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Research Article

THE INHIBITION TEST OF SECONDARY METABOLITES COMPOUNDS FROM ENDOPHYTE BACTERIA IN BANANA PEEL AGAINST PATHOGENIC BACTERIA AND FUNGI

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ABSTRACT

Background: The abundant banana production in Southeast Sulawesi causes environmental problems due to the large amount of banana peel waste. Banana peels contain nutrients that are food for microbes, especially endophytic bacteria. Endophytic bacteria from banana peels can produce secondary metabolite compounds that are used as agents to inhibit the growth of pathogenic bacteria and fungi. The purpose of this study was to determine the inhibitory power of secondary metabolite compounds from bacterial isolate code KPM2 (Kulit Pisang Mas 2) on bacteria and fungi.

Methods: The inhibition zones of secondary metabolite compounds from bacterial isolate code KPM2 against Propionibacterium acne and Staphylococcus aureus bacteria were 14 mm and 12 mm, respectively, while the inhibition zones of Erythromycin and Cefoxitin were 28 mm and 17.5 mm, respectively.

Results: The inhibition zone test of secondary metabolite compounds from KPM2 bacterial isolates against Aspergillus niger and Candida albicans fungi were obtained at 19.5 mm and 27 mm respectively, while the inhibition zone of Ketoconazole antifungal was 21.5 mm and 45 mm respectively.

Conclusion: This study was concluded that the secondary metabolite compounds produced by KPM2 bacterial isolates were able to inhibit the growth of Propionibacterium acne and Staphylococcus aureus bacteria as well as on pathogenic fungi Aspergillus Niger and Candida albicans.

Keywords: *Endophytic bacteria, Metabolite compounds, Pathogenic bacteria, Pathogenic fungi*

INTRODUCTION

Banana (Musa paradisiaca) is one of the leading agricultural commodities in Southeast Sulawesi Province. Based on data from the Central Statistics Agency of Southeast Sulawesi Province in 2020, the highest banana production was in Muna Regency at 70,066 quintals per year, followed by North Kolaka Regency and Konawe Regency at 58,984 quintals/year and 42,099 quintals/year (1).

The high carbohydrate content allows the discovery of bacteria that have the potential to degrade carbohydrates. Bacteria that are symbiotic with banana peels will produce types of metabolites that are used to help the carbohydrate metabolism process and protect themselves from external disturbances. Several bacteria such as Enterococcus durans, E. gallinarum, E. hirae, E. faecium, Lactobacillus plantarum, L. curieae, Weissella cibaria and Pediococcus acidilactici are found in bananas (2).

Based on several studies, banana peels contain high antioxidants, and this antioxidant content is very good for the skin, especially facial skin. Banana peels are a rich source of starch (3%), protein (6-9%), fat (3.8-11%), total fiber (43.2-49.7%), and unsaturated fatty acids, pectin, amino acids and micronutrients (K, P, Ca, Mg) (). In addition, banana peels are also rich in various antioxidants (3).

All parts of the banana plant have a role in medical applications (4)Amit and Shailandra, 2006). Antifungal and antibacterial are found in the skin and flesh of ripe bananas (5) Brook et al., 2005). Omojasola and Jilani (2009) reported the antimicrobial action of tetracycline, erythromycin, doxycycline, and clindamycin. In addition, benzoyl peroxide, acetic acid and retinoids are also often used, but these drugs have side effects in their use as anti-acne, including irritation, while long-term use of antibiotics in addition to causing resistance can also cause organ damage and immunohypersensitivity

Endophytic bacteria are bacteria that live in plant tissues without causing harm and even provide many benefits to their host plants. Endophytic bacteria colonize the same ecological niche as plant pathogens (especially vascular wilt pathogens), making them more suitable as candidates for biological control agents. Endophytic bacteria have many beneficial effects on their host plants, including stimulating plant growth (6).

Several types of bacteria that have the potential to be found are the Lactobasillus bacteria group. Lactobasillus is one of the probiotic bacteria that is widely used in the health, food, and pharmaceutical industries. Nurdyansyah et al., (2018) stated that banana peel flour fermented with Lactobasillus casei can produce metabolites/free cells. So far, the need for drugs and therapies has increased. Therefore, alternative drugs or therapies are needed by utilizing waste in nature to reduce environmental impacts (7). Microbes that are spread in nature are producers of drugs/therapies that are widely used commercially. Although many drugs/therapies have been created through chemical synthesis and compound engineering, nature remains the main source for finding new drugs (8)

METHODS

Preparation of control antibiotics Erythromycin, Cefoxitin and Ketoconazole

The antibiotics erythromycin and cefoxitin were made at a concentration of 1%

while the antifungal ketoconazole was made at a concentration of 2% (9).

Pathogenic Bacteria Inhibition Test

A total of 100 µl of liquid culture of pathogenic bacteria Propionibacterium acne and Staphylococcus aureus were mixed into sterile NA (Nutrient Agar) agar media. Then the liquid culture of pathogenic bacteria was slowly spread in a petri dish. After that, the media was allowed to solidify, then the disc paper was placed on the surface of the media and 100 µl of supernatant from KPM2 bacteria could be put into it. The NA (Nutrient Agar) agar media was then incubated for 2x24 hours. Measurement of results was carried out by measuring the clear zone which indicates the zone that inhibits the sample from the growth of pathogenic bacteria (10).

Pathogenic Fungal Inhibition Test

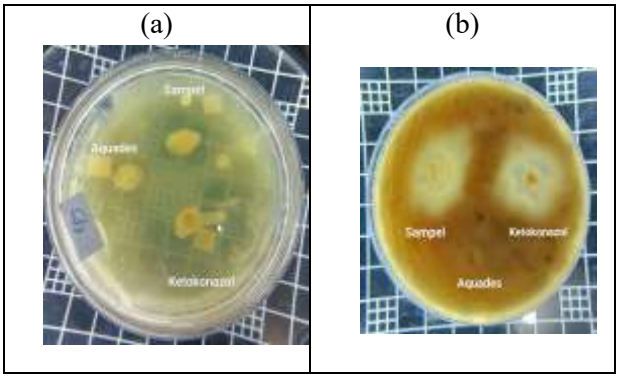
A total of 100 µl of liquid culture of pathogenic fungi Candida albicans and Aspergillus niger were mixed into sterile PDA (Potato Dextrose Agar) media that had been warmed by nails. Then the liquid culture of pathogenic fungi was slowly spread into a petri dish. After that, the media was allowed to solidify, then the disc paper was placed on the surface of the media and 100 µl of KPM2 bacterial supernatant was added to it. The PDA (Potato Dextrose Agar) agar media was then incubated for 3x24 hours. Measurement of the results was carried out by measuring the clear zone which indicates the zone that inhibits the sample from the growth of pathogenic fungi. The test was carried out in a triple way (10).

RESULTS AND DISCUSION

Inhibitoryof KPM2 Bacterial Supernatant Against Pathogenic Bacteria

Table 1. Inhibition zone of Propionibacterium acne and Staphylococcus aureus bacteria

Test bacteria	Inhibition zone diameter (mm)	
	Secondary Metabolic Compounds	Positive control (Erythromycin and Cefoxitin)
Propionibacterium acnes	14mm	28mm (S)
Staphylococcus aureus	12mm	17.5 mm (R)



Figur 1: The results of the inhibitory power of KPM2 bacterial supernatant against Propionibacterium acne bacteria (a) the inhibitory power of KPM2 bacterial supernatant against Staphylococcus aureus bacteria (b)

test fungus	Inhibition zone diameter (mm)	
	Secondary metabolite compounds	Positive control (Ketoconazole)
Aspergillus niger fungus	19.5mm	21.5mm (S)
Candida albicans	27 mm	45mm (S)

(a) (b)

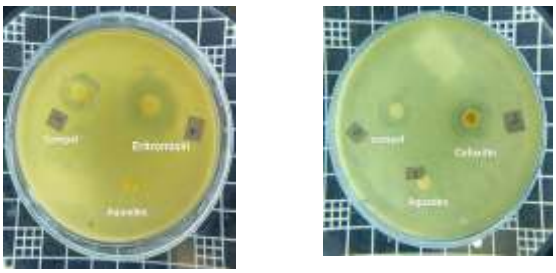


Figure 2. The results of the inhibitory power of KPM2 bacterial supernatant against Aspergillus niger fungus (a) the inhibitory power of KPM2 bacterial supernatant against Candida albicans fungus (b)

Based on the research of Sanatang and Titi (2022), secondary metabolite compounds found in the supernatant of KPM2 endophytic bacteria were obtained in the form of flavonoids, saponins, alkaloids and triterpenoids. The pathogenic bacteria used in this study were Propionibacterium acne and Staphylococcus aureus. While the pathogenic fungi used were Aspergillus niger and Candida Albicans (11).

Secondary metabolite compounds used as antibacterial and antifungal agents in this study include Flavonoids, flavonoids have been known to have antibacterial activity. Flavonoids have many structures, Flavonoids with their antibacterial structures are known to have multiple cell targets and not just one specific target action (12).

Saponin compounds can act as antibacterial by damaging cell membranes. Damage to the cell membrane causes important substances to leave the cell and can also prevent important substances from entering the cell. If the function of the cell membrane is damaged, it will cause cell death (13) Antibacterial tannins can kill bacterial growth because they have antibacterial properties by precipitating proteins and causing the bacterial cell membrane to shrink, resulting in decreased cell permeability (14).

In the inhibition test study, secondary metabolite compounds from endophytic bacteria KPM2 and antibiotics were used which are believed to inhibit the growth of pathogenic bacteria and fungi. The results of the inhibition zone diameter in table 1 show different results in Propionibacterium acne and Staphylococcus aureus bacteria. The diameter of the inhibition zone of the secondary metabolite compound is 14 mm while the 1% Erythromycin antibiotic used produces an inhibition zone of 28 mm which is included in the sensitive category. Erythromycin antibiotics are antibiotics that are effective against several anaerobic bacteria (15).

The inhibition test conducted on Staphylococcus aureus bacteria obtained the results of the inhibition zone diameter using the secondary metabolite compound KPM2 of 12 mm while the diameter of the inhibition zone using the 1% Cefoxitin Antibiotic was 17.5 mm included in the resistant category. From both inhibition results, it can be concluded that the inhibition of the Cefoxitin antibiotic is greater than that of the secondary metabolite compound. Cefoxitin antibiotic is a second generation Cephalosparin antibiotic included

in the β-lactam producing antibiotics used to treat bacterial infections (16).

According to CLSI (2017), the inhibition zone is divided into three categories, including the antibacterial strength criteria, the diameter of the inhibition zone is less than 12 mm which is considered resistant, the inhibition zone of 13-14 mm is considered moderate and the inhibition zone of more than 15 mm is considered sensitive (17). In this study, using the endophytic bacterial isolate KPM2 which produces secondary metabolite compounds, the results were the same as the study conducted by namely that the secondary metabolite compounds contained in Mas banana peel (Musa synthesis) such as Flavanoid, saponins, tannins and triterpenoids can inhibit the growth of pathogenic bacteria (18).

The results of the inhibition test based on table 2, show the inhibition of the pathogenic fungus Aspergillus niger using secondary metabolite compounds from endophytic bacteria KPM2 of 19.5 mm, different from the 2% Ketoconazole antibiotic used, showing an inhibition result of 21.5 mm which is included in the Sensitive category. From the two results, it can be concluded that the inhibition of the 2% Ketoconazole antibiotic used is greater than the secondary metabolite compound from the banana peel endophytic bacterial isolate KPM2. While the inhibition of Candida albicans fungus using secondary metabolite compounds from the KPM2 bacterial isolate produced 27 mm, different from the inhibition of the 2% ketoconazole antibiotic used, producing 45 mm which is included in the sensitive category. The results of this study have similarities with the study conducted by Where the saponin compound of banana tree trunk can inhibit the growth of pathogenic fungi, one of which is Candida albicans (19).

From the research results, the inhibitory power of Propionibacterium acne and Staphylococcus aureus bacteria has a different level of sensitivity because the secondary metabolite compound bacteria used to inhibit the growth of Propionibacterium acne bacteria has a high level of sensitivity compared to Staphylococcus aureus bacteria. While the results obtained in the inhibition test of Aspergillus niger and Candida albicans fungi have a high level of sensitivity, but the inhibition of Candida albicans fungi is higher than the inhibition of Aspergillus niger fungi. This level of sensitivity is characterized by the high level of inhibition produced by a particular antimicrobial compound. This difference in sensitivity levels causes an inhibition zone produced by secondary metabolite compounds from KPM2 endophytic bacterial isolates in different pathogenic bacteria and fungi, this is due to differences in the structure of the cell walls owned by each bacteria and fungus (20).

The parameters used to measure the activity or strength of bioactive compounds contained in banana peel endophytic bacteria can be seen in the width or extent of the inhibition zone formed. The wider or wider the inhibition zone formed, the stronger the bioactive compounds contained in the banana peel endophytic bacterial extract in inhibiting the growth of pathogenic bacteria and fungi.

CONCLUTION

Based on the research results, it can be concluded that the supernatant from KPM2 bacteria can inhibit the growth of pathogenic bacteria such as Propionibacterium acne and Staphylococcus aureus and pathogenic fungi

such as Aspergillus niger and Candida albicans.

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