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Isolation of Endophytic Bacteria from Bark, Leaf, and Pericarp of Mangosteen (*Garcinia mangostana* L.) and Testing of The Antimicrobial Activity.

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ABSTRACT

A research on antimicrobial activity testing of the endophytic bacteria which was symbiosis with mangosteen has been conducted. About fourteen isolates of bacteria from mangosteen have been isolated. Each bacterial isolates were fermented in aqueous media for 24 hours on a rotary shaker incubator at temperature of 30 °C and speed of 120 rpm. Each fermentation of bacterial isolates were tested their antimicrobial activity against microbes *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* with the agar diffusion method. There were two bacterial isolates that active against *Escherichia coli* and five bacterial isolates that active against *Staphylococcus aureus*.

Keywords: *Garcinia mangostana* L., endophytic bacteria, mangosteen, antimicrobial activity

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INTRODUCTION

Search for the source of bioactive compounds is continued to be done along with the many new diseases are emerging. One source of bioactive compounds derived from microbes is endophytic microbes. Endophytic microbes can produce bioactive compounds that are potential to be developed into drugs. One of plant that is used to treat diarrhea, dysentery, and ulcers is mangosteen. The extract from mangosteen pericarp was effective against *Staphylococcus aureus*, *Staphylococcus albus*, and *Micrococcus lutus*. The strong anti bacterial activity of the extract suggests that it is a good drug of choice for which might be helpful in preventing the progress of various diseases and it can be used in alternative system of medicine [1]. There is a need to search new ecological niches for potential of natural bioactive agents for different pharmaceutical, agriculture and industrial application; these should be renewable, eco-friendly and easily obtainable natural products discovery in the search for new drugs, and is the most potent source for the discovery of novel bioactive compounds. Therefore, a large number of bioactive compounds are isolated from the plants, bacteria, fungi and many other organisms. Endophytic microbes being the most promising of these have been a source of various such bioactive compounds. Many of these compounds are being used for the treatment of a number of diseases [2]. The purpose of this study was to test the antimicrobial activity of the compounds isolated from endophytic microbes of mangosteen.

EXPERIMENTAL SECTION

Test drug and chemicals

The leaves, bark and pericarp of the mangosteen (*Garcinia mangostana* L.) were obtained from Lubuk Alung, West Sumatra, Indonesia. All other chemicals used were of analytical grade.

Isolation of endophytic bacteria

Each fresh plant was washed with running water. Then each part of the plant was cut to a size of 1 x 1 cm². Furthermore, in the laminar air flow cabinet, each organ of the plant was disinfected of their surfaces by immersing the organ by successively in 70 % ethanol for 30 seconds, a solution of 5 % sodium hypochlorite for 5 minutes, 70 % ethanol for 30 seconds and rinse with distilled water for 3 minutes [3,4].

Each organ of the plant was planted using tweezers into a Petri dish containing media Nutrient Agar (NA) by splitting parts of the plant and put in prone position. Each Petri dish was planted 2-3 slices of the organ, incubation at temperature 37 °C for 18-24 hours. Bacteria that grow gradually were purified one by one. Colonies that have a different form with each other colonies can be considered different colonies [5, 6].

Antimicrobial activity test

Medium for production of antibiotics was prepared with the composition of corn soaking water (3 %), glucose (3 %), calcium carbonate (0.5 %), ferrous sulfate (0.1 %), magnesium sulfate (0.2 %), zinc sulfate (0.01%), and sterile distilled water added up to 100% [5]. The medium was heated to boiling and sterilized by autoclaving at 121 °C for 15 minutes. Each of isolates that had been purified in previous experiments was inoculated on the liquid medium that has been prepared. Each of the isolates was fermented. The fermentation process was done in 250-mL Erlenmeyer flask and incubated at temperature of 30 °C for 24 hours on a rotary shaker incubator at 120 rpm. Then the fermentation solution was centrifuged at a speed of 5000 rpm for 15 minutes. Supernatant was tested of antimicrobial activity against microbes using paper disc method. Paper discs was dipped in the supernatant and planted in the medium NA containing bacteria and in the medium PDA containing fungi. Then it was incubated at temperature of 37 °C for 18-24 hours. Barriers to growth were observed and measured its diameter by using a caliper. The results of centrifugation of each microbe were washed 3 times with distilled water, dried by air, so that the biomass was obtained [7].

Characterization of endophytic bacteria isolates

Characterization of the isolates was carried out against isolates that show significant antibacterial activity. Observations were made macroscopically, colonies form, color, and surface condition, while the shape of the cell was observed microscopically by Gram stain and using biochemical reaction [7].

RESULTS AND DISCUSSION

In the process of isolation of endophytic bacteria, it was used direct planting method in which pieces of plant organs which have been disinfected its surface were affixed to the media Nutrient Agar (NA) in the position of organ surface sticks to the media. This method is chosen because it is more practical and faster process [4, 6]. Bacteria that grow in media was purified and fermented to determine its ability to produce antimicrobial compounds. In this study, it was obtained 14 isolates of endophytic bacteria and biochemical test results were presented in Table11-14.

Table 1: Characteristics of endophytic bacteria isolated with BG code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil	<i>Bacillus</i> sp1
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 2: Characteristics of endophytic bacteria isolated with BH code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil	<i>Bacillus</i> sp2
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 3: Characteristics of endophytic bacteria isolated with B-P₁ code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp3
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 4: Characteristics of endophytic bacteria isolated with B-P₂ code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp4
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 5: Characteristics of endophytic bacteria isolated with B-T₁ code

No.	Treatment	Observation	Genus
1.	Gram stain	- Coccus	<i>Enterobacter aerogenes</i>
2.	Aerobe/ Anaerobe	Aerob	

3.	TSIA	m/k
4.	Gas	-
5.	H ₂ S	-
6.	Catalase	±
7.	Oxidase	-
8.	Motility	+
9.	Indole	-
10.	Urea	+
11.	Citrate	+
12.	Lactose	-
13.	Glucose	+
14.	Sucrose	-
15.	Mannitol	-
16.	MR	-
17.	VP	+
18.	OF	±
19.	KCN	+
20.	Arginine	-
21.	Lysine	-
22.	Ornithin	+
23.	Phenylalanine	-
24.	Aesculin	-
25.	Arabinose	-
26.	Raffinose	-
27.	Sorbitol	-
28.	Trehalase	-
29.	Xylose	-
30.	Dulcitol	-
31.	Malonate broth	-
32.	Nitrate	-
33.	Gelatin	+

Table 6: Characteristics of endophytic bacteria isolated with B-T₂ code

No.	Treatment	Observation	Genus
1.	Gram stain	- coccus	<i>Klebsiella</i> sp
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	m/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	±	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	+	
11.	Citrate	+	
12.	Lactose	-	
13.	Glucose	+	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	OF	±	
19.	KCN	+	
20.	Arginine	-	
21.	Lysine	-	
22.	Ornithin	+	
23.	Phenylalanine	-	
24.	Aesculin	-	

25.	Arabinose	-	
26.	Raffinose	-	
27.	Sorbitol	-	
28.	Trehalase	-	
29.	Xylose	-	
30.	Dulcitol	-	
31.	Malonate broth	-	
32.	Nitrate	-	
33.	Gelatin	+	

Table 7: Characteristics of endophytic bacteria isolated with B-U₁ code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp5
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 8: Characteristics of endophytic bacteria isolated with B-U₂ code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp6
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 9: Characteristics of endophytic bacteria isolated with B-K₅1 code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp7
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 10: Characteristics of endophytic bacteria isolated with B-K₅2 code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil Spore	<i>Bacillus</i> sp8
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 11: Characteristics of endophytic bacteria isolated with B-K₁₀1 code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil	<i>Bacillus</i> sp9
2.	Aerobe/ Anaerobe	Aerob	

3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 12: Characteristics of endophytic bacteria isolated with B-K₁₀2 code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil	<i>Bacillus</i> sp10
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

Table 13: Characteristics of endophytic bacteria isolated with B-K₁1 code

No.	Treatment	Observation	Genus
1.	Gram stain	- coccus	<i>Alcaligenes</i> sp
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	m/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	±	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	+	
11.	Citrate	+	
12.	Lactose	-	
13.	Glucose	+	

14.	Sucrose	-
15.	Mannitol	-
16.	MR	-
17.	VP	+
18.	OF	±
19.	KCN	+
20.	Arginine	-
21.	Lysine	-
22.	Ornithin	+
23.	Phenylalanine	-
24.	Aesculin	-
25.	Arabinose	-
26.	Raffinose	-
27.	Sorbitol	-
28.	Trehalase	-
29.	Xylose	-
30.	Dulcitol	-
31.	Malonate broth	-
32.	Nitrate	-
33.	Gelatin	+

Table 14: Characteristics of endophytic bacteria isolated with B-K₁2 code

No.	Treatment	Observation	Genus
1.	Gram stain	+ Basil	<i>Bacillus</i> sp11
2.	Aerobe/ Anaerobe	Aerob	
3.	TSIA	k/k	
4.	Gas	-	
5.	H ₂ S	-	
6.	Catalase	-	
7.	Oxidase	-	
8.	Motility	+	
9.	Indole	-	
10.	Urea	-	
11.	Citrate	-	
12.	Lactose	-	
13.	Glucose	-	
14.	Sucrose	-	
15.	Mannitol	-	
16.	MR	-	
17.	VP	+	
18.	Nitrate	+	
19.	Gelatin	+	

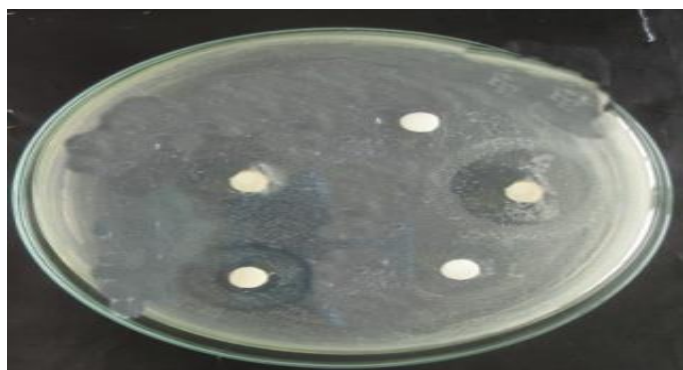


Figure 1: The profile of inhibition zone caused by fermentation liquid of endophytic bacteria against testing bacteria

Table 15: Inhibition caused by fermentation liquid of endophytic bacteria

No	Code of isolate	Genus	Inhibition Diameter		
			<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>C. albicans</i> (mm)
1.	B-G	<i>Bacillus</i> sp1	-	14	-
2.	B-H	<i>Bacillus</i> sp2	-	11	-
3.	B-P ₁	<i>Bacillus</i> sp3	-	15	-
4.	B-P ₂	<i>Bacillus</i> sp4	-	-	-
5.	B-T ₁	<i>Enterobacter aerogenes</i> sp	-	-	-
6.	B-T ₂	<i>Klebsiella</i> sp	13	-	-
7.	B-U ₁	<i>Bacillus</i> sp5	-	-	-
8.	B-U ₂	<i>Bacillus</i> sp6	-	-	-
9.	B-K ₅ 1	<i>Bacillus</i> sp7	18	-	-
10.	B-K ₅ 2	<i>Bacillus</i> sp8	-	-	-
11.	B-K ₁₀ 1	<i>Bacillus</i> sp9	-	16	-
12.	B-K ₁₀ 2	<i>Bacillus</i> sp10	-	15	-
13.	B-K ₁ 1	<i>Alkaligenes</i> sp	-	-	-
14.	B-K ₁ 2	<i>Bacillus</i> sp11	-	-	-

Table 16: pH of Media and Biomass of Bacteria after 48 hours cultivation

Isolate	pH of Media	Biomass (gram)
B-H	6.71	0.080
B-G	6.72	0.076
B-P ₁	6.61	0.095
B-P ₂	6.80	0.065
B-T ₁	6.82	0.059
B-T ₂	6.78	0.109
B-U ₁	6.85	0.069
B-U ₂	6.61	0.063
B-K ₅ 1	6.72	0.078
B-K ₅ 2	6.81	0.065
B-K ₁₀ 1	6.77	0.080
B-K ₁₀ 2	6.67	0.078
B-K ₁ 1	6.67	0.058
B-K ₁ 2	6.69	0.050

Table 15 showed that there were two bacterial isolates (B-T₂ and B-K₅1) resulted in inhibition against test bacteria *E. coli* and five isolates (BH, BG, B-P₁, B-K₁₀1 and B-K₁₀2) resulted in inhibition against *S. aureus*.

In testing of antimicrobial activity of compound produced by endophytic bacteria against fungi *Candida albicans* there was no inhibition due to *Candida albicans* including eukaryotic microorganisms, whereas pathogenic microbes used to test antimicrobial activity were Gram-positive and Gram-negative including prokaryotic microbes. There are difference of structure of cell wall components in eukaryotic especially *Candida albicans*. In eukaryotic the cell wall structural components contain chitin, cellulose or glucans, whereas in prokaryotic the cell wall component contain peptidoglycan. In addition metabolite produced was not suitable for eukaryotic microorganisms as secondary metabolites produced cannot damage the cells of *Candida albicans* [7]. In addition the limitations of antifungal drug discovery than antibacterial drugs this is due to the fungus associated with complex cell structure than bacteria [8].

The activity of endophytic bacteria was generally greater against Gram-positive bacteria than Gram-negative bacteria. This was due to the structure of the cell wall of Gram-negative bacteria was complicated and also the external lipopolysaccharide layer was found in Gram-negative bacteria. This resistance was also associated with the permeability and pore bacterial cell wall. The pores of the cell wall of Gram-negative bacteria were highly selective for molecules that can get into the cell [9,10]. There were several isolates that

do not have antimicrobial activity and the possibility of these isolates have other activities such as poison, giving color or pigment, growth agents, pesticides [7].

Table 16 showed that the pH of Media and Biomass of Bacteria after 48 hours cultivation. There was the influence of pH changes on filtrate endophytic microbes. Endophytic microbes decreasing in pH of the filtrate showed the role of the production of antimicrobial which can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* [9]. The addition of biomass of each endophytic microbe also has an influence on the growth of *Escherichia coli* and *Staphylococcus aureus*. The results showed that the antimicrobial synthesized in its infancy. Initial growth was seen when a microbial cell inoculated on nutrient agar was enlarging in size, volume and weight of the cell. The cells were continued to divide exponentially. As long as conditions allow, the growth and division of cells takes up a number of established cell population. The more cells that grow, the antimicrobial activity was increasing along with the metabolites excreted in the form of antimicrobial compounds out of the cell [9,10].

CONCLUSION

About fourteen isolates of bacteria from leaves, bark, and fruit peel of mangosteen (*Garcinia mangostana* L.) have been isolated. There were two bacterial isolates that active against *Escherichia coli* and five bacterial isolates that active against *Staphylococcus aureus*. There was no bacterial isolate that active against *Candida albicans*.

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