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Inhibition Test of *Uncaria cordata* (Lour.) Merr Extract Against Pathogenic Bacteria of Freshwater Fish In Vitro

Maryani[1]*, Mohamad Rozik[1], Milad Madiyawati[1], Yuli[2]

[1]Faculty of Agriculture, University of Palangka Raya
[2]State Vocational School 8 Sidrap

***Correspondence**:
maryani@fish.upr.ac.id

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ABSTRACT

Uncaria is a genus of plants that have medicinal properties and chemical content. *Uncaria cordata* (Lour.) Merr is a herbal plant that is still rarely known for its efficacy in herbal medicinal ingredients. *Uncaria cordata* (Lour.) Merr it self contains metabolic compounds in the form of flavonoid, phenolic, steroid, saponin, terpenoid and alkonoid. This study aims to test the effectiveness of *Uncaria cordata* (Lour.) Merr as an antimicrobial on *Aeromonas hydrophila*, *Pseudomonas* sp. and *Edwardsiella* ect.) Merr as an antimicrobial on *Aeromonas hydrophila*, *Pseudomonas* sp. and *Edwardsiella ictaluri* bacteria and the type of bacteria that are more effective in using *Uncaria cordata* (Lour.) Merr in inhibiting the proliferation of disease-causing bacteria in cultured fish. The results showed that *Uncaria cordata* (Lour.) Merr, was able to inhibit the proliferation of bacteria *Aeromonas hydrophila*, *Pseudomonas* sp. and *Edwardsiella ictaluri* with an average diameter of the inhibition zone of *Aeromonas hydrophila* bacteria at a concentration of 100% forming an inhibition zone of 21.7 mm with a very strong inhibition zone category, in the bacteria *Pseudomonas* sp. at 100% concentration formed an inhibition zone of 21 mm with a very strong inhibition zone category, and in *Edwardsiella ictaluri* bacteria at 100% concentration formed an inhibition zone of 21.3 mm with a very strong inhibition zone category. The 100% concentration of *Uncaria cordata* (Lour.) Merr is the most effective concentration to inhibit bacterial proliferation.

INTRODUCTION

Disease attack in fish is one of the main obstacles that can threaten because it can harm aquaculture businesses such as decreased production, decreased water quality and even total death. (Ashari et al., 2014). Diseases in fish do not just appear and attack fish directly. Fish diseases arise due to a mismatch between fish as pathogen hosts (disease-causing microorganisms) and the environment (Iqbal, 2016). The defense system of the fish body can be disrupted due to environmental changes and the development of pathogens in aquaculture containers. Some pathogens that attack fish are Aeromonas hydrophila, Pseudomonas sp. and

Edwardsiella ictaluri. These bacteria cause disease in fish in the form of systemic disorders that cause high mortality rates in fish, and can attack cultured fish. This disease can spread to other areas in a short time (Sari, 2015).

Efficient efforts can be made in handling diseases caused by pathogenic bacteria in aquaculture activities by using natural ingredients in the surrounding environment. Uncaria is a genus of plants that have medicinal properties and chemical content because some of them have been used in traditional medicine. The part of this plant that is widely used in medicine is its root. The root (Bajakah in Dayak language) is widely used by the Dayak people in medicine, some Uncaria species are also known as bajakah. Based on the results of a literature search, it was found that there are at least five species of Uncaria found in Kalimantan. The four Uncaria species are *Uncaria cordata* (Lour.) Merr, *Uncaria longiflora*, *Uncaria nervosa*, and *Uncaria tomentosa* (Erwin, 2020).

Uncaria cordata (Lour.) Merr is a herbal plant that is still rarely known for its efficacy in herbal medicine. Uncaria cordata (Lour.) Merr itself contains metabolite compounds such as flavonoids, phenolics, steroids, saponins, terpenoids and alkaloids (Saputra, 2018). In addition, phytochemical tests that have been carried out on Uncaria cordata (Lour.) Merr root bark and wood extracts show that this plant contains secondary metabolites in the form of alkaloids, flavonoids, and terpenoids. These compounds have potential as antibacterials (Maulina *et al*., 2019). However, there has been no research on the antibacterial activity test of Uncaria cordata (Lour.) Merr) against bacteria that cause fish diseases in aquaculture activities. To prove this scientifically, antimicrobial testing was carried out against three pathogenic bacteria namely *A. hydrophila*, *Pseudomonas* sp. and *E. ictaluri*. This study aims to test the effectiveness of *Uncaria cordata* (Lour.) Merr as an antimicrobial scientifically tested against these pathogenic bacteria.

METHODS

Time and Place of Research

The research was conducted from July to September 2023 and was carried out at the Fish Quarantine Station, Quality Control and Safety of Fishery Products on Jl. Adonis Samad, Panarung, Kec. Pahandut, Palangka Raya City, Central Kalimantan.

Research Tools and Materials

The tools used in the study are as follows: Analytical Balance, paper discs, Petri dishes, test tubes, autoclave, oven, laminary flow, ose needle, Bunsen, incubator, caliper, micropipette, blender, stationery, camera, vortex and Mc Farland 0.5, while the materials used were: *Uncaria cordata* (Lour.) Merr, *Aeromonas hydrophila*, *Pseudomonas* sp., *Edwardsiella ictaluri*, Nutrient Agar (NA), TSA, Mueller Hinton Agar (MHA), Aquadest, Cotton Swab.

Research Procedure

Media Preparation

Agar media is used as a medium for growing bacteria, agar media powder NA (Nutrient Agar) as much as 8 g dissolved with 400 ml of distilled water, MHA (Mueller Hinton Agar Medium) as much as 38 g dissolved with 1 liter of distilled water. Each ingredient is dissolved in an erlenmeyer, then stirred and heated using a hot plate. After the media is completely dissolved, it is then sterilized using an autoclave at 121°C for 15 minutes. After completion, wait for the media temperature to drop to 40-50 ℃ and then ready to be poured into the cup. The ready media is left at room temperature for 24 hours and then put into the refrigerator.

Preparation of *Uncaria cordata (*Lour.) Merr*)* Extract (Maceration Method)

Uncaria cordata (Lour.) Merr) in fresh form was cleaned then dried in the sun with a black cloth covering. The dried *Uncaria cordata* (Lour.) Merr) was then blended into powder. A total of 100 g of *Uncaria cordata* (Lour.) Merr) powder was put into a dark vessel, then 750 mL of 96% ethanol solvent was added and sealed. Soak the *Uncaria cordata* (Lour.) Merr) solution for 3 days while stirring every 8 hours and then filtered and obtained maserat 1. The dregs of the filtered maserat 1 were soaked again with 250 mL of 96% ethanol for 1 day, filtered again and obtained maserat 2. Maserat 1 and 2 were mixed and precipitated overnight then separated from the residue and concentrated by evaporation to obtain a thick ethanol extract (Puspitasari, 2016).

Preparation of Test Bacteria Solution

Pure culture of bacteria dissolved into 10 ml of 0.85% sodium chloride (NaCl) solution, then homogenized. The solution was compared with the McFarland standard turbidity of 0.5 to obtain a standardized inoculum suspension of 10^8 cfu/ml (Asifa, 2014).

Effectiveness Test of *Uncaria cordata* (Lour.) Merr Extract

Test the effectiveness of *Uncaria cordata* (Lour.) Merr in inhibiting the proliferation of bacteria *A. hydrophila, Pseudomonas* sp. and *E. ictaluri*. using the Kirby Bauer method or paper disc agar diffusion method. This method is to determine the activity of antimicrobial agents. Discs containing antimicrobial agents are placed on agar media that have been planted with microorganisms that will diffuse on the agar media. A clear area indicates the inhibition of microorganism proliferation by the antimicrobial agent on the agar surface.

Observation

Observations in the study were made after 1x24 hours of incubation period. The diameter of the inhibition zone was measured using a caliper in millimeters (mm). Measurement of the inhibition zone distance starts from the edge of the test well to the circular boundary of the inhibition zone. The diameter of the inhibition zone was categorized based on its anti-bacterial strength according to Susanto *et al*. (2012).

Table 1. Category of Bacterial Inhibition Zone according to Susanto *et al*. (2012)

Diameter	Strength Inhibition
≤ 5 mm	Weak
6-10 mm	Medium
11-20 mm	Strong
≥ 21 mm	Very Strong

Research Design

The study used the Randomized Group Design method with 3 groups, 5 treatments of *Uncaria cordata* (Lour.) Merr), and 3 replicates. The treatment used was by placing paper discs containing *Uncaria cordata* (Lour.) Merr) on media containing bacteria *A. hydrophila, Pseudomonas sp.,* and *E. ictaluri*.

Treatment:

P1: 0% concentration
P2: 25% concentration
P3: 50% concentration
P4: 75% concentration
P5: 100% concentration

Group:

Group 1: *Aeromonas hydrophila bacteria*
Group 2: *Pseudomonas sp. bacteria*
Group 3: *Edwardsiella ictaluri bacteria*

Data Analysis

Data were obtained descriptively-quantitatively through recording the results of bacteria *A. hydrophila, Pseudomonas* sp. and *E. ictaluri* after being treated with *Uncaria cordata* (Lour.) Merr. Data were presented descriptively in the form of tables and figures. Data were processed with computer software SPSS (Statistical Program for Social Science) for windows. Data analysis used One Way Anova statistical test with α=0.05 and LSD (Least Significant Difference).

RESULTS

From the results of research that has been carried out on the effectiveness of *Uncaria cordata* (Lour.) Merr, in inhibiting the proliferation of bacteria *A. hydrophila, Pseudomonas* sp., *E. ictaluri* with the treatment of P1 (0% concentration), P2 (25% concentration), P3 (50% concentration), P4 (75% concentration), and P5 (100% concentration), the results of the inhibition zone are obtained in Figure 1 below.

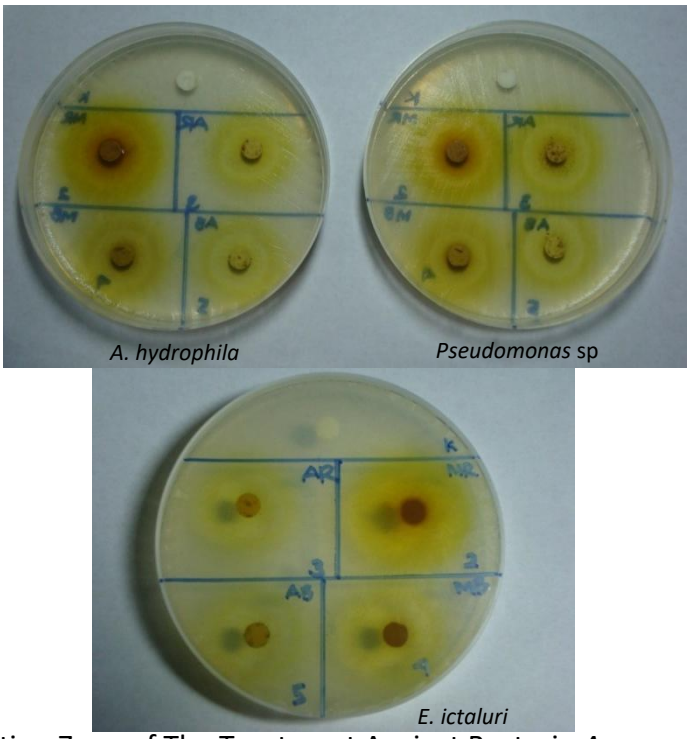

Figure 1. Inhibition Zone of The Treatment Against Bacteria *Aeromonas hydrophila, Pseudomonas sp, Edwardsiella ictaluri*

Figure 1. shows the inhibition zone formed by the treatment of *Uncaria cordata* (Lour.) Merr) against the proliferation of bacteria *A. hydrophila, Pseudomonas sp.,* and *E. ictaluri*. Measurement of the inhibition zone formed from the concentration of *Uncaria cordata* (Lour.) Merr) can be seen in Table 2 below.

Table 2. Average Diameter of Inhibition Zone of *A. hydrophila, Pseudomonas sp.* and *E. ictaluri*

Bacteria	No	Concentration	Diameter (mm)			Average Diameter (mm)	Categories
			I	II	III		
Aeromonas hydrophila	1	Aquades	0	0	0	0	Weak
	2	25%	17	17	18	17,3	Strong
	3	50%	18	16	18	17,3	Strong
	4	75%	19	21	20	20	Strong
	5	100%	22	22	21	21,7	Very Strong
Pseudomonas sp.	1	Aquades	0	0	0	0	Weak
	2	25%	14	14	13	13,7	Strong
	3	50%	16	15	15	15,3	Strong
	4	75%	18	18	17	17,7	Strong
	5	100%	21	21	21	21	Very Strong
Edwardsiella ictaluri	1	Aquades	0	0	0	0	Weak
	2	25%	13	14	12	13	Strong
	3	50%	14	14	15	14,3	Strong
	4	75%	17	16	17	16,7	Strong
	5	100%	22	21	21	21,3	Very Strong

Table 2 above shows that the average diameter of the highest inhibition zone is in treatment P5 with a concentration of 100% on bacteria *A. hydrophila* by 21.7 mm, on bacteria *Pseudomonas* sp. by 21 mm, and on bacteria *E. ictaluri* by 21.3 mm, while the lowest average is in treatment P1 by 0 mm.

The results of the analysis of variance (ANOVA) to determine the effect of the treatment given that bacteria and treatment significantly affect the inhibition zone (P value <0.05). There is a significant interaction between the type of bacteria and the treatment given to the bacterial inhibition zone. So, it was concluded that the effectiveness of *Uncaria cordata* (Lour.) Merr) had a significant effect in inhibiting the proliferation of bacteria *A. hydrophila, Pseudomonas* sp., and *E. ictaluri* (H0 rejected, H1 accepted), then continued with Duncan's further test.

The results of Duncan's further test on the average diameter value of the inhibition zone in the treatment showed the sig. value of each treatment which was greater than $\alpha = 0.05$. The P2, P3, P4 and P5 treatments gave the same effect on the inhibition zone and were different from the P1 treatment. The results of Duncan's further test for groups showed no significant difference, indicated by the formation of one subset and each rp value that filled in each subset for measurement at the $\alpha = 0.05$ level.

DISCUSSION

In Figure 1 and Table 2 above, it can be seen that *Uncaria cordata* (Lour.) Merr at all concentrations can inhibit the proliferation of bacteria *A. hydrophila. Pseudomonas* sp. and *E. ictaluri*. The zone of inhibition formed on each disc paper containing *Uncaria cordata* (Lour.) Merr has inhibitory power on these 3 bacteria. The largest zone of inhibition was produced at a concentration of 100%, both on *A. hydrophila* bacteria and on *Pseudomonas sp*. and *E. ictaluri* bacteria. The diameter of the inhibition zone from the extract treatment at 100%

concentration, *A. hydrophila* bacteria had a higher diameter compared to *Pseudomonas* sp. and *E. ictaluri* bacteria. Referrence to Susanto *et al.* (2012) in Soedrajat *et al.* (2022), showed that the 100% concentration extract is included in the very strong category in all types of bacteria, and the treatment of 75%, 50% and 25% concentration extracts is included in the strong category, while the treatment of 0% concentration extract, is included in the weak category.

The antibacterial activity of *Uncaria cordata* (Lour.) Merr) against *A. hydrophyila* bacteria was higher than that against *Pseudomonas* sp. and *E. ictaluri* bacteria at all concentrations. This shows that each bacterium has a different response to antibacterial compounds. As stated by Rio (2012) that each bacterium has the ability to respond to each stimulus depending on internal and environmental factors. The results showed that the greater the concentration of *Uncaria cordata* (Lour.) Merr), the greater the diameter of the inhibition zone formed. In line with the opinion of Sugiantina *et al*. (2022) stated that the higher the concentration, the more active substances contained therein and the greater the potential of the extract in inhibiting bacterial proliferation and vice versa, if the lower the concentration of the extract used, the lower the potential of the extract in inhibiting bacterial proliferation. This is because the increase in concentration will be followed by an increase in the concentration of bioactive compounds with higher antibacterial effects (Simanungkalit *et al*., 2020; Rahayu *et al*., 2023).

The existence of antibacterial activity of *Uncaria cordata* (Lour.) Merr, because it contains indole alkaloid compounds, triterpenes, flavanoids, phenols and phenylpropanoids (Zhang *et al*., 2015). The same thing in research conducted by Abdullah et al, (2016) found that U. cordata obtained 10 compounds with diverse structures consisting of three flavanoids: quercetin (1), kaempferol (2) and taxifolin (3), three phenolic acids: 2-hydroxybenzoic acid or (4) 2,4 dihydroxybenzoic acid (5), 3,4-dihydroxybenzoic acid (6), two coumarins: scopotelin (7), 3,4 dihydroxy-7-methoxycoumarin (8), 1 iridoid glycoside: loganin (9) and 1 sterol: β-sitosterol (10). In addition, the results of research by Rahmawati *et al.* (2016) state that the compounds obtained from isolation of *Uncaria cordata* are terpenoids and have very strong category cytotoxic activity, namely 2.57 pµg/mL.

Alkaloid as anti-bacterial is by disrupting the components that make up the peptidolikan in bacterial cells, so that the bacterial cell wall layer is not formed intact and causes cell death. The mechanism of flavonoid compounds as antibacterial is by denaturing bacterial cell proteins, damaging the cytoplasmic membrane, and inhibiting bacterial energy metabolism by inhibiting the use of oxygen by bacteria (Sadino, 2017; Sapara *et al*., 2016; Apriyanto *et al*., 2013). Phenols and proteins form hydrogen bonds that cause the protein structure to be damaged. Where hydrogen bonds will affect the permeability of cell walls and cytoplasmic membranes, so that the permeability of cell walls and cytoplasmic membranes is disrupted there will be an imbalance in the cell which causes the cell to lysis (Rijayanti, 2014).

Saponin compounds then interact with cell phospholipid membranes that are impermeable (objects do not allow to pass through) to lipophilic or non-polar compounds, causing the integrity of the cell membrane to decrease, changes in the morphology of the bacterial cell membrane, and finally the cell membrane will be fragile and eventually die (Rinawati, 2011).

The mechanism of triterpenoids contained in the Kaik-Kaik Root plant (*Uncaria cordata* (Lour.) Merr, which reacts with porins or transmembrane proteins located on the outer membrane of the bacterial cell wall, then forms strong polymer bonds and causes damage to porins. Damaged porins will reduce the permeability of the bacterial cell membrane and cause

bacterial cells to lack nutrients, so that bacterial proliferation is inhibited or dies (Wulandari *et al*., 2020). Triterpenoid compounds that have antimicrobial activity include borneol, sineol, pinene, kamfene, and kamfor (Conner *et al*. 1993).

CONCLUSION

The conclusions of this study are:

1. *Uncaria cordata* (Lour.) Merr, is able to inhibit the proliferation of bacteria *Aeromonas hydrophila*, *Pseudomonas* sp. and *Edwardsiella ictaluri* with the average diameter of the inhibition zone of Aeromonas hydrophila bacteria at 100% concentration forming an inhibition zone of 21.7 mm with a very strong inhibition zone category, in Pseudomonas sp. bacteria. at 100% concentration formed an inhibition zone of 21 mm with a very strong inhibition zone category, and *in Edwardsiella ictaluri* bacteria at 100% concentration formed an inhibition zone of 21.3 mm with a very strong inhibition zone category.
2. The 100% concentration of *Uncaria cordata* (Lour.) Merr is the most effective concentration to inhibit bacterial proliferation.

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