Rhizome
The anatomical examination was conducted by making thin transverse sections of the rhizome using a sharp knife. The sections were then placed on a microscope slide, a drop of distilled water was added, and the slide was covered with a cover slip. The preparations were observed under a light microscope at 10x magnification to identify the characteristic tissue structures of the rhizome.

d. Phytochemical Screening
The *Boesenbergia rotunda* rhizome extract was then tested for secondary metabolites through phytochemical screening, which included a flavonoid test using the Shinoda method (Satria et al., 2022), a tannin test using $FeCl_3$ reagent (Harahap et al., 2024), alkaloids using Mayer's, Dragendorff's, and Wagner's reagents (Parbuntari et al., 2018), saponins, and terpenoids. Each test was conducted using specific reagents that produced a color change or the formation of a precipitate as an indicator of the presence of these secondary metabolites.

e. Antibacterial Activity Test
The antibacterial activity test was performed using the disk diffusion method. Prepared Nutrient Agar medium was poured into sterile Petri dishes, which were then inoculated with *Staphylococcus aureus* bacteria via smear. Sterile filter paper disks impregnated with rhizome extract at a specific concentration were then placed on the surface of the medium. The dishes were then incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones formed around the discs was measured using a vernier caliper. The value

4. Results and Discussion

Microscopic Anatomy of the *Boesenbergia rotunda* Rhizome

Microscopic observations of the anatomical structure of the *Boesenbergia rotunda* rhizome at 10x magnification reveal the presence of storage parenchyma tissue with starch granules scattered throughout the ground tissue. Microscopic observations also revealed secretory structures believed to be associated with the storage of secondary metabolites, such as essential oils and phenolic compounds. These microscopic findings support the pharmacological potential of the *Boesenbergia rotunda* rhizome as a medicinal plant.

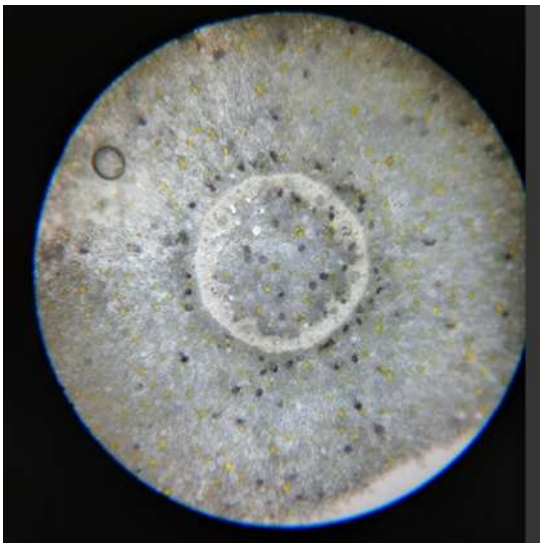

Figure 1. Microscopic anatomical structure of the *Boesenbergia rotunda* rhizome (10×)

Based on Figure 1, it can be seen that the parenchyma tissue in the rhizome of *Boesenbergia rotunda* dominates the basic tissue structure of the rhizome. The parenchyma cells are polygonal in shape and arranged relatively densely, with scattered starch granules. Parenchyma tissue functions to store nutrients and secondary metabolites such as alkaloids, flavonoids, terpenoids, and other phenolic compounds. According to Andini et al. (2020), this genus is a major genus within the Zingiberaceae family that is widely used medicinally due to its aromatic compounds. Previous research has shown that plants in the Zingiberaceae family are known to possess secretory cells and essential oil-storing tissues that contribute to the plants' pharmacological activities, including antibacterial activity (Liu et al., 2019). These anatomical characteristics support the results of phytochemical screening, which revealed the presence of various active secondary metabolites. Thus, the anatomical structure of the rhizome is closely related to the plant's biological activity, particularly as a natural antibacterial agent.

Phytochemical Screening

The results of phytochemical screening indicate that the rhizome extract of *Boesenbergia rotunda* tested positive for flavonoids, alkaloids, terpenoids, saponins, and tannins using the $FeCl_3$ reagent. The presence of these secondary metabolites is believed to play a key role in the antibacterial activity against *Staphylococcus aureus*.

Table 1. Results of the Phytochemical Screening of *Boesenbergia rotunda* Rhizome Extract

No.	Compound Group	Reagent/Test	Positive Indicator	Observation Result	Interpretation
1.	Flavonoids	Shinoda Test	Formation of red/orange color	The solution changed to red color	Positive
2.	Tannins	1% $FeCl_3$ Test	Formation of greenish-black/dark blue color	The solution showed a greenish-black color	Positive
3.	Alkaloids	Wagner's Reagent Test	Formation of brown precipitate	The solution showed a light brown to blackish color	Positive
4.	Alkaloids	Mayer's Reagent Test	Formation of yellowish-white precipitate	Formation of light brown precipitate	Positive

No.	Compound Group	Reagent/Test	Positive Indicator	Observation Result	Interpretation
5.	Alkaloids	Dragendorff's Reagent Test	Formation of orange-brown precipitate	The solution showed a blackish-brown color	Positive
6.	Terpenoids	Liebermann–Burchard Test	Formation of red/green color	The solution changed to red color	Positive
7.	Saponins	Foam Test	Formation of stable foam	Stable foam was formed	Positive

(+) = Contains secondary metabolites
(–) = Does not contain secondary metabolites

Based on the results of the phytochemical screening in Table 1, the *Boesenbergia rotunda* rhizome extract was found to contain several important classes of secondary metabolites, namely flavonoids, alkaloids, terpenoids, and saponins. A positive result for flavonoids was indicated by a color change in the solution to red after the addition of Shinoda's reagent. Alkaloid tests using the Wagner, Mayer, and Dragendorff reagents showed color changes and the formation of precipitates, indicating the presence of alkaloids in the extract.

The presence of terpenoids was indicated by the formation of a red color in the Liebermann–Burchard test, while saponins yielded a positive result through the formation of a stable foam after shaking. In the tannin test, no specific color change was observed, so the result was negative. These phytochemical screening results indicate that the rhizomes of *Boesenbergia rotunda* contain secondary metabolites with potential as antibacterial bioactive compounds. The combination of flavonoids, alkaloids, terpenoids, and saponins is thought to contribute to the growth-inhibitory activity against *Staphylococcus aureus* demonstrated in the antibacterial test (Cowan, 1999; Cushnie & Lamb, 2011).

Flavonoids

A positive result for flavonoids is indicated by the appearance of a red color during the test. Flavonoids are known to exhibit antibacterial activity through several mechanisms, including inhibiting nucleic acid synthesis, damaging the cytoplasmic membrane, and inhibiting bacterial energy metabolism (Cushnie & Lamb, 2011). The phenolic groups in flavonoids can interact with cell membrane proteins, causing protein denaturation and increased permeability of the bacterial cell membrane (Harborne, 1998). Additionally, flavonoids in plants of the Zingiberaceae family are known to possess high antioxidant activity, which can help inhibit oxidative stress in cells and enhance the pharmacological potential of the plants (Panche et al., 2016).

Alkaloids

Alkaloid tests using the Wagner, Mayer, and Dragendorff reagents yielded positive results, indicated by the formation of brown to dark brown precipitates. Positive results with all three reagents indicate the presence of a significant amount of alkaloids in the rhizome extract. Alkaloids exhibit antibacterial activity by inhibiting the formation of peptidoglycan in bacterial cell walls, thereby causing bacterial cells to undergo lysis (Dewick, 2009). Alkaloid compounds are also known to interfere with bacterial DNA replication and protein synthesis (Cowan, 1999). These activities inhibit bacterial growth and can even lead to cell death.

Terpenoids

A positive result for terpenoids is indicated by a color change to red. Terpenoids are one of the main components of essential oils in the Zingiberaceae family. These compounds are lipophilic, enabling them to

interact with bacterial lipid membranes and increase cell membrane permeability (Guimarães et al., 2019). Damage to the cell membrane causes the leakage of intracellular components such as proteins and ions, thereby disrupting bacterial metabolism. The activity of terpenoids against Gram-positive bacteria is generally higher because their cell wall structure is simpler than that of Gram-negative bacteria (Burt, 2004).

Saponins

The saponin test yields a positive result with the formation of stable foam. Saponins are known to possess natural surfactant properties that can reduce the surface tension of bacterial cell membranes (Sparg et al., 2004). The antibacterial mechanism of saponins involves the formation of complexes with membrane sterols, leading to damage to the integrity of the cell membrane. This damage results in the leakage of essential components from inside the bacterial cell and ultimately leads to cell death (Morrissey & Osbourn, 1999).

Tannins

Negative results in the tannin test indicate that the *Boesenbergia rotunda* rhizome extract does not contain detectable levels of tannins. However, the absence of tannins does not diminish the extract's antibacterial potential, as antibacterial activity can still be generated by a combination of flavonoids, alkaloids, terpenoids, and saponins.

Phytochemical screening results indicate that the rhizome extract of *Boesenbergia rotunda* contains flavonoids, alkaloids, terpenoids, and saponins. The presence of these secondary metabolites is thought to contribute to the extract's antibacterial activity against *Staphylococcus aureus*. Flavonoids are phenolic compounds known to exhibit antibacterial activity through mechanisms such as inhibition of nucleic acid synthesis, disruption of cytoplasmic membrane function, and inhibition of bacterial cellular energy metabolism (Cushnie & Lamb, 2011). The hydroxyl groups on flavonoids can form complexes with extracellular proteins and membrane proteins, thereby damaging the integrity of bacterial cell membranes (Harborne, 1998). Additionally, flavonoids possess antioxidant activity that can enhance the biological stability of plant extracts (Panche et al., 2016).

Positive results for alkaloids in the Wagner, Mayer, and Dragendorff tests indicate the presence of a significant amount of alkaloids in the rhizome extract. Alkaloids are known to act by disrupting peptidoglycan formation in bacterial cell walls, thereby causing cell lysis (Dewick, 2009). Some alkaloid compounds have also been reported to inhibit bacterial DNA replication and protein synthesis (Cowan, 1999).

The presence of terpenoids in the extract was indicated by the formation of a red color during testing. Terpenoids are the main components of essential oils; they are hydrophobic and thus able to interact with the lipid layer of the bacterial membrane. This interaction causes an increase in membrane permeability and the leakage of intracellular components, ultimately leading to bacterial cell death (Guimarães et al., 2019).

Saponins, characterized by the formation of stable foam, are known to act as natural surfactants. The antibacterial activity of saponins occurs through the formation of complexes with membrane sterols, leading to damage to the cytoplasmic membrane and the release of essential components from inside the bacterial cell (Morrissey & Osbourn, 1999).

Meanwhile, the negative results from the tannin test indicate that tannins were not detected in the extract used. The absence of detectable tannins may be influenced by the type of solvent, the extraction method, or the concentration of the compounds in the sample. Nevertheless, antibacterial activity can still be produced through the synergistic effects of other secondary metabolites.

Discussion of Antibacterial Activity

The results of the antibacterial testing showed that the *Boesenbergia rotunda* rhizome extract produced an inhibition zone diameter of 17 mm against Staphylococcus aureus. Based on the classification of antibacterial inhibitory activity, an inhibition zone diameter of 10–20 mm is categorized as strong.

Table 2. Results of the Antibacterial Activity Test of Boesenbergia rotunda Rhizome Extract

Bacterial Test Organism	Diameter of Inhibition	Category
Staphylococcus aureus	17,00 ± 0,00 mm	Strong

Test results for the antibacterial activity of *Boesenbergia rotunda* rhizome extract against *Staphylococcus aureus* showed an average inhibition zone diameter of 17.00 ± 0.00 mm, which falls into the "strong" category according to the Davis and Stout classification. Inhibition zone diameters in the 10–20 mm range indicate that the extract has a good ability to inhibit the growth of the test bacteria (Davis & Stout, 1971). These results suggest that the bioactive compounds in the extract are able to diffuse effectively in agar medium and exert an inhibitory effect on bacterial growth.

From a descriptive statistical perspective, the strong inhibition zone diameters suggest that the secondary metabolites in the extract exhibit effective antibacterial activity against Gram-positive bacteria. This activity is believed to result from the synergistic action of flavonoids, alkaloids, terpenoids, and saponins, which were previously detected in phytochemical screening results. This combination of secondary metabolites is known to damage cell wall structure, increase membrane permeability, and inhibit bacterial cell metabolism (Cowan, 1999; Cushnie & Lamb, 2011).

If the test is conducted in multiple replicates, the inhibition zone results should be presented as the mean ± standard deviation to indicate the level of homogeneity of the test results. A small standard deviation indicates that the antibacterial activity of the extract exhibits a good level of consistency. According to Walpole (2015), presenting data as a mean and standard deviation is important for statistically describing the distribution of measurement results.

The antibacterial activity of *Boesenbergia rotunda* rhizome extract is thought to stem from the synergistic effects of the various secondary metabolites it contains. Flavonoids work by inhibiting nucleic acid synthesis, damaging the cytoplasmic membrane, and inhibiting bacterial energy metabolism (Cushnie & Lamb, 2011). Alkaloids are known to disrupt peptidoglycan formation in the bacterial cell wall, thereby causing cell lysis (Dewick, 2009). Meanwhile, lipophilic terpenoids can interact with the lipid layer of the cell membrane and increase bacterial membrane permeability (Guimarães et al., 2019). Saponins act as natural surfactants that can disrupt the integrity of bacterial cytoplasmic membranes by forming complexes with membrane sterols (Morrissey & Osbourn, 1999).

Staphylococcus aureus is a Gram-positive bacterium that has a thick peptidoglycan layer but lacks an outer membrane, unlike Gram-negative bacteria. This structure allows antibacterial compounds from plants to penetrate the bacterial cell wall more easily, resulting in more optimal inhibitory activity (Madigan et al., 2018). Therefore, Gram-positive bacteria are generally more sensitive to natural-based antibacterial compounds than Gram-negative bacteria.

The results of this study are consistent with several previous studies reporting that plants from the Zingiberaceae family exhibit strong antibacterial activity against *Staphylococcus aureus*. The flavonoids, essential oils, and other phenolic compounds in plants of the Zingiberaceae family are known to play a key role in their antibacterial activity against pathogenic bacteria (Burt, 2004; Rahman et al., 2014).

This strong antibacterial activity indicates that the rhizomes of *Boesenbergia rotunda* have the potential for further development as a source of herbal antibacterial agents. However, antibacterial activity can also be

influenced by several factors, such as extract concentration, solvent type, extraction method, and the sensitivity of the test bacteria (Harborne, 1998). Therefore, further research such as determining the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and identifying specific active compounds is needed to support the development of this plant as a natural antibacterial candidate.

The Relationship Between Anatomy, Phytochemical Composition, and Antibacterial Activity

The results of this study indicate a close relationship between the microscopic anatomical characteristics of the rhizome, the content of secondary metabolites, and the antibacterial activity of Boesenbergia rotunda rhizome extract against Staphylococcus aureus. The anatomical structure of the rhizome is known to play a role in the biosynthesis and storage of secondary metabolites that contribute to the plant's pharmacological activity.

Anatomical observations indicate that the rhizome is dominated by parenchyma tissue with starch granules scattered throughout the ground tissue. According to Fahn (1991), the parenchyma tissue in rhizomes functions as a food reserve and a site for the accumulation of secondary metabolites. In plants of the Zingiberaceae family, this tissue is generally associated with the presence of secretory cells and essential oil spaces that play a role in the synthesis of bioactive compounds.

The presence of a significant number of starch granules indicates high metabolic activity in the rhizome tissue. This metabolic activity supports the formation of secondary metabolites, which plants require as a natural defense mechanism against attacks by microorganisms (Taiz & Zeiger, 2010). Thus, the anatomical characteristics of the rhizome are not only related to its physiological function as a food reserve organ but are also linked to the plant's ability to produce bioactive compounds.

This relationship is reinforced by the results of phytochemical screening, which revealed the presence of flavonoids, alkaloids, terpenoids, and saponins in the rhizome extract. These compounds are known to possess distinct yet complementary antibacterial mechanisms. The synergy among these secondary metabolites is believed to be the primary factor behind the extract's antibacterial activity against *Staphylococcus aureus*, as evidenced by an inhibition zone diameter of 17 mm, which falls into the "strong" category.

In addition to being influenced by the content of secondary metabolites, the sensitivity of *Staphylococcus aureus* to the extract is also related to the structural characteristics of the cell wall of Gram-positive bacteria. The absence of an outer membrane allows active compounds to diffuse more easily and damage the bacterial cell structure compared to Gram-negative bacteria (Madigan et al., 2018).

Thus, this study demonstrates that the anatomical structure of the *Boesenbergia rotunda* rhizome supports the accumulation of secondary metabolites that contribute to its antibacterial activity. The relationship between anatomy, phytochemical composition, and antibacterial bioactivity reinforces the potential of this plant as a source of natural, herb-based antibacterial agents that can be further developed in the fields of pharmacy and health.

5. Conclusion

Based on the research results, it can be concluded that the rhizome of *Boesenbergia rotunda* has microscopic anatomical characteristics dominated by parenchyma tissue with a distribution of starch granules in the ground tissue, which serves as a storage site for food reserves and secondary metabolites. Phytochemical screening results indicate that *Boesenbergia rotunda* rhizome extract contains flavonoids, alkaloids, terpenoids, and saponins that have potential as antibacterial compounds.

Antibacterial activity testing against *Staphylococcus aureus* showed that the *Boesenbergia rotunda* rhizome extract was able to inhibit bacterial growth, with an inhibition zone diameter of 17.00 ± 0.00 mm, which falls into the "strong" category. This antibacterial activity is believed to result from the synergistic action of various secondary metabolites that work through mechanisms such as cell membrane damage, increased membrane permeability, disruption of cell wall synthesis, and inhibition of bacterial metabolism.

The research results indicate a correlation between the anatomical characteristics of the rhizome, the content of secondary metabolites, and the resulting antibacterial activity. Thus, the rhizome of *Boesenbergia rotunda* has the potential to be developed as a natural, herbal-based source of antibacterial agents. Further research is needed to determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and to identify the active compounds that play the most significant role in this antibacterial activity.

6. References

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