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ANTIBACTERIAL ACTIVITY OF WATER HYACINTH (*Eichhornia crassipes*) EXTRACTS AGAINST *Aeromonas hydrophila IN-VITRO* AND *IN-VIVO*

***JANET O. SATURNO, MISSAEL T. MAXION AND RODORA M. BARTOLOME**

College of Fisheries- Freshwater Aquaculture Center, Central Luzon State University, Science City of Munoz, Nueva Ecija 3120, Philippines

***Corresponding Author: Janet O. Saturno: E Mail: jsaturno_orden@yahoo.com**

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ABSTRACT

This paper demonstrated the antibacterial activity water hyacinth (*Eichhornia crassipes*) extracts against *Aeromonas hydrophila in-vitro* and *in-vivo*. The active components of the plant were obtained using ethanolic, methanolic, and water as extracting solvents. Among extracts, ethanol as extracting solvent recorded the highest zone of inhibition at 11.33mm, which is not significantly different to water extract (9.33mm). In addition, determination of the activity index from the *in-vitro* antibacterial experiments revealed that ethanolic (0.723) and water (0.596) extracts exhibited significant activity. The test bacteria are moderately susceptible to both extracts. In contrast, methanol extract had the lowest antibacterial activity. However, in-vivo assay revealed no significant difference in terms of survival between the treated feeds with positive control (90.333) and the feeds treated with ethanol extract (86.667). The present study found that water hyacinth exhibits potential as an antibiotic agent in aquaculture.

Keywords: *Aeromonas hydrophila*, Solvent extraction, Susceptibility testing, Water Hyacinth (*Eichhornia crassipes*)

INTRODUCTION

Infectious diseases are always a hazard and may cause significant stock losses and problems with animal welfare [1] where synthetic antibiotics was the easy recourse leading to the development of resistance among the pathogens [2]. Addressing this problem requires continuous structural modification on drugs, but due to the

inability on continuously making modifications on drugs, plant derived antimicrobials had gained popularity [2].

Curative nature of plants has been exploited with significance however, many studies focuses on medicinal plants as source of phytochemicals, which are already economically important [2]. Water hyacinth is widely recognized as the world's worst weed because it forms dense impenetrable mats across water surfaces that cause obstruction in fishing activities, irrigation, and drainage clogging [3]. This macrophyte can also threaten local native species diversity, change the physical and chemical aquatic environment leading to alteration of ecosystem structure and function [4]. These conditions have negative effects on environment, human health and economic development [5]. On a lighter note, water hyacinth contains alkaloids and flavonoids [2] that exhibit several biological activities such as wound healing and inhibits illnesses [2] [5].

Water hyacinth is a rooted macrophyte which due to this could pause several problems like drainage clogging lead to flooding and entrapping fish movement in the water that limits their ability to look for food and to avoid predator. In terms of water quality, when densely abundant covers the water surface preventing sunlight penetration reducing the photosynthetic activity that may redound to oxygen depletion or low productivity, worst is mortality. Here in, the present work reported that water hyacinth was screened on its biological activity to prevent infections from *A. hydrophila* with red tilapia (FAST).

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MATERIALS AND METHODS

Source of Plants

Water hyacinth used in the experiment was collected from the Freshwater Aquaculture Center, Central Luzon State University. The stolon and lamina of the plant were washed thoroughly to remove dirt then processed to powder form [2].

Extraction of Plant Sample

Extract was obtained through decoction method [6] with the use of methanol and ethanol solvents. Concentrated extract was filtered with filter paper.

In-vitro Antibacterial Assay

In-vitro Antibacterial Experiment was conducted to test antimicrobial activity against *A. hydrophila* in-vitro with five treatments following CRD in culture media Mueller-Hinton agar. T1 - water extract, T2- ethanol extract, T3- methanol extract, T4- chloramphenicol and T5- negative

control. The zone of inhibition was determined following the disk method [2]. The zone of inhibition was measured using a Vernier caliper and data were expressed in millimeter. Level of zone inhibition followed were >13 - highly sensitive or susceptible, 8-moderately sensitive or intermediate and <8- resistant [2]. The activity index of the extracts of water hyacinth was calculated using the formula provided with a ≥0.5 activity index were considered as significant activities [2]. Treatments from in-vitro experiment that showed significant activity index were used in the in-vivo experiment.

In-vivo Screening

A total of 120 red tilapia (FAST) of the same weight group (3-5 g) was obtained from the FAC–CLSU, Science City of Muñoz, Nueva Ecija and conditioned appropriately. The study was carried out in 24 polyethylene plastic bags each measuring 18" x 32" and wooden frames each measuring 2 m x 0.5 m x 1 m stocked with red tilapia 10 fish/bag. Intramuscular injection of 0.05 mL pure culture of *A. hydrophila* was followed [7]. This experiment was done for two weeks. Extracts of water hyacinth with a concentration of 0.5g/mL were diluted in 500 ml of 95% ethyl alcohol. The mixture was sprayed to the commercial feeds weighing 1 kg/treatment while mixing continuously to homogenize then air dried. Treated feeds were fed to the fish daily at the rate of 3% body weight twice a day (9:00 am and 4:00 pm). The number of dead fish was examined for any sign of bacterial infection such as hemorrhage of the skin, fins, oral cavity, and muscles daily. At the end of the study, the wastewater and experimental fish were disposed properly to avoid biohazards. Fish were treated with NaCl to kill some of the bacteria on fish bodies prior burying underground.

Statistical Analysis

Analysis of Variance (ANOVA) was performed to assess the effects of different solvent extracts on the survival of FAC Red tilapia (FAST). A significance level of $P<0.05$ was used. Duncan multiple range test was used in comparing means of the different treatments IBM SPSS Statistics software version 21.0.

RESULTS AND DISCUSSION

Antibacterial Activity In-vitro

The diameter of the zone of inhibitions suggested the relative susceptibility of the test microorganism to the different extracts from water using three different solvents (ethanol, methanol and water). In the present study, the susceptibility of every treatment was evaluated through the zone of inhibition on bacterial growth in culture plates. Table 1 presents the zone of inhibition of *A. hydrophila.*

Table 1: Zone of inhibition of *A. hydrophila* obtained in the in-vitro antibacterial experiment

Extract	Zone of inhibition (mm)	Remarks	Activity Index
Water extract	9.33[b]	Moderately susceptible	0.60
Ethanol extract	11.33[b]	Moderately susceptible	0.72
Methanol extract	4.33[c]	Resistant	0.28
Chloramphenicol	15.67[a]	Susceptible	n/a
Negative control	0.00[d]	Resistant	0

Note: Means with different superscript letters are significantly different from each other at 5% probability level by DMRT

Moderate susceptibility was recorded on water and ethanol extracts. The highest susceptibility was recorded in chloramphenicol. *A. hydrophila* in fish are sensitive to a number of antibiotics [8]. Inhibitions of chloramphenicol against *A. hydrophila* in petri plates ranged from 12-18 mm [9] and current study was within that range.

Negative control (distilled water only) obtained the lowest zone of inhibition followed by methanol extract thus, indicating resistance of *A. hydrophila* in these treatments. Statistical analysis showed that T1 and T2 had significant zone of inhibition than the other treatments. Plant-based constituents or phytochemicals exhibit different modes of action against bacterial strains which range from interference with the phospholipoidal cell membranes leading to increase permeability profile and loss of cellular constituents, damage of enzyme and destruction or inactivation of genetic material [10]. Zone of inhibition in T3 and T5 were comparable and significantly higher than T1, T2 and T4.

Both ethanol (0.723) and water (0.596) extracts obtained significant activity indexes (Table 1). More phytochemicals from dry form of water hyacinth could be extracted with the use of water [11] and ethanol [2]. Hence, both treatments were used in the in-vivo antibacterial experiment.

Antibacterial Activity In-vivo

Per cent mortality and per cent survival were observed during the in-vivo experiment (Table 2). After 11 days of exposure to the different treatments negative treatment registered the highest mortality (40%) while fish treated with chloramphenicol were all protected thus no mortality was observed. After 13 days of observation chloramphenicol had 93.33 per cent survival as compared to 50 per cent for distilled water. Fish with skin ulcerations, lesions and other signs that have died are shown on Figure 1.

Table 2: Average daily mortality recorded in four treatments during in-vivo experiment

Treatments	Mortality (%) after 11 days	Survival (%) after 13 days
Chloramphenicol (T1)	0.00	93.33[a]
Distilled water (T2)	40.00	50.00[b]
Ethanolic extract (T3)	6.67	86.67[a]
Aqueous extract (T4)	30.00	56.67[b]

Note: Means with different superscript are significantly different from each other at 5% probability level by DMRT

Figure 1: Experimental fish showing positive signs of Motile Aeromonas Septicemia

A hydrophila is an opportunistic bacterium thus mostly infects non-healthy individuals and individuals with weakened immune system. This can be the reason why high mortality occurred during day 11 wherein experimental fish were more stressed due to water contamination by feeding and fecal excretion, thus, making them more susceptible. However, the decrease in the mortality from day 12 to day 14 can be attributed to the effect of the treatments. Results coincide with a study where infection from *A. hydrophila* could be treated usually within a period of 12-15 [7].

Fish that have died during the in-vivo experiments showed positive signs of Motile Aeromonas Septicemia (MAS). According to several scientists [12-14] the signs of this infection varied from bloated appearance, gill rot, skin ulcerations or surface lesions, abdominal swelling and bulging of eyes.

ANOVA showed that survival on chloramphenicol and ethanolic extract were comparable, but significantly higher than distilled water and aqueous extract. However, T2 and T4 were comparable.

Results of the study showed that use of antibiotics as treatment for fish infection was more effective. However, the use of antibiotics has several disadvantages such as being expensive, presence of antibiotic residue in water [9] and the intense use or misuse of antibiotics also leads to the development of antibiotic resistance genes in pathogenic bacteria.

In the in-vitro antibacterial experiment, there was no significant difference detected between extracts from ethanol and extracts from water, however, in the in-vivo experiment, significant difference was observed. Results of the study showed that water as an extraction solvent of antimicrobial phytochemicals might not be a good option, on the other hand, extracts from ethanol exhibits stronger effect than that of water.

Positive signs of infection after the experiment were exhibited only by distilled water (Table 3). This suggests that fish fed with chloramphenicol, ehtanolic extract and aqueous extract completely recovered from infection, thus, the extracts could be used as treatment for Motile *Aeromonas Septicemia* caused by *A. hydrophilla*.

Table 3: Observation of fish for the presence and absence of infection after the in-vivo antibacterial experiment

Treatment	No. of Fish Survived	Bloated Appearance		Pale Gills		Skin Ulcerations	
		+	-	+	-	+	-
1	28	0	28	0	28	0	28
2	15	1	14	1	14	0	14
3	26	0	26	0	26	0	26
4	13	0	13	0	13	0	13

Water hyacinth was proven to have the following phytochemicals: alkaloids, sterols, terpenoids, anthocyanins, anthroquinones, flavonoids, phenols and terpenoids that are mainly responsible for the medicinal and therapeutic effects of plant extracts [5] [2]. However, the extractions of these phytochemicals are greatly affected by the extraction solvent to be used.

Effectiveness of ethanol as an extraction solvent can be attributed to the presence of higher activity and higher amounts of polyphenols compared to other solvents [6]. Also, ethanol could easily penetrate cellular membrane to extract the intracellular ingredients or chemicals from plant materials.

Methanol exhibits higher polarity than ethanol and almost all the identified phytochemicals that exhibits antibacterial property are saturated organic compounds [6] thus, methanol should have exhibited better results. However, methanol is cytotoxic in nature making it unsuitable for extraction in the studies [6].

Water is a better medium for extraction of antimicrobial phytochemicals compared with ethanol [6] however, in the present study, 70% ethanol is used which is composed of 30% water and 70% ethanol. Therefore, addition of water that exhibits high

dissolving activity to ethanol that exhibits fast penetration to cellular cells would make a good extraction solvent. Additionally, extracts from 70% ethanol has been found to give a more consistent antimicrobial property [6].

Follow up study on the preparation of water hyacinth extract to make it commercially available to treat *A. hydrophila* is currently being conceptualized.

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