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Antibacterial Activity Test of Elephant Ginger (*Zingiber officinale* Rosc.) Endophytic Fungi Variation of Elephants Against Bacteria That Cause Skin Infections

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Abstract. Elephant ginger rhizome has the potential as an antibacterial, therefore the research was conducted to test the antibacterial activity of elephant ginger endophytic fungi against bacteria that cause skin infections, determine test bacteria that can be inhibited by elephant ginger endophytic fungi and bioautogram profile for its antibacterial activity. Isolation of endophytic ginger elephant fungi using the direct planting method. Screening of endophytic fungi isolates was placed on the surface of NA medium containing test bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acne*. The screening results obtained were 2 isolates namely isolates which had the highest activity namely IFDRJG03 and IFDRJG04. For IFDRJG03 isolates can inhibit the antibacterial activity of *Staphylococcus aureus* 25.26 mm, *Staphylococcus epidermidis* 26.89 mm and *Propionibacterium acne* 27.12 mm, IFDRJG04 isolates can inhibit the antibacterial activity of *Staphylococcus aureus* 15.87 mm, *Staphylococcus epidisidis* 18 and 96 mm. 27.43 mm. Then the TLC-Bioautography test was carried out, the results of IFDRJG03 isolates were obtained using chloroform eluents: methanol (1: 1) had antibacterial activity against *Staphylococcus aureus* with Rf1 = 0.9 and Rf2 = 0.5, *Staphylococcus epidermidis* with Rf1 value = 0.8 and Rf2 = 0.5 and *Propionibacterium acne* with Rf1 value = 0.9 and Rf2 = 0.5 and isolation of IFDRJG04 isolates with eluent of ethyl acetate : ethanol : water (8:2:1) has antibacterial activity against *Staphylococcus aureus* Rf1 values = 0.9 Rf2 = 0.7 Rf3 = 0.6, *Staphylococcus epidermidis* with Rf1 value = 0.7 Rf2 = 0.6, Rf3 = 0.5 and *Propionibacterium acne* with Rf1 value = 0.9 and Rf2 = 0.7.

Introduction

Infectious disease is a problem in the health sector which from time to time continues to grow. Infection is a disease that can be transmitted from humans to humans and from animals to humans. These infections can be caused by bacteria, fungi, viruses, and parasites. One part of the human body that is very sensitive to various kinds of infections is the skin. Skin infection is a skin disease caused by bacteria including *Staphylococcus aureus* (Mishra, Yadav and Mishra, 2016).

Skin diseases in Indonesia are generally caused more by bacterial, fungal, parasitic, and allergic-based infections. This is in contrast to western countries which are more often affected by degenerative diseases, a disease that arises due to deterioration of body cell function. Synthesis drugs such as antibiotics can cause high resistance. One way to overcome the problem of resistance to antibiotics is to look for new antibacterial compounds, one of which is the utilization of endophytic fungi.

Endophytes can produce bioactive secondary metabolites, such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins, and terpenoids (Gouda *et al.*,

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2016). Khusnul et al. (2017) succeeded in isolating endophytic fungi of the genus *Aspergillus sp* and *Genus Absidia sp* from leaves of grass jelly (*Cyclea barbata Miers*.) which are antibacterial against *Salmonella tiphy* (Khusnul, Wahyuni and Virgianti, 2015). Hamzah et al. (2018) isolated endophytic fungi from mangrove plants (*Rhizophora mucronata*) and successfully obtained isolates of *Fusarium lateritium* and *Xylaria sp.* which are antibacterial against *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Hamzah *et al.*, 2018).

One of the plants that has traditional medicinal properties is the elephant ginger rhizome. Elephant ginger is efficacious to relieve symptoms of sore throat, relieve heart problems and treat vomiting, ascites, cough, anorexia, fever, anemia, flatulence, colic, constipation, swelling, elephantiation, anti-inflammatory, anti-cancer, antimicrobial and dysuria besides ginger is also used in ginger the treatment of diarrhea, cholera, dyspepsia, diabetes and ginger content also has an effect on bacteria. The chemical content that has been investigated and known to be responsible for the antibacterial effect are terpene compounds, terpene compounds are bacteriostatic agents. In addition to having uses as a basic ingredient in the manufacture of traditional and modern medicines, antioxidants and antibacterial compounds of secondary metabolites produced by ginger can inhibit the growth of pathogenic bacteria that harm human life including *Staphylococcus aureus* and *Escherichia coli* (Arifin, 2012).

The results of research conducted by Sari and Nasir (2013) reported that fresh extracts of ginger rhizome have antimicrobial activity against the bacteria *Staphylococcus aureus, Escherichia coli and Candida albicans*. (Sari, Kartika Indah Permata, Periadnadi, Nasir, 2013). Dianasari, et al (2020) reported that elephant ginger in the n-hexane fraction with a concentration of 20% had a inhibition of 9.78 mm against *Staphylococcus aureus* bacteria (Dianasari *et al.*, 2020). Kaitu, et al (2013) reported that red ginger endophytic fungi have antibacterial activity against *Escherichia coli* and *Streptococcus pyogenes*. Based on the description above, the purpose of this study is to test the antibacterial activity of endophytic fungi in the ginger rhizome of elephant (*Zingiber officinale Rosc*.) against bacteria that cause skin infections (Kaitu, Boy Rahardjo Sidharta and Atmodjo, 2013).

Experimental

Material and Methods

The materials used in this study were distilled water, 70% alcohol, test cultures (*Staphylococcus aureus*, *Staphylococcus epidermidis* and *Propioni bacterium acne*), chloramphenicol, chromatographic plates Thin Layer (TLC), medium Maltosa Yeast Broth (MYB), Nutrient medium medium Agar (NA), Potato dextrose Agar (PDA) medium, capillary tube, elephant ginger (*Zingiber officinale Rosc.*) Var. Elephant.

The tools used in this study were autoclaves (SMIC Model YX-280 B), petri dishes (Normax), Enkas, erlenmeyer glasses (Iwaki Pyrex), chemical beakers (Iwaki pyrex), incubators (Memmert), spiritus lamps, lamps UV 254 and 366 nm (Philips), ose, oven, knife, sheker, test tube, analytical balance (Chyo) and vial.

Procedures

The elephant ginger rhizome is cleaned and washed with running water to remove dirt. After washing, the sample is soaked with 70% alcohol for 2 minutes, then the sample is rinsed with sterile aquadest ± 1 minute and repeated several times (Yunus, 2015).

Isolation of endophytic fungi was carried out using the direct planting method of the elephant ginger rhizome, cut using a sterile knife then the pieces were placed on the surface of the Potato Dextrose Agar Chloramphenicol (PDAC) medium. Then incubated for 3 days at room temperature. Purification of endophytic fungi is carried out by preparing a PDA medium in a compact, sterile petri dish. 1 ose of endophytic fungal isolates were taken using a needle preparation. Placed in the middle of a petri dish containing a PDA then incubated at 27°C for 3 x 24 hours (Widowati *et al.*, 2016).

Macroscopic examination of endophytic fungi includes morphological observations (Adriani, 2015). Macroscopic examination was carried out by observing pure fungi isolates including color, colony shape, and elevation (Reckow, V., Widayat, W., Rijai, 2016). All isolates from elephant ginger endophytic fungi were grown into a PDA medium, then the endophytic fungi isolate was cut into small ± 1 cm, placed on the surface of the Nutrient agar (NA) medium containing test bacteria namely *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propinibacterium acne* then incubated for 1 x 24 hours at 37°C then observed inhibition zones formed (Pratiwi, 2016).

Rejuvenation of the test bacteria was carried out by taking the test bacteria each one ose and then inoculated by being etched on an oblique NA medium then incubated at 37◦C for 1 × 24 hours (Noverita, Fitria and Sinaga, 2009). The rejuvenated bacteria were inoculated with ose from an oblique NA medium and suspended in a tube containing 5 mL of sterile physiological NaCl (Gazali, Anam and Khumaidi, 2016). Endophytic fungi fermentation is done using MYB medium. Endophytic colonies that have been

incubated on PDA medium for 3 x 24 hours at 25°C, are taken into small pieces and put into an erlenmeyer glass containing 25 mL. MYB medium was then fermented using a 200 rpm rotary shaker at room temperature for 21 days. Microbial growth medium was filtered to separate the fermented liquid from the supernatant and mycelia obtained then used for antimicrobial testing (Yunus, 2015). TLC identification was carried out by bottling IFDRJG 3 and IFDRJG 4 endophytic fungi extracts on the TLC plate and eluted for IFDRJG 3 chloroform: methanol (1:1), IFDRJG 4 eluent ethyl acetate: ethanol: water (8: 2: 1) then observed under UV light at length of chloroform: methanol (1: 1), IFDRJG 4 eluent ethyl acetate: ethanol: water (8: 2: 1) then observed under UV light at length of chloroform: methanol (1: 1), IFDRJG04 eluent ethyl acetate: ethanol: water (8: 2: 1) waves of 254 nm and 366 nm.

The TLC-Bioautographic antibacterial activity test was carried out by pouring NA medium into a 10 mL petri dish and adding the test bacteria suspension as much as 0.2 mL, then homogenized. The eluted TLC lemeng is placed on the surface of the medium and left for 60 minutes, then the plate is removed. Incubated for 24 hours at 37°C then the inhibition zone was observed (Mani *et al.*, 2014).

Result and Discussion

From the results of the isolation of endophytic fungi, the elephant ginger (*Zingiber officinale Rosc.*) Elephant variant obtained 5 isolates. The results of isolation can be seen in table 1.

Table 1. Results of isolation of endophytic fungi isolates from ginger rhizomes.

No	Isolate code	Fungi culture
1	IFDRJG 1	1st ginger endophytic fungi isolate elephant
2	IFDRJG 2	2st ginger endophytic fungi isolate elephant
3	IFDRJG 3	3st ginger endophytic fungi isolate elephant
4	IFDRJG 4	4st ginger endophytic fungi isolate elephant
5	IFDRJG 5	5st ginger endophytic fungi isolate elephant

In this research, planting was carried out first using Pepton Dexstrosa Agar + Cloramfenikol (PDAC) medium. The addition of cloramphenicol is intended to ensure that the growth is fungi instead of bacteria then isolation is carried out to obtain endophytic fungi isolates. From the results of the isolation of the elephant ginger rhizome (*Zingiber officinale Rosc.*) Var. elephants obtained 5 isolates. The isolates were then purified, to obtain pure endophytic fungi isolates which were then subjected to macroscopic testing. Macroscopic examination was carried out to determine the shape of the morphology of the isolates so that it can be known with certainty that the endophytic fungi isolates obtained were not the same.

Furthermore, an antibacterial activity screening test is performed. From 5 isolates obtained, there were only 2 isolates which had the highest antibacterial activity, namely IFDRJG 3 isolate and IFDRJG 4 isolate. The screening test results showed that IFDRJG 3 isolate could inhibit the activity of *Staphylococcus aureus* by 25.26 mm, *Staphylococcus epidermidis* 26.89 mm and *Propionibacterium acne* 27.12 mm and isolate IFDRJG 4 can inhibit the activity of *Staphylococcus aureus* by 15.87 mm, *Staphylococcus epidermidis* 18.96 mm and Propionibacterium *acne* 27.43 mm. Both IFDRJG 3 and IFDRJG 4 isolates were continued in the fermentation process. The fermentation process is carried out for 21 days because, in that phase the fungi secrete secondary metabolites to the maximum (Pratiwi, 2016).

Table 2. Macroscopic tests of endophytic fungal isolates of ginger elephant rhizomes.

Microbial code	Color	Shape	Edge	Elevation
IFDURJG 1	White	Konsetrik	Tread-like	Flat
IFDURJG 2	White	Irregular and spreading	wavy (undulat)	Crateriform
IFDURJG 3	White	Irregular and spreading	Lobate	Hilly
IFDURJG 4	White	L-form	Iiregullar (erose)	Hilly
IFDURJG 5	Brown	Filamentous	Breaking	Umbenate

Table 3. Screening tests and measurement of inhibition zones of endophytic fungal isolates of elephant ginger.

	Inhibitory zone diameter (mm)		
Isolate fungi	*P. acne* (mm)	*S. epidermidis* (mm)	*S. aureus* (mm)
IFDRJG 1	12,65	12,95	13,23
IFDRJG 2	13,84	13,52	21,40
IFDRJG 3	27,12	26,89	25,26
IFDRJG 4	15,87	18,96	27,43
IFDRJG 5	14,38	15,43	19,03

Furthermore, the fermentation results are filtered to separate the supernatant and mycelia by adding ethyl as a solvent and then evaporated to obtain ethyl acetate extract

from the supernatant and mycelia. The selection of ethyl solvent as a solvent because ethyl acetate is semi-polar so that it can attract polar compounds and non-polar compounds besides that ethyl acetate also has low toxicity (Pratiwi, 2016). After the extract is obtained then proceed to identify TLC. The ethyl acetate extract was bottled on the TLC plate and eluted using chloroform methanol (1: 1) eluent for IFDRJG 3 and water ethyl acetate (8: 2: 1) for IFDRJG 4.

Table 4. Rf value of TLC-Bioautography test extract of endophytic fungi number 3 (IFDRJG 3).

Spot	Bacteria code test	Rf value	Color	
			UV 254 nm	UV 366 nm
2	*S. aureus*	Rf_1= 0,9 Rf_2= 0,5	Green	Fluorescent purple
2	*S. epidermidis*	Rf_1= 0,8 Rf_2= 0,5	Green	Fluorescent purple
2	*P. acne*	Rf_1= 0,9 Rf_2= 0,5	Green	Fluorescent purple

The results of TLC testing using chloroform: methanol (1: 1) eluent with UV spot 254 and UV 366 light spot appearance obtained several spots. In the elephant ginger rhizome isolate IFDRJG 3 code obtained 2 active spots that can inhibit the growth of *Staphylococcus aureus* with Rf1 value = 0.9 and Rf2 = 0.5, then 2 active spots that can inhibit the growth of *Staphylococcus epidermidis* with Rf1 value = 0.8 and Rf2 = 0.5 and there are 2 active spots that can inhibit the growth of *Propionibacterium acne* with Rf1 value = 0.9 and Rf2 = 0.5.

Then the ginger rhizome isolate IFDRJG 4 code with eluen ethyl acetate: ethanol: water (8: 2: 1) obtained 3 active spots that can inhibit the growth of *Staphylococcus aureus* with a value of Rf1 = 0.9 Rf2 = 0.7 and Rf3 = 0.6, then 3 active spots that can inhibit the growth of *Staphylococcus epidermidis* with a value of Rf1 = 0.7 Rf2 = 0.6 and Rf3 = 0.4 and there are 2 active spots that can inhibit the growth of *Propionibacterium acne* with an Rf1 value of 0.9 and Rf2 = 0.7.

Antibacterial activity is characterized by the formation of clear zones on the surface of the medium where the spots diffuse. And based on the results of tests conducted using the TLC method, bioautography proves that elephant ginger rhizome fungi isolates have the potential to be antibacterial.

Table 5. Rf value of TLC-Bioautography test extract of endophytic fungi number 4 (IFDRJG 4).

Spot	Bacteria code test	Rf value	Color	
			UV 254 nm	UV 366 nm
3	*S. aureus*	Rf_1= 0,9 Rf_2= 0,7 Rf_3= 0,6	Green	Fluorescent purple
3	*S. epidermidis*	Rf_1= 0,7 Rf_2= 0,6 Rf_3= 0,4	Green	Fluorescent purple
2	*P. acne*	Rf_1= 0,9 Rf_2= 0,7	Green	Fluorescent purple

Conclusion

Based on the research results obtained, it can be concluded that from the isolation results of endophytic fungi rhizome of elephant ginger (Zingiber officinale Rosc.) Var. elephants were found 2 isolates which had the highest activity as antibacterial, namely IFDRJG03 isolate and IFDRJG04 isolate. Both of these isolates have the potential to be antibacterial.

Conflict of Interest

The authors declare that there is no conflict of interest.

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