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Catharanthus Roseus Leaf Extract Antimicrobial Efficacy Against *V.Harveyi* Infection

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ABSTRACT

Diseases are a significant stumbling block to aquaculture expansion. Chemotherapeutants have been used to cure or prevent disease in the past. One of the most important bacteria is V.harveyi. It resulted in significant losses in cultured fish output all over the world. Current treatment procedures are ineffective and hazardous to the environment. To establish alternative approaches, researchers should focus their efforts on discovering new medications, particularly those derived from plants. Natural goods have been used as a source of medicinal substances for many years and have demonstrated to be useful. Many herbal substances have been discovered to have non-specific immune-stimulating activities in fish with V.harveyi. The current study looks on screening C.roseus herbal for antibacterial efficacy against V.harveyi infection. Total protein analysis was performed in both the control and experimental groups in this investigation. In the control, D-1, D-2, and D-3 diet fed groups, the Carassius auratus survived 65, 75, 85, and 80% of the time, respectively. In the D2 and D3 groups, the carbohydrate proportion increased significantly (P 0.01) to 120 and 140 mg/100ml of blood, respectively. Because the bacterial load in the blood is larger in infected animals, the protein values are higher.

Keywords: *Medicinal plants; Catharanthus roseus; Vibrio harveyi; aquaculture; health.*

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INTRODUCTION

Aquaculture has emerged as a popular food-producing sub-sector that complements agriculture [1] and has become the fastest-growing food-producing business, rising more than five times faster than the global population. The aquaculture sector, particularly fish farming, is one of the most remarkable commercial success stories in Asian aquaculture history, with Asia producing more than 80% of the world's cultured fish. Diseases in aquaculture are currently a major stumbling block to the industry's expansion, affecting both socioeconomic development and rural livelihoods in some countries [2]. Traditional Indian doctors claim to have successfully employed the plant to treat a variety of ailments. C.roseus' antibacterial properties, on the other hand, have yet to be thoroughly documented. We present fresh information on C.roseus' antibacterial properties utilising recognized microbial pathogens as test organisms in this research. Bacterial pathogens are the most economically important infections in cultured fishes around the world.

Traditional remedies rely heavily on medicinal plants as a source of active ingredients. The development of novel antibiotics that minimise the rising resistance among microorganisms has gotten a lot of attention [3]. For the isolation of novel compounds that lead to drug discovery, phytochemical and biological screening is required. Medicinal plants often include a number of chemicals that may be possible natural antimicrobial agents that could be used to treat common microbial illnesses as an alternative, effective, less expensive, and safe antimicrobial agent [4]. In the late 1990s, the use of plant extracts in medicinal therapy gained a lot of traction [5]. The best antibacterial activities were found in an ethanolic extract of *Catharanthus roseus* Linn leaves [6]. *V.harveyi* bacteria were used to assess sensitivity.

Antibiotics and other chemicals used in aquaculture have their own complicated or grow-out system, which can contribute to the growth of antibiotic-resistant bacteria in both fish and shrimp, as well as microorganisms that cause human disease. The absorption and distribution of antibiotics in fish and shrimp, as well as the durability of residues and the effects of antibiotics in the environment, are yet unknown. Antibacterial extracts, in addition to chemotherapeutic medicines and vaccines, have been extensively accepted by fish farmers, albeit there are still numerous reservations about their efficacy from users. Kumaran et al. [7] demonstrate how herbal products have antibacterial, antiviral, immune stimulant, and anti-stress properties that have a substantial impact on shrimp aquaculture. Several traditional medicines demonstrated antiviral and antibacterial efficacy against harmful bacteria and viruses found in fish and shrimp. The phytotherapeutic techniques will be environmentally safe, cost-effective, and have less negative effects in aquaculture.

MATERIALS AND METHODS

Collection and Processing of *Catharanthus roseus*

Catharanthus roseus, for example, was obtained in the rural area of Lekshmipuram, Kanyakumari District. The collected plant materials were shade dried at temperatures ranging from 28 to 35^0 C, and the drying process was continued until the moisture content was less than 14%. After drying, the plant materials were minced with a wooden knife before being fed into a grinder. The minced materials were ground into powder using teeth mills and sieved, then stored in an airtight container at room temperature until used.

Extraction

Hexane was used three times to remove the powdered plant components. The residue was then treated with ethyl acetate and extracted three times before being treated with methanol and extracted three times. Each extraction was carried out by soaking the sample overnight. To avoid the evaporation of plant components, each of these solvent extracts was concentrated in a rotatory evaporator at decreased pressure at 45^0 C to 50^0 C. The aqueous extract was lyophilized and kept at 4^0 C.

Phytochemical analysis

To screen for the presence of various chemical ingredients, phytochemical screening of the methanol and ethyl acetate extracts was carried out using established procedures [8].

Experiment set-up and feeding

Carassius auratus fish weighing 9±1 g were obtained from a local vast aquarium in Azhigiyamandapam, Tamil Nadu, India, and acclimatised in the lab. They were kept in a laboratory FRP tank with a capacity of 3000 litres. *Carassius auratus* of uniform size were chosen from the stock culture and put into separate experimental fibre glass tanks (100 litre capacity) for three experimental groups: D-1, D-2, D-3, and Control. In each group, triplicate cultures (n = 10 x 3 = 30) were maintained with steady aeration and continuous flow-through water. Every day, water quality measures such as temperature (27±1.0°C), salinity (25±1.5%), and pH (8±0.1) were monitored. The fish were fed 10 percent of their body weight three times a day at 8.00, 13.00, and 18.00. Before feeding, any uneaten food and waste materials were removed. The fish from both the experimental and control groups were injected intramuscularly with *V.harveyi* after 25 days of eating. Individual groups' blood and muscles were collected for additional biochemical research.

Cumulative mortality and Growth parameters

After the 25^{th} day after immunisation, the percentage of cumulative mortality was calculated. By subtracting the original weight from the end weight, the weight gain (wet) was computed.

Biochemical analysis

Blood samples from control and experimental groups challenged *Carassius auratus* were analysed for biochemical markers such as total protein [9] and total carbohydrate [10].

RESULTS

Phytochemical analysis

The antibacterial activity of *Catharanthus roseus* extract was tested against *V.harveyi*. Steroids, Saponins, Cardiac glycosides, tannins, titerpenoids, flavonoids, coumarin, and other phytochemicals were discovered during the phytochemical screening of the plants. Flavonoids, alkaloids, and coumarin were found to be absent in *Catharanthus roseus*. Steroids, Saponins, Cardiac glycosides, tannins, and titerpenoids were found in abundance in *Catharanthus roseus* (Table 1).

Sl No	Phytochemical constituent	*Catharanthus roseus*
1.	Steroids	+
2.	Saponins	+
3.	Flavanoids	-
4.	Coumarin	-
5.	Alkaloids	-
6.	Cardiac glycosides	+

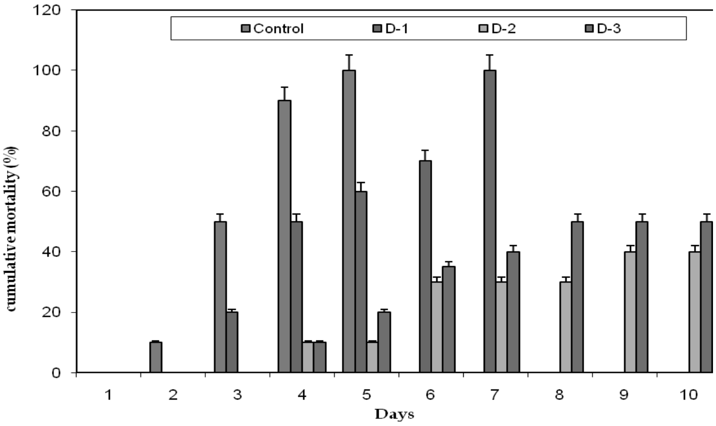
7.	Titerpenoids		+
8.	Tannins		+

Table 1. Phytochemical constituent for the antibacterial plant *Catharanthus roseus*

Cumulative mortality after V.harveyi Challenging

Following the culture period, the Carassius auratus were challenged by injecting *V.harveyi* intramuscularly. When no immunostimulant or antibacterial properties herbal extract is given in the meals, there is a 100 percent cumulative mortality within 5 days. The percentage of cumulative mortality was lower in the D2 and D3 groups. That indicates the groups in the aforementioned groupings each had a 60% and 50% chance of surviving. Immunostimulant herbals were found to be greatly impacted in their ability to fight *V.harveyi* (Fig 1).

Fig. 1 Cumulative mortality of herbal diets fed with *Carassius auratus*



Survival & Growth parameters

Table 2 shows the survival, weight gain, and specific growth rates. In the control, D-1, D-2, and D-3 diet fed groups, the *Carassius auratus* survived 65, 75, 85, and 80% of the time, respectively. The control diet resulted in a minor weight gain of 1 mg/d. In the D-1, D-2, and D-3 diet fed groups, the gain was dramatically raised to 2.5, 2.2, and 2.6 mg/day, respectively. The control and D-3 groups had the lowest specific growth of 0.17 percent and the highest specific growth of 0.35 percent, respectively.

Table 2. Growth characteristics of herbal antibacterial active principle incorporated diets fed *Carassius auratus*

Treatment	Length (mm)		Wet weight (mg)		Weight gain (mg/day)	Specific growth rate (%)	Survival (%)
	Initial	Final	Initial	Final			
Control	8.6 ± 0.16	11.2 ± 0.16	10.6 ± 0.12	12.1 ± 0.35	1.3[a] ± 0.12	0.17[a] ± 0.16	65
D-1	8.4 ± 0.29	11.3 ± 0.12	10.4 ± 0.24	12.7 ± 0.16	2.5[b] ± 0.20	0.28[b] ± 0. 08	75
D-2	7.9 ± 0.12	11.6 ± 0.16	10 ± 0.18	12.4 ± 0.18	2.2[b] ± 0.16	0.31[c] ± 0.12	85
D-3	8.2 ± 0.29	11.6 ± 0.16	9.6 ± 0.14	12.2 ± 0.24	2.6[b] ± 0.18	0.33[c] ± 0.18	80

Means with the same superscripts (a-c) do not differ from each other ($P < 0.01$).

Biochemical Changes of *V.harveyi* treated *Carassius auratus*

In both the control and experimental groups, total protein analysis was done. Because the bacterial load in the blood is larger in infected animals, the protein values are higher. The protein level in the blood of the control group after a V.harveyi challenge is 115 mg ml-1. In the D1, D2, and D 3 blood groups, the value was reduced to 110, 99, and 95 mg ml-1, respectively (Table 3). As recorded, the carbohydrate level of V.harveyi challenged. In the D2 and D3 groups, the carbohydrate proportion increased significantly (P 0.01) to 120 and 140 mg/100ml of blood, respectively. Table 3 summarises the findings.

Table 3. Biochemical changes in the blood of *Carassius auratus* fed herbal antibacterial diets after *V.harveyi* challenge

Treatments	**Biochemical changes**	
	Protein (mg/ml)	**Carbohydrate (mg/100ml)**
Control	115.4 ± 0.32[a]	117.7 ± 1.24[a]
D-1	110.2 ± 0.43[b]	98.3 ± 1.24[b]
D-2	99.6 ± 0.47[c]	120.4 ± 0.32[c]
D-3	95.5 ± 0.37[d]	140.4 ± 0.32[d]

Means with the same superscripts (a-e) do not differ from each other (P < 0.01).

DISCUSSION & CONCLUSION

Natural compounds from the plant kingdom have been studied in recent years for their immune modulating potential in the treatment of infectious and neoplastic disorders. Herbal treatment, often known as "phytomedicine," is a type of supplementary medicine that involves the use of plants, plant parts, or plant-derived chemicals for therapeutic purposes [11]. Volatile oils, tannins, phenolics, saponins, alkaloids, polysaccharides, and polypeptides have all been proved to be efficient antibiotic alternatives. The screening of plant extracts and natural products for antimicrobial activity has revealed that higher plants could be a source of new anti-infective drugs [12] as well as a source of primary lead compounds for drug discovery from natural products.

Steroids, saponins, cardiac glycosides, tannins, titerpenoids, flavonoids, coumarin, and a variety of other phytochemical substances were investigated in this study. Phytochemical examination revealed the presence of physiologically active chemicals such as phenols and tannins in the extracts, according to Hangerman and Bulter [13]. It has long been recognised that certain chemical molecules have antibacterial properties. In the goldfish Carassius auratus, herbal antibacterial extracts boosted the immune system and reduced microbial infection, while Magdelin [14] used herbal immunostimunants on the ornamental fish Poecilia sphenops in a similar study. The protein values in the control and experimental groups are greater in the control groups for biochemical characteristics. It's primarily owing to the infected Carassius auratus's high bacterial burden. Because they are less infected, the D1 and D2 groups had lower bacterial loads.

According to Beckage [15], baculoviruses encode a range of proteases and other enzymes that 'melt' the tissues, resulting in an increase in protein content in the haemolymph and a decrease in muscle and hepatopancreas of infected shrimps. In compared to fish, total carbohydrate and glucose levels increased in the blood and decreased in the muscle and gut of *V.harveyi* infected *Carassius auratus*. Infected or stressed animals' glucose levels typically rise to ward off infection or stress [7]. The transfer of glucose and carbohydrate from the gut and muscle to the blood could result in high amounts of glucose and total carbohydrate in the blood.

According to the findings of this study, there is some resistance to *V.harveyi* infections. The biochemical findings revealed a distinct pattern of resistance. The herbal *Catharanthus roseus*, which has antimicrobial properties, has a superior ability.

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