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Antibacterial Activity of *Bauhinia sirindhorniae* Extract Against *Aeromonas hydrophila* Isolated from Hybrid Catfish

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Abstract

The studies on antibacterial activity of *Bauhinia sirindhorniae* extract, an indigenous plant of Nong Khai province, against *Aeromonas hydrophila* isolated hybrid catfish were assayed. Root, stem, leaves and flowers of *B. sirindhorniae* were extracted by acetone, ethanol 50 %, ethanol 95 % and methanol. In antibacterial screening performed by disc method was conducted. The results revealed that the highest activity against *A. hydrophila*, found in roots extract using acetone with an inhibition zone, was 20.33 ± 0.58 mm. The minimum inhibitory concentration (MIC) for *B. sirindhorniae* extracts were also determined by the broth dilution method. The lowest MIC value with high efficacy against *A. hydrophila* was 97.66 ppm which could be obtained from the root extract of *B. sirindhorniae* from acetone. The minimum bactericidal concentrations (MBC) of *B. sirindhorniae* extracts were also determined. The determination of MBC was not observed in any concentration of *B. sirindhorniae* extracts.

Keywords: Antibacterial, *Bauhinia sirindhorniae* extract, indigenous plant, *Aeromonas hydrophila*, hybrid catfish

Introduction

Hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) is one of the most important commercial fish species in the aquaculture industry in Thailand. Disease outbreaks are recognized as a significant constraint to aquaculture production and trade, affecting both the economic development and socioeconomic revenue of the sector in many countries in the world. *Aeromonas hydrophila* is among the most common bacteria in freshwater fish culture throughout the world [1]. *A. hydrophila* is an opportunistic pathogen, Gram negative bacteria and facultative anaerobic short bacillus, and causes high mortality in cultured and feral fish. Haemorrhagic septicemia, ulcer disease or red-sore disease is caused by *A. hydrophila*. It has been various symptoms such as hemorrhagic septicemia, scale protrusion, ulceration and infectious abdominal dropsy [2]. Antibiotic treatment is the most common way of controlling disease problems especially regarding bacterial infection including diseases caused by *A. hydrophila*. However, over-use and inappropriate usage of antibiotics in aquaculture are an increasing risk and potentially damaging to aquatic animals, consumers and environment. Antibiotic residues in animal tissues, aquatic environment and the development of antibiotic resistant strains of bacteria, for instance, causes increased difficulty in treating diseases of aquaculture stock.

Nowadays, aquaculturists have paid attention to extracts and biologically active compounds isolated from herbal medicine. Antimicrobial activities of medicinal herbs are being increasingly reported from different parts of the world [3]. Herb extracts are becoming more popular due to them being safer than chemical products. Herbs also play the role of an immunostimulant, conferring early activation to the non-specific defense mechanisms and elevating the specific immune response [4]. Additionally, herbs extracts have a

potential application as an immunostimulant in fish culture because they can be easily obtained, can be given orally, and are not expensive and act against a broad spectrum of pathogens [5].

Bauhinia sirindhorniae K. & SS. Larsen, a tendrilled liana, locally known in Thai as Sirindhorn-vallee, is an indigenous plant of northeastern of Thailand especially in Nong Khai province [6,7]. The antibacterial activity of *B. sirindhorniae* extracts and its pure compounds against *Bacillus subtilis* and *Staphylococcus aureus* were assayed. The results revealed that *B. sirindhorniae* extracts and its active compounds exhibited antibacterial activity as evident from MIC and MBC values [8,9]. The antibacterial studies of this herb were investigated against some common human pathogenic bacteria. The aim of present study was to analyze the antibacterial activities of *B. sirindhorniae* against *Aeromonas hydrophila* (fish pathogenic bacteria) isolated hybrid catfish.

Materials and methods

Herbal extract

Bauhinia sirindhorniae K. & SS. Larsen were collected from Nong Khai province, Thailand. The samples of *B. sirindhorniae* were identified by morphology followed by Larsen and Larsen [6]. Stems, roots, leaves and flowers were dried, powered and immersed in 4 different solvents including acetone, 50 % ethanol, 95 % ethanol and methanol (1:6 w/v) and kept at room temperature for 7 days. The extracts of each sample were centrifuged and then filtered using Whatman no: 1 filter paper. The filtrate of each sample was collected and then evaporated using a rotary vacuum evaporator and stored at 4 °C until needed.

Bacteria preparation

Aeromonas hydrophila was isolated from hybrid catfish by streak plate technique on Tryptic Soy Agar (TSA; Himedia). The species of bacteria was identified by morphology using Gram's stain and biochemical properties using API 20E strip (Biomerieux). The bacteria strain was cultured on TSA at 37 °C overnight. The isolated colonies were cultivated in Tryptic Soy Broth (TSB; Himedia) and incubated with a shaker at 34 °C for 18 h. The turbidity of bacteria was adjusted to equivalent to 0.5 McFarland turbidity standards No.1 (3.0×10^8 CFU/ml).

Antimicrobial activity by disc diffusion method

Antibacterial activity of the extracts against *A. hydrophila* was performed by disc diffusion method [10]. Briefly, the prepared bacteria were inoculated onto a Mueller Hinton Agar (MHA; Himedia) surface and distributed evenly with a sterile L-shaped glass rod. The sterile paper discs of 5 mm diameter were placed on inoculated agar plate and then 20 µl of each herbal extract was impregnated on the disc. In the same way positive control disc was also prepared by using each solvent (acetone, 50 % ethanol, 95 % ethanol and methanol). The antimicrobial activity was evaluated by measuring the diameter of inhibition zone surrounding the discs where no growth occurred. All tests were carried out in triplicate and expressed in millimeters.

Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of crude extracts against *A. hydrophila* were determined by serial 2 fold dilution [10]. Briefly, 1 ml of TSB and 1 ml of graded doses of crude extracts were added to each test tube. After that, 1 ml of suspended *A. hydrophila* was inoculated to these test tubes followed by incubation at 37 °C for 24 h. The test tubes showed as well as clear content were selected that showing suppressed growth of bacteria. The lowest or minimum concentration was considered as MIC. Two test tubes including extract and bacteria and medium and bacteria were used as negative control and positive control, respectively. All inhibitory concentrations were analyzed by adding each test tube showing activity into agar plate and incubated at 37 °C for 24 h. The lowest concentration of the extract which kills *A. hydrophila* is defined as MBC.

Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) test. Significant level was set at $P < 0.05$.

Results and discussion

Antimicrobial activity by disc diffusion method

The results of antimicrobial activity assay of all crude extracts with different solvents against *A. hydrophila* are presented in **Table 1**. Highly significant antibacterial activity was observed in the roots extract using all solvents. The acetone extract showed the highest activity with an inhibition zone of 20.33 ± 0.58 mm followed by 50 % ethanol, 95 % ethanol and methanol with inhibition zones of 15.67 ± 0.58, 15.00 ± 2.00 and 15.00 ± 1.00 mm, respectively. The lowest activity was found in the acetone extract of the flowers.

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The MIC of *B. sirindhorniae* extracts with different solvents against *A. hydrophila* is shown in **Table 2**. The results revealed that the MIC of the roots extract using acetone was 97.66 ppm followed by the acetone extract of the stems, 781.25 ppm. The determination of MBC was not observed in any concentration of the extracts.

Table 1 Antimicrobial activity of B. *sirindhorniae* extracts with different solvents against *A. hydrophila*.

Herbs	Solvents			
	Diameter of inhibition zone (mean ± SD, mm)			
	Acetone	**50 % Ethanol**	**95 % Ethanol**	**Methanol**
Roots	20.33 ± 0.58^{c}	15.67 ± 0.58^{c}	15.00 ± 2.00^{c}	15.00 ± 1.00^{c}
Stems	13.67 ± 0.58^{b}	11.67 ± 0.58^{b}	14.67 ± 0.58^{c}	14.67 ± 0.58^{c}
Leaves	12.67 ± 0.58^{b}	11.67 ± 0.58^{b}	10.67 ± 0.58^{a}	13.67 ± 1.15^{bc}
Flowers	10.00 ± 1.00^{a}	11.00 ± 0.00^{b}	11.67 ± 0.58^{ab}	12.67 ± 0.58^{b}
Control	13.00 ± 0.00^{b}	9.33 ± 0.58^{a}	13.00 ± 1.00^{bc}	10.67 ± 0.58^{a}

Remark: Different letters in the vertical line are different significantly ($P < 0.05$)

Table 2 The minimal inhibitory concentration of B. *sirindhorniae* extracts with different solvents against *A. hydrophila*.

Herbs	Solvents			
	Minimal inhibitory concentration (ppm)			
	Acetone	**50 % Ethanol**	**95 % Ethanol**	**Methanol**
Roots	97.66	6,250.00	3,125.00	3,125.00
Stems	781.25	6,250.00	1,562.50	1,562.50
Leaves	3,125.00	12,500.00	6,250.00	3,125.00
Flowers	12,500.00	25,000.00	6,250.00	6,250.00

Previous studies of many herbs extracts successfully investigated antimicrobial activity against some fish pathogenic bacteria including *Aeromonas hydrophila*. For examples, pomegranate peels (*Punica granatum* L. var.), Indian almond leaf (*Terminalia catappa* L.) [11,12], fresh garlic (*Allium sativum* Linn.), Japanese green tea (*Camellia sinensis*) and piper leaf (*Piper sarmentosum*) extracts [12] and garlic extract [13]. However, there was not much information in the study of antimicrobial activity of the *Bauhinia sirindhorniae* extract, especially activity against fish pathogens. In this present study, the antibacterial activities of *B. sirindhorniae* extracts against *A. hydrophila* (fish pathogenic bacteria) were applied. Sirivan [8] studied the biological evaluations of the stems and roots of *B. sirindhorniae*. The results revealed that the isolated compounds from *B. sirindhorniae* showed activity against *Bacillus subtilis* and *Staphylococcos aureus*. More importantly, the compounds of *B. sirindhorniae* which exhibited

activity against bacteria including (2S)-eridodictyol, isoliquiritigenin, flavonoids, (2S)-naringenin and luteolin can be isolated. In the same way, the leaves, roots and stems of the *B. sirindhorniae* crude extract from 95 % ethanol showed activities against *B. subtilis* and *S. aureus* as evidenced from the MIC and MBC values [9]. Furthermore, the highest activity against *Streptococus agalactiae* (Gram positive, fish pathogenic bacteria) was found in the root extract using acetone with an inhibition zones of 18 ± 3.46 mm. MIC values against *S. agalactiae* were 1562.5 ppm, which was obtained from the root and leaves extracts of *B. sirindhorniae* from acetone, ethanol 95 % and methanol [14].

This study demonstrated that, *in vitro* experiment, the power of antibacterial activity of *B. sirindhorniae* extracts showed strong activity against *A. hydrophila* as described above. All findings of this study suggest that all extracts of *B. sirindhorniae* contain some active components which possess antibacterial properties. It can be concluded that *B. sirindhorniae* extracts are active specifically against fish pathogenic bacteria as well. However, the antibacterial activity of *B. sirindhorniae* extracts against bacteria in fish, are in an *in vivo* experiment, and further studies are recommended. The application of *B. sirindhorniae* extracts highlighting the importance of herbs as immunostimulants and alternative therapeutic agents to control fish diseases was also required. Additionally, the study of purification of individual bioactive components and their antibacterial activity will need to be studied.

Conclusion

The acetone extract of *Bauhinia sirindhorniae* roots showed the highest antibacterial properties against *Aeromonas hydrophila* with the diameter of the inhibition zone 20.33 ± 0.58 mm and a MIC value of 97.66 ppm. The lowest inhibitory activity was found in the flowers extract with acetone and 50 % ethanol with an inhibition zone of 10.00 ± 1.00 and a MIC value of 25,000 ppm, respectively.

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