



IN VITRO SCREENING OF ACACIA (*Samanea saman*) and ANNATTO (*Bixa orellana*) AGAINST *Aeromonas hydrophila*

**ARREN CHRISTIAN M. DE GUIA¹, SOMAR ISRAEL D. FERNANDO² AND
ALVIN T. REYES¹**

¹Soil and Water Quality Laboratory, College of Fisheries- Fresh Water Aquaculture Center, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

²Department of Biological Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines

*Corresponding author: Arren Christian M. De Guia: E-mail: arrenchristian@gmail.com

Received 25th Feb. 2018; Revised 17th March 2018; Accepted 29th March 2018; Available online 1st June 2018

DOI: <https://doi.org/10.31032/IJBPAS/2018/7.6.4470>

ABSTRACT

This study determined the antimicrobial activity of Acacia (*Samanea saman*) and Annatto (*Bixa orellana*) against *A. hydrophila* through In vitro antibacterial testing. Result showed that *Samanea saman* and *Bixa orellana* has prevalent antibacterial activity against the fish pathogen *A. hydrophila* with mean values of 14.00 ± 1.67 and 10.17 ± 0.75 and comparable to vancomycin. The minimum inhibitory concentration (MIC) was 100 mg/ml for *S. saman* and 50 mg/ml for *B.orellana* in which inhibits the bacterial growth. Thus, *Samanea saman* and *Bixa Orellana* found to have pronounced potential as an herbal antibacterial medicine that can be used to treat fish related bacterial diseases.

Keywords: In vitro, *Samanea saman*, *Bixa orellana*, *Aeromonas hydrophila*

INTRODUCTION

Tilapia, as a popular aquaculture species in the Philippines is reared in hatcheries and by grow-out operators

because of its high market demand in the country [1]. Tilapia offers economic and social benefits for rural communities. It also plays vital role in terms of

worldwide employment [2]. However, in the early 1990s, severe disease outbreak threatened the growing tilapia industry in the country which was then attributed to *Aeromonas hydrophila* [3].

Aeromonas are water-borne pathogens that are common in almost all aquatic environments including fresh, brackish and marine waters [4]. *Aeromonas* species are the cause of diseases in cultured and feral fishes in Europe [5]. “Motile *Aeromonas* Septicemia” (MAS), “Hemorrhagic Septicemia”, “Ulcer Disease”, or “Red-Sore Disease” are some of the common diseases caused by *A. hydrophila* (Swann and White, 1995). *Aeromonas* has been found to produce exotoxin such as aerolysin-like hemolysin (ALH), *Aeromonas* Serine Protease (ASP) and *Aeromonas* Metalloprotease (AMP) to survive undesirable condition [4]. The study also showed that ALH’s primary action is to rupture the cells of the victim; however, until today its mechanism has been a mystery, while ASP has been found to promote plasma coagulation by means of activating prothrombin [4]. ASP is a common and deadly consequence to sepsis victims while the mode-of-action of AMP has

not yet been determined by the experts [4].

Philippines has thousands of plants are known to have medicinal value and the different plant parts are used to cure specific ailments since ancient times[6]. The antimicrobial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant that include alkaloids, steroids, tannins, phenol compounds, flavonoids and resins fatty acids gums. Thus, this study determined the efficacy of plant samples against the fish pathogenic bacteria.

MATERIALS AND METHODS

Collection of Plant Samples

Collection of Plant Samples
Leaves of Acacia (*Samanea saman*) and Annatto (*Bixa orellana*) were collected by hand picking inside the Central Luzon State University, Philippines. Pressed plant parts served as voucher specimens and were used for the authentication.

Ethanol Extraction

Plant leaves were cleaned and disinfected to remove foreign bodies and cut into small pieces (2-3 cm) and air dried to make it suitable for grinding then pulverized [7]. Twenty-

five grams of pulverized leaves were soaked in 100 ml 70% ethanol in a stoppered flask for 72 hours and filtered subsequently. Rotary evaporator was used to remove ethanol from the extract. Extracts obtained were stored in a tightly stoppered sterile amber bottle and kept refrigerated until use.

Preparation of *Aeromonas hydrophila*

Pure culture of *A. hydrophila* was obtained from the University of the Philippines, Los Baños–National Institute of Molecular Biology and Biotechnology (UPLB-BIOTECH). From the pure bacterial culture four colonies were transferred to 5 ml Trypticase soy broth. The broth was incubated at 30 °C or at an optimum growth temperature until achieves or exceeds the turbidity of 0.5 McFarland standards. The turbidity of the test bacterial suspension with that of 0.5 McFarland was compared against a white background with contrasting black line under adequate light [8].

Disc diffusion Assay

Five concentrations of 25, 50, 100, 500 and 800 mg/ml [9] of ethanol extracts of Acacia (*Samanea saman*) and Annatto (*Bixa orellana*)

individually were used. Sterile paper discs (5 mm) were soaked and air dried on sterile petri plates with individually dispensed leaf extracts under a biosafety laminar flow. Prepared media on petri plates of Mueller-Hinton agar, swabbed with bacterial culture were used. Air-dried disc with five concentrations of each plant were individually seeded on plates in a five -concentration per plant manner.

Vancomycin served as positive control while sterile distilled water served as negative control. Plates were incubated at 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

In a sterilized agar plate, 1 ml of the ethanolic extract (25, 50, 100, 500 and 800 mg/ml) and 9 ml of the MHA was pipetted and mixed thoroughly. The agar was allowed to solidify at room temperature. Four isolated colonies were sub-cultured to a tube with 3 ml Mueller Hinton Broth. The broth was incubated at 30 °C for 18-24 hours until it achieves or exceeds the turbidity of 0.5 McFarland standards. The standardized inoculum was diluted at a dilution ratio of 1:10 in sterile saline solution to obtain the desired concentration of 10⁶ CFU/ml.

From the standardized inoculum, 0.1 ml was streaked to the surface of the prepared agar. The agar plates were incubated at 30 °C for 18-24 hours. The MIC was taken as the lowest concentration that completely inhibits the growth of the organism [8].

Statistical Analysis

Significant differences in the diameter of the zone of were analyzed using One-Way Analysis of Variance (ANOVA) under the Statistical Package of Predictive Analytics Software (PASW) Statistics Version 18.

RESULTS AND DISCUSSION

Antibacterial Testing

The antimicrobial activity of the plant extracts and antibiotics discs was determined through measuring the zone of inhibition. Acacia and Annatto showed zone of inhibitions indicating the antibacterial activity of the plants phytochemical components at 24 hours of incubation against the fish pathogen *A. hydrophila*.

Minimum Inhibitory Concentration

The minimum inhibitory concentration for Acacia and Annatto that inhibited bacterial growth were recorded at 100 mg/ml and 50 mg/ml respectively.

Antibacterial Activities of Plant Extracts

Chemical components which are classified into some major groups like alkaloids, acids, essential oils, steroids, saponins and tannins provide the medicinal property of the plants, and getting these chemicals out of the plants depend upon the solubility of these compounds in various solvents [10]. The observed protective effect of ethanol extract may correspond to a synergic participation of several of the above-cited compounds [11].

Usually, the plant's active components hinder with growth and metabolism of microorganisms and prevent them from spreading [12]. In addition, the antibacterial activity is most likely due to the combined effects of adsorption of polyphenols to bacterial membranes with membrane disruption and subsequent leakage of cellular contents [13, 14] and the generation of hydroperoxides from polyphenols [15]. Acacia, which had comparable effect to Vancomycin against *A. hydrophila*, is a known producer of secondary metabolites with significant biological activity such as antitumor, anti-inflammatory, antibacterial and anti-

parasitic [16]. This plant offers variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, naringenin-7-O-b-D-(6-O-galloyl) glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives. Some authors also claimed that acacia had spasmogenic, vasoconstrictor, anti-hypertensive, -mutagenic, -carcinogenic, -spasmodic, -inflammatory, -oxidant and

-platelet aggregatory properties [17, 18, 19, 20].

CONCLUSION

The threat in the growing tilapia industry in the country of water-borne pathogens that are common in almost all aquatic environments including fresh, brackish and marine waters brought interest to find natural way of treating the diseases. *Samanea saman* and *Bixa Orellana* found to have pronounced potential as an herbal antibacterial medicine that can be used to treat fish related bacterial diseases.

Table 1: Antimicrobial activity of plant extracts and antibiotics against *A. hydrophila*

Plant Extracts/Antibiotics	Zone of Inhibition (mm)		
	12-hr	18-hr	24-hr
Acacia	14.00±1.79 ^b	14.00±1.77 ^b	14.00±1.67 ^b
Annatto	9.17±0.41 ^a	10.11±0.75 ^a	10.17±0.75 ^a
Vancomycin	15.17±1.51 ^c	15.23±1.37 ^c	15.37±1.47 ^c

Variable having different letter superscripts are significantly different.

Table 2: MIC of plant extracts against *A. hydrophila*.

Plants	Concentrations (mg/ml)				
	25	50	100	500	800
Annatto	-	+	+	+	+
Acacia	-	-	+	+	+

+ no growth; - intense growth

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