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Research Article

Antibacterial Activity of Actinomycetes Isolates from The Rhizosphere of The Turate Kasumba Plant (*Carthamus Tinctorius* L.) Against Bacteria Causing Skin Infections

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ABSTRACT: Actinomycetes is one group of microorganisms capable of producing secondary metabolites that function as antibacterials. Actinomycetes isolates used were isolated from the rhizosphere of the kasumba turate plant (*Carthamus tinctorius* L.). The purpose of this study was to determine the antibacterial activity of Actinomycetes isolates from the rhizosphere of kasumba turate (*Carthamus tinctorius* L.) which is the largest and most against skin infection bacteria. The results of the study obtained 2 isolates that are most active. IARK-6 obtained spot with Rf value = 0.70 for *Staphylococcus aureus* bacteria, Rf1 = 0.70 and Rf2 = 0.38 for *Staphylococcus epidermidis* bacteria, Rf = 0.70 for *Pseudomonas aeruginosa* bacteria. While the results of KLT-Bioautography testing for isolate IARK-7 obtained spots with a value of Rf = 0.38 for *Staphylococcus aureus* bacteria, Rf = 0.70 for *Staphylococcus epidermidis* bacteria, Rf = 0.38 for *Pseudomonas aeruginosa* bacteria.

KEYWORDS: Antibacterial; Actinomycetes; Kasumba Turate Rhizosphere; KLT-Bioautography; *Carthamus tinctorius* L.

1. INTRODUCTION

Infectious diseases are the most common disease factor in developed and developing countries, including in Indonesia (Rusli et al., 2020). One of them is a skin infection, skin health is very important for humans, but there are still many who often ignore and underestimate this disease (Djata et al., 2022). Skin diseases in Indonesia are generally caused more by bacterial, fungal, and viral infections, and because of allergic bases. Other factors are people's habits and unclean environment (Agustina et al., 2017).

To overcome infectious diseases used antibiotics, which are substances that can inhibit the growth of a microorganism. Antibiotics that are initially sensitive to microorganisms can become insensitive or called resistance, which is caused by several factors, such as the intensity of exposure to an area and irrational use of antibiotics (Rusli et. al, 2020). Antibiotic-producing microbes are mostly obtained from soil microbes, one of which is Actinomycetes, which is the largest producer of active compounds compared to bacteria or molds, such as antimicrobial, anticancer, antiviral, and anticholesterol compounds (Fitriana et al., 2018).

Several studies have succeeded in isolating Actinomycetes from the rhizosphere which has the potential to produce antibiotics and is able to inhibit bacterial skin infections. Among them are (Ambarwati et al., 2010) succeeded in isolating Streptomyces from the corn rhizosphere (*Zea mays*) and managed to find 23 isolates, 5 of which were able to inhibit *Staphylococcus aureus.* Based on the description above, the antibacterial activity of Actinomycetes isolates from the rhizosphere of the kasumba turate plant (*Carthamus tinctorius* L.) was conducted against test bacteria that cause skin infections.

2. EXPERIMENTAL SECTION

The research method used is a laboratory experimental method by testing the antibacterial activity of Actinomycetes *isolates* from the rhizosphere of the kasumba turate plant (*Carthamus tinctorius* L.) against test bacteria that cause skin infections by the KLT-Bioautography method. This research was conducted at the Laboratory of Pharmaceutical Microbiology of the Bachelor of Pharmacy Study Program, Faculty of Pharmacy, Universitas Muslim Indonesia. The population used in this study was the turate kasumba plant (*Carthamus tinctorius* L.) and the sample used was the rhizosphere of the turate kasumba plant ((*Carthamus tinctorius* L.) from Galesong District, Kabupaten Takalar, South Sulawesi.

2.1. Tools

The tools used in this study include beakers (Iwaky Pyrex), autoclaves (SIMC Model YX280 B), Petri dishes (Normax), rotavapor, incubator (Memmert), stainless steel spoons, Erlenmeyer (Iwaki Pyrex), calipers, Laminar Air Flow (LAF), microscopes, ovens (Memmert), rotary shakers, analytical balances (Chyo), test tubes, split funnels, object glasses, 254 nm and 366 nm UV lamps, capillary pipes, chambers, spirit lamps, and vials.

2.2. Materials

The materials used in this study include aquades, *Staphylococcus aureus* test bacteria (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990), *Pseudomonas aeruginosa* (ATCC 27853), 70% ethanol, ethyl acetate, KLT plate, methanol, SNA (Starch Nitrite Agar), SNB (Starch Nitrite Broth), NA (Nutrient Agar), disc blank, Gram A paint (Violet Crystal, Gram B paint (Iodine), Gram C paint (Alcohol 96%), paint Gram D (Safranin) and rhizosphere samples of the turate kasumba plant (*Carthamus tinctorius* L.).

3. RESULTS AND DISCUSSION

Actinomycetes isolates used in this study are the results of isolation from the rhizosphere of the turate kasumba plant (*Carthamus tinctorius* L.) to determine its antibacterial activity against test bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *and Pseudomonas aeruginosa*. Actinomycetes isolates were then rejuvenated on Starch Nitrate Agar (SNA) medium. SNA is a selective medium used for the isolation of Actinomycetes and can minimize contamination of other bacteria (Riyanti et al., 2012). Rejuvenation of Actinomycetes bacteria is carried out in an incubator at a temperature of 37 ºC for 3 x 24 hours.

After the next rejuvenation process microscopic observations were made, this aims to see the shape of cells and the properties of Gram by making preparations from each pure isolate of bacterial samples then staining (Oka et al., 2019). Gram painting/staining is done to color microorganisms or their background because microorganisms are difficult to see with a light microscope because they do not adsorb or refract light. Gram staining also aims to determine whether endophytic bacteria are Gram-positive or Gram-negative (Dwijoseputro et al., 1998). The results of microscopic tests with gram painting can be seen in **Table 1**.

The isolates obtained were then tested for antagonists against bacteria *Staphylococcus aureus, Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* by inoculating on Nutrient Agar (NA) medium that had been inoculated with test bacteria and then observed its activity. The antagonist test results can be seen in **Table 2**.

Based on the results obtained from testing antagonists of antibacterial isolates of Actinomycetes rhizosphere kasumba turate (*Carthamus tinctorius* L.) against test bacteria, IARK-6 and IARK-7 codes provide the highest inhibitory zone activity against test bacteria. The inhibitory zone is a clear area around the growth medium of test bacteria that is not overgrown with bacteria (Putri et al., 2016). The criteria for antibacterial power strength are as follows: the diameter of the inhibitory zone of 5 mm or less is categorized as weak, the diameter of 5-10 mm is categorized as medium, the diameter of 10-20 mm is categorized as strong and the last diameter of 20 mm or more is categorized as very strong (Davis et al., 1971). Based on the criteria mentioned, the antibacterial power of isolates with IARK-6 and IARK-7 codes against test bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* is included in the strong category.

Furthermore, the fermentation process of isolates with the highest inhibitory zones, namely IARK-6 and IARK-7 using Starch Nitrate Broth (SNB) medium at room temperature for 14 days with a cornering speed of 150 rpm. The use of SNB as a medium because it is rich in carbon and mineral content. Good growth media is media that is able to provide a source of carbon and other minerals needed for the growth and activity of microorganisms (Todar et al., 2009). The source of SNB carbon comes from soluble starch which contains various C elements from starch and glycerol (Ali et al., 2009). SNB medium also contains KNO_3 as an inorganic source and is rich in minerals such as magnesium, sodium, iron, and potassium.

After the fermentation process is carried out, the fermentate is filtered using filter paper to separate biomass and supernatant. Then the supernatant is extracted by liquid-liquid extraction method using ethyl acetate solvent in a ratio of 1:1. Ethyl acetate solvent is able to attract the most secondary metabolites and has the highest activity compared to other extracts (Sulistyani et al., 2014). After being cornered for 30 minutes and allowed to stand for a while, after that 2 layers will form in the split funnel, the top layer is evaporated until a fermented isolate extract is obtained and then the next test is carried out.

Next, the identification test of Thin Layer Chromatography (KLT). Ethyl acetate fermentate extracts IARK-6 and IARK-7 were tolerated onto the KLT plate after which they were eluted using chloroform eluent: methanol (5: 1). To see the pattern of separation of chromatographic stains detected by visible detection, namely with the direct eye, under UV lamps 254 nm and 366 nm.

In KLT-Bioautography testing the contact method is used because it is easier, simpler, and most commonly used. This method can also transfer active compounds to the medium in order can produce greater inhibitory power compared to direct bioautography methods that are easily contaminated and overlay bioautography (immersion) which is difficult to observe the inhibitory zone (Djije et al., 2008). The results of the antibacterial activity test of Actinomycetes isolates of the rhizosphere of kasumba turate (*Carthamus tinctorius* L.) by KLT-Bioautography can be seen in **Table 3**.

KLT-Bioautography test results for isolates with IARK-6 obtained spots with Rf = 0.70 values for *Staphylococcus aureus* bacteria, Rf_1 = 0.70, and Rf_2 = 0.38 for *Staphylococcus epidermidis* bacteria, Rf = 0.70 for *Pseudomonas aeruginosa bacteria.* While the results of KLT-Bioautography testing for IARK-7 isolates obtained spots with a value of Rf = 0.38 for *Staphylococcus aureus* bacteria, Rf = 0.70 for *Staphylococcus epidermidis* bacteria, Rf = 0.38 for *Pseudomonas aeruginosa bacteria.* Rf values that have met the requirements of a good Rf value are between 0.2-0.8 (Rohman et al., 2009).

From the results obtained, it can be concluded that two isolates of Actinomycetes rhizosphere kasumba turate (*Carthamus tinctorius* L.) namely IARK-6 and IARK-7 have antibacterial activity so they have potential antibacterial activity.

Table 1. Microscopic test results of isolates of Actinomycetes rhizosphere kasumba turate (*Carthamus tinctorius* L.) with Gram painting.

Isolate Code	Gram Staining
IARK -1	Gram Positive
IARK - 2	Gram Positive
IARK - 3	Gram Positive
IARK - 4	Gram Positive
IARK - 5	Gram Positive
IARK - 6	Gram Positive
IARK - 7	Gram Positive

Table 2. Antagonist test results of antibacterial isolates of Actinomycetes rhizosphere kasumba turate (*Carthamus tinctorius L.*)

Code Isolates	Inhibitory Zone Diameter (mm)		
	SA	ONE	PA
IARK - 1	17,69	16,45	13,39
IARK - 2	12,30	13,19	15,94
IARK - 3	16,67	10,67	16,34
IARK - 4	16,71	17,51	14,26
IARK - 5	16,85	17,67	15,18
IARK - 6	17,00	17,68	17,80
IARK - 7	17,16	17,52	17,75

Table 3. Antibacterial activity test results of Actinomycetes isolates of the rhizosphere of kasumba turate (*Carthamus tinctorius* L.) by KLT-Bioautography using chloroform eluent: methanol (5: 1)

Isolate Code	Bacteria	Patches	Rf	Color of spotting appearance	
				UV 254 nm	UV 366 nm
IARK 6	*Staphylococcus aureus*	1	0,70	Blue	Purple
	Staphylococcus epidermidis	1 2	0,70 0,38	Blue Fluorescent blue	Purple Fluorescent purple
	Pseudomonas aeruginosa	1	0.70	Blue	Purple
IARK 7	*Staphylococcus aureus*	1	0,38	Fluorescent blue	Fluorescent purple
	Staphylococcus epidermidis	1	0,70	Blue	Purple
	Pseudomonas aeruginosa	1	0,38	Fluorescent blue	Fluorescent purple

4. CONCLUSION

Based on the results of research on the antibacterial activity of Actinomycetes isolates from the rhizosphere of the kasumba turate plant (*Carthamus tinctorius* L.) against bacteria tests that cause skin infections that have been carried out, it can be concluded that: Antibacterial isolates Actinomycetes rhizosphere of kasumba turate (*Carthamus tinctorius* L.) as many as seven isolates, of which two namely IARK-6 and IARK-7 have the greatest antibacterial activity in inhibiting the growth of test bacteria that cause skin infections, namely *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa.* Bioautogram profile of fermented antibacterial isolate of Actinomycetes rhizosphere of kasumba turate (*Carthamus tinctorius* L.) IARK-6 obtained spots with Rf = 0.70 values for *Staphylococcus* aureus bacteria, Rf_1 = 0.70 and Rf_2 = 0.38 for *Staphylococcus epidermidis* bacteria, Rf = 0.70 for *Pseudomonas aeruginosa bacteria.* KLT-Bioautography test results for IARK-7 isolates obtained spots with Rf = 0.38 values for *Staphylococcus aureus* bacteria, Rf = 0.70 for *Staphylococcus epidermidis* bacteria, Rf = 0.38 for *Pseudomonas aeruginosa* bacteria.

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Ethical Approval: -

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