

Antibacterial effect of *Decalepis hamiltonii* leaf extract enriched diet on fish pathogen in *Carassius auratus*

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Abstract

Medicinal plants are vital sources of natural bioactive compounds, providing safe and effective alternatives for managing a wide range of diseases. *Decalepis hamiltonii*, a medicinally important plant, is known for its broad-spectrum antibacterial properties, particularly against pathogens responsible for common bacterial infections. The present study investigates the phytochemical composition and antibacterial potential of *D. hamiltonii* leaf extract when incorporated into the diet of *Carassius auratus* (goldfish). Experimental fish were challenged with *Aeromonas hydrophila*, a major pathogen in aquaculture. The results demonstrated that the supplemented feed enhanced the immune response and survival rate of the fish, significantly reducing the impact of bacterial infection. These findings support the use of *D. hamiltonii* as a sustainable and eco-friendly dietary supplement for disease management in the ornamental fish industry.

Keywords: *Decalepis hamiltonii*, phytochemicals, *Carassius auratus*, bacterial infection.

1. Introduction:

Medicinal plants have been explored therapeutically in traditional medicines to alleviate human ailments for several millennia. Medicinal plants are defined as those which produce one or more active constituents capable of preventing or curing an illness. Natural products play a major role as active substances, model molecules for the discovery and validation of drug

targets. *Decalepis hamiltonii* Wight & Arn., locally known as “Magalikizhangu,” is a rare, endemic medicinal plant species naturally distributed in the Western Ghats, including the forests of Kanyakumari district in Tamil Nadu. It thrives in dry and moist deciduous forests, especially on rocky slopes and crevices, and is well adapted to the environmental conditions of the southern Western Ghats (Baskaran et al., 2012). The roots of this species are traditionally used by the indigenous Kani tribes of Kanyakumari for treating gastrointestinal, respiratory, and circulatory ailments. They also prepare rejuvenating tonics from the root extracts due to their adaptogenic and antioxidant properties (Rajan et al., 2002). However, overharvesting for medicinal use has led to a significant decline in its wild populations, resulting in its inclusion on the IUCN Red List of Endangered Species (IUCN, 2014). Conservation strategies involving both in-situ and ex-situ measures, along with community awareness, are essential to ensure the sustainable use and protection of *D. hamiltonii* in its native habitat. Leaf extract of *Decalepis hamiltonii* is used in the treatment of various bacterial infectious diseases such as pneumonia, diarrhoea, urinary tract infection and even some skin disease.

A. hydrophila causes a disease known as haemorrhagic septicaemia or ulcer disease in fish and belongs to the most common bacteria present in aquatic environments throughout the world. The bacterium is naturally found in the intestinal tract of the fish and does not cause the disease under natural conditions (Swann and White 1989). *Decalepis hamiltonii* extract contains a wide spectrum of activity against a group of bacteria that are responsible for the most common bacterial diseases. Thus, the plant possesses an abundance of the antibacterial compounds. The aim of the present study was to investigate the photochemical and antimicrobial activities of the extract from *D. hamiltonii* using experimental animal models.

2. Methodology:

Ten-gram dried powders of *Decalepis hamiltonii* plant leaf material filtering the extracts were prepared. In order to study the functional groups of the active extracts, they will be analysed through Phytochemicals, TLC and FTIR. After structural elucidation of the active compounds, they will be incorporated to the artificial diets with different concentrations and combinations such as 100, 200 and 400 mg Kg⁻¹ feeding to the fish culture experiments. After a

regular interval such as 20-, 40-, 60- and 80-days random sampling will be made and challenge and monitor the cumulative mortality, haematological and biochemical diagnosis and improvement will be analyzed.

3. Results:

The result of the phytochemical analysis showed that the *D. Hamiltonii* had the presence of tannin, saponin, steroids and flavanoids (Table.1). Thin layer chromatographic analysis of the hot water extract of *D. hamiltonii* revealed that, the R_f value spot 0.164, 0.275 and 0.611 (Fig.1) was confirmed as the active compounds. The active fraction of *D. hamiltonii* extract have the functional groups in the I-R spectrum. The broad peak around 2922 cm^{-1} may be the -OH stretching or -NH stretching the one peak at 1041 cm^{-1} may be due to C-O stretching. The one at 673 cm^{-1} may be due to $\text{C}\equiv\text{C-H}$. The one at 1624 cm^{-1} may be due to RONO_2 . The observation revealed that it may be inferred that the compound is alkenes or ketones. Thus, the extract may contain a free carbonyl group where the OH group is hydrogen bonded. The extract is also suspected to contain a carbonyl species in conjugation with O= bond (Fig 2).

The antibiogram studies of the *Aeromonas hydrophila* against the selected Antibiotics (zone of inhibition in cm) the maximum values were got for strain by using the antibiotic Chloramphenicol at 1.5 cm in diameter. *D. hamiltonii* were effectively suppressed the pathogens at 0.7, 0.8 and 2 cm of zone of inhibition to *A. hydrophila* (Fig 3). The goldfish *C. auratus* succumbed to death cent percent within six days after *A. hydrophila* challenge when no vaccination was given. The *D. hamiltonii* extract incorporated diet fed fishes survived of 90 % after ten days of challenges in *A. hydrophila* respectively in 25 days of post (dpv) vaccination (Fig 4).

The biochemical parameters including serum albumin, globulin, and proteins of treated *C. auratus*. The serum albumin level of vaccinated groups was increased from the control fishes. The amount of globulin was found to be higher in all the *D. hamiltonii* extract incorporated diet fed groups when compared with the control group. The total serum protein analysis was performed in the control and vaccinated groups. The total serum protein was increased in the experimental groups. The RBC level was decreased in the control groups (no vaccination)

when compared to the *D. hamiltonii* extract incorporated diet fed groups (Table.2). This diet may be useful to solve the problems of *A. hydrophila* infection in ornamental fish industry.

Table No.1. Phytochemical analysis of hot water extract of *D. hamiltonii* by standard protocols

Sr. No	Phytochemical constituents	Hot water extract
1	Alkaloid	-
2	Saponin	+
3	Steroids	+
4	Tannin	+
5	Terpenoids	-
6	Flavonoids	+

Table No.2. Biochemical parameters of the gold fish *C. auratus* treated with *D. hamiltonii* herbal antibacterial diet and challenged with *A. hydrophila* after 25dpv

Sr. No	Treatment & Challenge	Biochemical parameters (µg/ml)		
		Serum albumin	Serum globulin	Serum protein
1	Blank control	232.45 ± 0.09	218.31 ± 0.06	436.23 ± 0.1
2	Control	224.69 ± 0.12	202.14 ± 0.1	432.74 ± 0.09
3	<i>D. hamiltonii</i> extract incorporated diet	241.78 ± 0.06	230.34 ± 0.09	461.20 ± 0.1



Fig 1. Thin Layer chromatogram of the *D. hamiltonii* extract by iodine development.

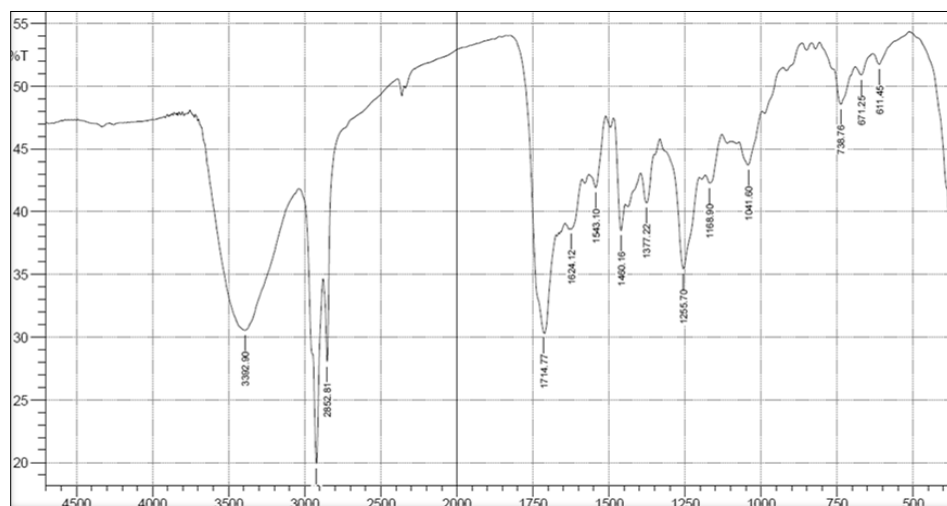


Fig 2. Fourier Transform Infrared Spectroscopy analysis for the *D. hamiltonii* extract

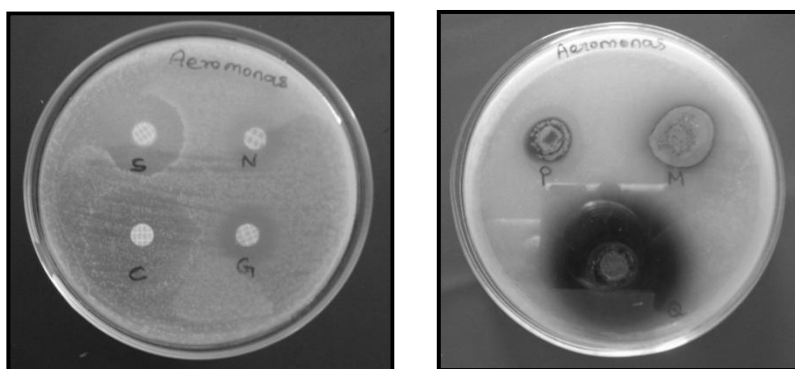


Fig 3. Antibacterial activity (zone of inhibition mm) of the antibiotics and *D. hamiltonii* extract

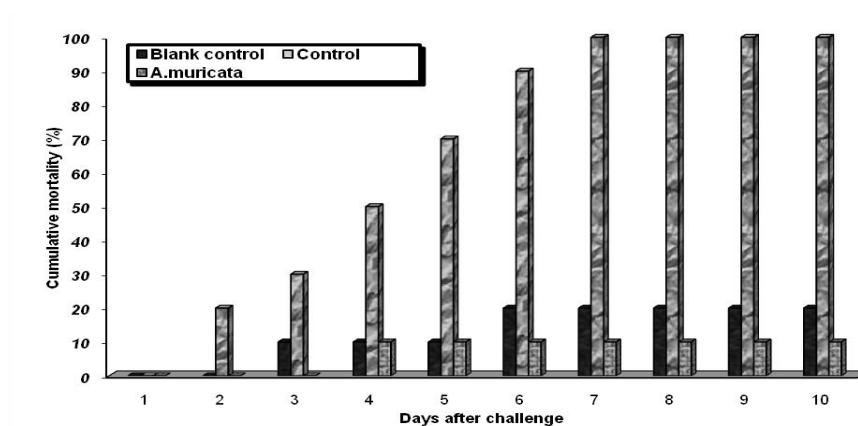


Fig 4. Cumulative mortality (%) of *D. hamiltonii* herbal antibacterial diet fed *C. auratus* challenged with *A. hydrophila*

4. Discussion:

Nowadays the chemical and synthetic vaccines have some demerits including high cost and some side effects. The antibacterial compound from herbal origin is advisable in aquaculture operations due to its versatile characterizers are safety, eco-friendly and create no side effects (Kumaran and Citarasu 2016). In the present study, the phytochemical analysis of the *D. hamiltonii* leaf extracts revealed the presence of saponin, steroid, tannin and flavanoids. The major active constituents of root extract *D. hamiltonii* are steroidal saponins namely shatavarins apart from alkaloids, flavonoids, sterols and terpenes (Kumaran and Citarasu, 2015). The extract of *D. hamiltonii* was separated into its constitutive fractions by preparative thin layer chromatography (TLC). The R_f value obtained was 0.164, 0.275 and 0.611 and the fractions may be active compounds. The FTIR study revealed that, *D. hamiltonii* had primary or secondary amine or an amide or substituted amide, olefinic band, cumulated system, C-F and C-Br bond (Bremer and Geesey, 1991).

Phytochemicals with adequate antibacterial efficacy can be used for the treatment of bacterial infections (Pathak *et al.*, 2010). The herbal antibacterial compound along with the *A. hydrophila* helped to increase the survival in juveniles of *C. auratus* after 25 dpv. The better weight gain (90 mg day⁻¹) was achieved in the highest doses antibacterial compound with inactivated *A. hydrophila* treated groups (Citarasu *et al.*, 2006). The influences of the *D. hamiltonii* extract improve the survival of the treated fishes by 90%. Like the serum biochemical parameters, the hematological parameters also improved the same way. In the present study, the high quality of *D. hamiltonii* extract incorporated diet helped to enhance the hematological parameters after the 25 dpv vaccination.

The present study demonstrated the significant antibacterial efficacy of *Decalepis hamiltonii* leaf extract when incorporated into the diet of *Carassius auratus* challenged with *Aeromonas hydrophila*, a common opportunistic fish pathogen responsible for hemorrhagic septicemia. The phytochemical constituents present in *D. hamiltonii*, particularly phenolics, flavonoids, and saponins, are known to exhibit strong antimicrobial activity, which likely contributed to the observed improvement in survival rate and reduction in infection symptoms among the treated fish groups (Baskaran *et al.*, 2012). The use of herbal immunostimulants such as *D. hamiltonii* offers a promising alternative to conventional antibiotics, especially in

ornamental fish aquaculture, where drug residues and pathogen resistance pose significant challenges. The increased levels of serum albumin, globulin, and total protein in the treated fish further confirm the immunomodulatory potential of *D. hamiltonii* extract, aligning with previous findings that support the bioactive role of this plant in enhancing fish health (Rajan et al., 2002). These results suggest that dietary supplementation with *D. hamiltonii* extract can be a sustainable and eco-friendly strategy to control bacterial infections in aquaculture systems.

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