ANTIMICROBIAL ACTIVITY OF KETAPANG LEAVES (*Terminalia cattapa* L.) ON GILLS DISEASE OF MUJAIR FISH (*Oreochromosis mossambicus*)

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Abstract

Antibiotic residue and resistance in cultivation of freshwater fish such as Mujair were problems that cause some experts to looking for a more natural and safe materials in dealing with it. Ketapang leaves (Terminalia cattapa L.) can be used empirically for microbial prevention in freshwater fish cultivation. The purpose of this study was to prove scientifically that fresh Ketapang leaves extract (Terminalia cattapa L.) may be used as an antimicrobial agent against infections on Mujair fish's gills. Stratified extraction using non-polar (n-hexane), semi-polar (ethyl acetate) and polar (ethanol) solvent was used to get viscous extracts of Ketapang leaves. Viscous extracts from each solvent were tested for antimicrobial activity against bacteria isolated from the gills, water pool and identified bacteria for comparison. The results showed that n-hexane extract has minimal inhibitory concentration of 2.5%, ethyl acetate extract of 0.6% and ethanol extract of 0.6% respectively. From this research, we can conclude that each ketapang fresh leaves extract has a good antimicrobial activity on microbes that cause gills disease in mujair fish.

Key word : *Antimicrobial, ketupang leaves*

ISBN : 978-979-18458-5-4

INTRODUCTION

Freshwater fish farming activities have difficulties in the maintenance of environmental pollution and food that may cause these fish to become sick. One of the existing fish disease is a disease derived from microorganisms such as bacteria and fungi. Common microbes that cause disease in freshwater fish (such as Mujair, Lele, Nila, Gurame, Patin and Mas) were Aeromonas sp., Pseudomonas sp., Staphylococcus sp. and Streptococcus sp.

To overcome the incidence of fish diseases due to microbes, several ways have been conducted, such as the use of antibiotics. The emergence of microbial resistance problems of freshwater fish farming due to the use of antibiotics that do not use the dosage regimen properly, encouraged some experts to seek antimicrobial compounds without resistency side effects. More natural ingredient were seek in overcoming fish illness due to microbial, such as using plants that have efficacy as an antimicrobial agent and safe in use.

Ketapang (Terminalia cattapa L.) is a plant that has antimicrobial compound. This plant empirically has been used to overcome the bacterial problem in freshwater fish farming. This research was to prove the antimicrobial activity of Ketapang leaves scientifically.

MITHODS

Materials

Ketapang fresh leaves (Terminalia cattapa L.), Mujair fish (infected in the gills), Nutrient Agar, phosphate buffer solution (LDF), Sabouraud Dextrose Agar (SDA), peptone broth, ethanol, n-hexane, ethyl acetate, phytochemical screening reagents .

Apparatus

Petri dishes, test tubes, Erlenmeyer, volume pipette, tweezers, sengkelit, Bunsen flame, analytical scales, blender, oven, autoclave, Laminar Air Flow, Vortex, filter paper, glass beaker, measuring cup, dropper drops the cup vaporizers, vacuum Rotavapor, extraction flask, stir bar, filter paper, incubator, filter paper, LAF, long slide.

Principles

Ketapang leaves (Terminalia cattapa L.) were determined in "Herbarium Bogorinse", Balitbang Botanical Center for Biology-LIPI, Bogor, Cibinong. Then The leaves extracted by maceration method, stratified by using 3 kinds of solvents: n-hexane (non polar), ethylacetate (semipolar), and ethanol (polar). Condensed extracts from each solvent were tested for its antibacterial activity against bacteria isolated from the gills of Mujair fish which were infected. n-hexane (non polar), ethylacetate (semipolar), and ethanol (polar) were used as blank. The agar diffusion method was used for antimicrobial activity assay. The diameter of inhibitory zones that resulted after the incubation time were then measured and recorded.

This research was to determine the antimicrobial activity of Ketapang leaves extracts using the well diffusion agar method against microbial isolated from Mujair fish gills. The plates were then incubated at 37°C for 24 hours. The diameter of clear zone that appear around the well were observed and recorded in millimeters (mm). Test results will be compared with the antibacterial activity of the solvent that used in extraction method as a negative control (blank).

Isolation of microbes on fish disease

Isolation of microbes from the gills of infected fish

Streak plate method procedure was used to isolated microbes from the infected fish's gills. The isolates were grown at Nutrient Agar (NA) media for bacteria and Sabouraud Dextrose Agar (SDA) media for fungi. The plates were then incubated at 35 ± 1 ? C for 24-48 h (bacteria) and at 20-25 ? C for 5 -7 days (fungi). After

incubation, each of the separated colony was streaked on a new agar plate to get pure isolates.

Isolation of microbes from the infected water pond

Pour plate method was done to get the isolates. A total of 25 ml of infected water pond was put into a sterile container which contained 225 ml of diluent solution LDF, and then homogenized. This procedure was done in duplicate. 1,0 mL of this mixture was then pour into 15-20 ml of Nutrient agar (bacteria) and Sabouraud Dextrose Agar (fungi), then homogenized. The plate were then incubated at 35 ± 1^{o} C for 24-48 h (bacteria) and at 20-25^{o}C for 5-7 days (fungi).

After incubation, each of the separated colony was streaked on a new agar plate to get pure isolates.

Antimicrobial activity test

To 1.0 mL of bacterial suspension with turbidity of 25% Transmittance was added NA (bacteria) and SDA (fungi) medium and homogenized. Make a hole wells at the solidified bacterial agar. Place the extracts and blanks at each hole. The plates were then incubated at 37°C for 48 h (bacteria) and 20-25°C for 5-7 days (fungi). All of the work were done in Laminar Air Flow (LAF) cabinet. After incubation, the presence or absence of clear zone (*inhibitory region*) on bacterial growth around the wells were observed. The antimicrobial activity of each extrats test and blanks were determined with the presence of clear zone/inhibition zone around the wells.

RESULTS AND DISCUSSION

Phytochemical screening of Ketapang fresh leaves (Terminalia cattapa L.)

Table I. athe Comporwid of Ketapang Leaves

	Fresh leaves	n-hexane extrt	ethyl acetate extrt	ethanol extract
Alkaloids	-	-	-	-
Flavanoid	+	+	+	+
Saponin	+	-	+	+
Tannins	+	-	+	+
Quinone Steroid /	+	-	+	+
Triterpenoid	+	+	+	+
Coumarin	-	-	-	-
Essential oils	-	-	-	-

Description:
+ : Indicates a positive result for the compound
- : Indicates a negative result of the compound

Extract	Micro-organisms			
	Isolates from the gills	Isolates from the water pond	Fungi	Bacteria
n-heksan	2,5 %	5%	7,5%	2,5%
Ethyl cetate	0,6%	0,6%	0,6%	0,6%
Ethanol	2,5 %	1,25%	7,5%	0,6%

Phytochemical screening:

Minimum Inhibitory Concentration (MIC)

From these results it could be seen that the whole leaves extracts of fresh Ketapang can be used as an antimicrobial agent against bacteria isolated from the fish's gill, water ponds, fungi (*Aspergilus niger* and *Candida albicans*) and identified comparative bacteria (Bacillus subtilis and Pseudomonas aeroginase)

CONCLUSION

Minimal Inhibitory Concentration of non-polar (n-hexane) extract of fresh Ketapang leaves to bacteria isolated from fish's gills and to identified comparative bacteria was of 2.5%, to the bacteria that isolated from pond water was 5%, and to the fungi was 7.5%.

Minimal Inhibitory Concentration of semipolar (ethyl acetate) extract of fresh Ketapang leaves to the bacteria that isolated from fish's gills, pond water, and comparative fungi and bacteria were 0.6%.

Minimal Inhibitory Concentration of polar (ethanol) extracts of fresh Ketapang leaves to bacteria that isolated from pond water was of 1.25% and to bacteria isolated from the fish's gills was 2.5%.

As conclusion, the non-polar (n-hexane), semipolar (ethyl acetate) and polar (ethanol) extracts of fresh Ketapang leaves have antimicrobial activity, not only to bacteria isolated from the gills of fish and pond water, but also to the comparative microbes.

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