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Preventive effect of seaweed *Padina tetrastromatica* against *Aeromonas hydrophila* infection in a freshwater fish

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Abstract
The pharmaceutical industry may be able to obtain bioactive metabolites from macroalgae for use in developing new drugs. It is a plentiful supply of all necessary nutrients and shows promise as a source of pharmacognosical value. Alkaloids, phenols, flavonoids, saponins, steroids, and associated active metabolites are of high medicinal value and are frequently used in the pharmaceutical and medical fields. The aqueous extracts of *Padina tetrastromatica* phytochemical components were investigated. The qualitative examination found alkaloids, steroids, tannin, phenols, saponins, terpenoids, and flavonoids to be present. Traditional antibiotics are becoming less effective in the present environment of rising and emerging drug resistance in a variety of microbial diseases, so research worldwide has concentrated on generating alternative therapy treatments with effective microbe abilities. The results of this investigation showed that extracts from the seaweed *Padina tetrastromatica* could be a possible replacement for the creation of bioactive leads in the treatment of aquaculture *A. hydrophila* infections.

Keywords: seaweeds, phytochemical, *Padina tetrastromatica*, *A. hydrophila*, aquaculture

Introduction

Macroscopic algae, such as seaweed, constitute a significant part of the marine biological resource. They are mainly found in shallow coastal waters where there is a surface that will help them to grow and thrive. The seaweeds are roughly divided into three types of macroalgae: green, brown, and red based on their colour. Since ancient times, people have been harvesting them, especially in China and Japan where they are a staple food. The most significant seaweeds in India in terms of their pervasive nature are Sargassum and Turbinaria among brown seaweeds, Hypnea and Kappaphycus among red seaweeds, and Ulva and Caulerpa among green seaweeds. The majority of seaweeds found in India are found around the beaches of Gujarat, Kerala, and Tamil Nadu (Subba Rao and Mantri, 2006) [14]. Algae are a class of plants that includes seaweed. Depending on their chemical and nutritional makeup, seaweeds are categorised as Rhodophyta (red algae), Phaeophyta (brown algae), or Chlorophyta (green algae). Seaweeds, like other plants, contain a variety of organic and inorganic compounds that are good for human health (Kuda *et al*, 2002) [6].

Seaweeds are rich sources of many trace elements, minerals, protein, iodine, bromine, vitamins, polysaccharides, bioactive substances and micronutrients (Chapman and Chapman, 1980). Seaweeds are used as food in many countries like Malaysia, Indonesia, Korea, Australia, Japan and Singapore especially in the form of salads, soups, jellies and in vinegar dishes and its use as food and medicine prior to 2000 BC has found a mention in ancient Chinese medicinal literature (Abbott, 1996). The species used as food includes *Caulerpa sp., Codiurn sp., Hydroclathurn sp., Sargassurn sp., Porphyra sp., Laurencia sp. Enterornorpha, Gradiaria, Sargassurn, Padina* and *Dictyota* sp. are used as feed for cattle and poultry (Qasim, 1998) [10]. The seaweeds have enormous biological potential and have been utilised in traditional medicine for a wide range of therapeutic purposes, including the treatment of dermatitis, gallstones, gout, scrofula, fevers, menstruation issues, renal issues, scabies, etc (Chapman, 1970; Hoppe, 1979) [4].

In brown, red, and green algae, substances having antioxidant, antiviral, antifungal, and antibacterial properties have been found (Bansemir *et al*, 2006) [2]. Seaweeds have a challenging habitat because they are subjected to both light and high oxygen levels. Free radicals and other potent oxidising agents can arise as a result of these conditions, yet seaweeds seldom sustain significant photodynamic damage during metabolism. This fact suggests that the cells of seaweed have certain defence mechanisms and substances. By chelating metal ions, limiting radical production, and enhancing the antioxidant endogenous system, phenolic substances can function as antioxidants. Several hundred molecules that are found in edible plants and include at least one hydroxyl group substituting for a benzene ring are known as "phenolic compounds" (Manach *et al*, 2004) [8]. Seaweeds and other plants frequently contain these phenolic chemicals. Flavonoids (also known as flavones, flavonols, flavanones, flavononols, chalcones, and flavan-3-ols), lignins, tocopherols, tannins, and phenolic acids are all included in the category of substances known as polyphenols (Shukla *et al*, 1997) [11].

In India, aquaculture has grown significantly over the past few decades. Intense aquaculture has resulted from the mass production of fish for human use. Additionally, unintentional wounds that develop during these cultivation techniques propagate contagious illnesses. Due to the economic losses brought on by inadequate growth and aquaculture illnesses, intensive freshwater aquaculture has taken on significant importance in India. The common carp

is prone to a variety of ailments, including red illness brought on by *Aeromonas hydrophilla* and hemorrhagic septicemia. Antibiotics are used to prevent infections in aquaculture that have negative impacts on the bacterial population in the aquatic ecosystem (Subasinghe, 2009) [13]. (Lalumera *et al*. 2004).In the present study, tested the potential seaweed *Padina tetrastromatica* extract as a means of providing growth efficiency and passive immunoprophylaxis.

Materials and Methods

1. Seaweed collection

Padina tetrastromatica seaweed was collected from the intertidal region of the Kadiyapattinam, Kanyakumari district, Tamilnadu. The seaweed samples were properly cleaned in fresh water to remove salt and other impurities before being air dried in the shade. The powdered dried seaweeds were divided into smaller pieces, kept at 4 °C pending analysis. In the field, seaweed is collected when the tide is low. According to tide tables, people must arrive for pickup one or two hours prior to low tide.

2. Identification of samples based on morphological criteria

Simple morphological criteria, reproductive structures, life history type, cross-sectional anatomical information, growth type, cytology, ultrastructural criteria, and increasingly molecular evidence are used to identify an organism. It is necessary to carefully study the colour and morphological distinctions among various genera, species, and taxonomic characteristics. The species were identified based on the encyclopedia resources. The identified samples were used for further bio chemical analysis.

3. Preparation of Extracts

After drying, the algae were weighed and then cut. Using a mixer grinder, the sample pieces were finely ground. Five grammes of the finely ground samples were weighed and then dissolved in a variety of organic solvents, including 80% ethanol, methanol, and chloroform. It was combined consistently over the course of 48 hours at room temperature. Using Whatman No1 filter paper, the sample that had been dissolved in each solvent was separated after 48 hours to be used for antimicrobial testing of algal samples.

4. Phytochemical screening (Sofowora, 1993; Trease, 1989) [12, 15].

Due to the demand for substances with potential for use in pharmaceuticals, research into the chemistry of seaweeds has grown dramatically in recent years. Many different species have been tested for their activity, and a number of biodynamic compounds have been isolated—often with hazardous characteristics and distinctive structural traits. It is sense to assume that secondary metabolites produced by marine creatures will differ dramatically from those produced by terrestrial animals given the stark differences in their environments. The presence or absence of active secondary metabolites like saponin, terpenoids, tannins, steroids, alkaloids, flavanoids, phlobatannins, anthraquinones, and phenol were checked by phytochemical analysis of an aqueous extract from specific algae, Padina tetrastromatica. The presence or absence of the chemicals in the Padina tetrastromatica algae extract was determined by general reaction in this investigation.

5. Fourier Transform Infra-Red (FTIR) Spectroscopic Analysis

The active components in the chosen hot water extract, such as *Padina tetrastromatica*, were qualitatively evaluated using Kemp's Fourier transform infrared (FTIR) method (1991). The procedure for sample preparation was followed as laid forth by Naumann *et al* (1991b) [9]. In a nutshell, a known weight of dry potassium bromide (KBr) (2.5 mg) was completely mixed with a known weight of dry algal sample (1 mg) in a smooth agate mortar. In order to get the diffuse reflectance infrared spectrum for replicate samples, the powder was placed within a microcup with a 2 mm internal diameter. Using the FTIR-8201PC, Fourier Transform Infrared Spectrometer, all IR spectra were captured in the mid infrared range (4000-400 cm^{-1}) at room temperature (26 °C ±1 °C) (Shimadzu).

6. Experimental Fish

The fish chosen for the present experiment were *Cyprinus carpio*. Its decision was justified by factors such as its excellent growth rate, ease of accessibility, widespread distribution, commercial significance, etc. It may thrive in a range of aquatic settings since it is a tolerant and hardy fish. Aqua-farmers have a strong demand for its seed for a range of uses, including monoculture and polyculture. Studies were thus conducted in aquaria and ponds due to the constant requirement for fry and fingerlings. For two weeks, the stock was acclimated in an aerated environment. The fingerlings were fed a baseline diet during the acclimation phase. Twenty-four hours prior to the start of the trial, the feeding was halted. Daily water quality monitoring and a 50% water exchange were conducted during throughout the experimental duration. The temperature was maintained at 16.5 ± 1 °C, dissolved oxygen concentration of 6.0 ± 1 mg l^{-1}, pH 7.5 ± 0.5.

7. Feeding

The experimental animals received 10% of their body weight of antibody-coated chow twice daily at 8 a.m. and 20 a.m. All experimental and control groups received their regular diets at 12:00 hours. The above-mentioned regimen was repeated three times for the control groups, who received uncoated feeds. Before feeding, the leftover food and waste were removed.

8. Treatments

After the period of 10 days acclimatization, different experimental treatments were started. In 18 plastic aqua's of 100 l capacity 50 fishes were distributed in triplicate, on each of the aqua at temperature of 28 °C, pH 7 and afterwards the following treatments were carried out:

Treatment 1: (Control group): The control fish groups were fed with commercial feed twice per day during the experimental period.

Treatment 2: (Experimental (E 2) fish group): The experimental (E 1) fish groups were fed with *P.tetrastromatica* extract supplemented diet (100 mg) feed twice per day during the experimental period.

Treatment 3: (Experimental (E 2) fish group): The experimental (E 2) fish groups were fed with

P.tetrastromatica extract supplemented diet (200 mg) feed twice per day during the experimental period.

9. Survival and growth parameters

Every 10 days throughout the culture period, the overall survival and death following challenge were evaluated. By subtracting the original weight from the end weight, the weight gain (wet) was computed. Through the use of the formula, the specific growth rate (SGR) was determined.

$$SGR\ (\%) = \frac{(\text{Ln } W_2 - \text{Ln } W_1)}{(t_2 - t_1)} \times 100$$

Where, Ln =Logarithmic number, W_2 = Final weight at time t_2, W_1 = Initial weight at time t_1.

10. Growth Inhibition Assay (Guimarães *et al.*, 2009) [13].

This assessment demonstrates that P. tetrastromatica extract's binding ability may prevent the growth of A. hydrophila in a liquid media. The same A. hydrophila strain that was employed as an antigen in the immunisation of hens was subcultured on tryptic soy agar plates with 1.5% NaCl added, and it is now suspended in TSB. 2 ml of TSB and 2 ml of prepared bacterial culture were combined, then incubated at 37 oC while being shaken. A spectrophotometer was used to quantify the culture's turbidity (optical density at 600 nm) at 1-h intervals. Up until the station stationary period, the growth curve was displayed.

Results

1. Distribution of seaweeds

Seaweeds were collected from Kadiyapattinam coastal region of Tamil Nadu during all seasons. Identified species belonged to Rhodophyceae (35.6%), Chlorophyceae (37.1%) and Phaeophyceae (28.3%). The substantial dominance of green algae over red and brown algae suggests that the existence of rocky coastlines is necessary for the attachment, which is predominantly found in Tamil Nadu.

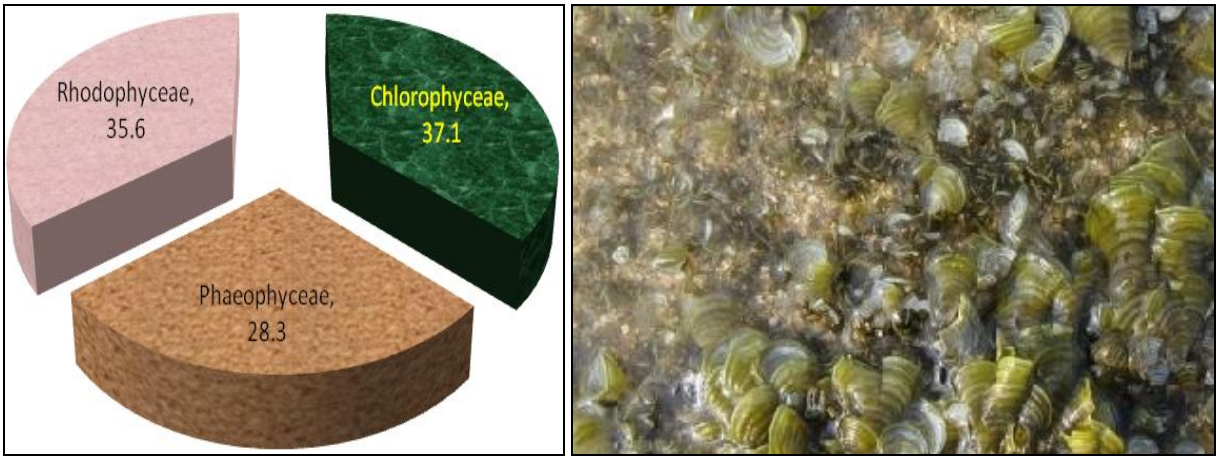

Fig 1: Percentage distribution of different groups of seaweeds at Kadiyapattinam coastal region of Tamil Nadu

2. *Padina tetrastromatica*

The fan-shaped, foliaceous thallus of *Padina tetrastromatica* is brown to yellowish brown in colour. The segments are dichotomously formed fans that are 5-55 cm long and 1-3 cm wide, irregularly branching. The thallus's growth is mediated by a marginal meristem, and its apical borders are involute. The thallus surface is covered in hairs, and calcium-rich marginal sori are the reproductive structures that appear there.

3. Phytochemical analysis

From the collected samples, specimens of the seaweed *Padina tetrastromatica* were chosen. The existence of phytochemicals presumed to be active chemical components in medications has been discovered by this investigation. The samples contained important therapeutic phytochemicals such saponin, titerpenoids, tannin, flavonoids, alkaloids, and glycosides. The seaweed *Padina tetrastromatica* had tannin, saponin, flavanoids, and alkaloids, according to the results of the phytochemical examination. (Table 4.1).

Table 1: Phytochemical Analysis of selected seaweeds by standard protocol

S. No	Phytochemical Compounds	*Padina tetrastromatica*
1	Tannins	Positive
2	Saponins	Positive
3	Steroids	Negative
4	Triterpenoids	Positive
5	Anthraquinones	_
6	Flavonoids	Positive/negative
7	Alkaloids	Positive
8	Cardiac glycosides	Negative

4. Analysis of functional groups

The active portions of the antibacterial extracts as determined by Fourier Transform Infrared Spectroscopy analyses are shown in figure 3.2. In wave numbers 500–4000/cm, the potential functional groupings of active principles were examined. The following peaks in the I-R spectrum were produced by the active fraction of hot water extracts of *Padina tetrastromatica*. The peaks represented the molecule's numerous functional groups. One peak at 1041 cm^{-1} may be caused by C-O stretching, while the broad peak about 2922 cm^{-1} may be caused by -OH stretching or -NH stretching. C-C-H might be the cause of one at 673 cm-1. One at 1624 cm-1 might result from RONO2. The observation showed that it is possible to assume that the compound is either an alkene or a ketone. Thus, where the OH group is hydrogen bound, the extract may contain a free carbonyl group. It's also possible that the extract contains a carbonyl species conjugated to an O= bond. (Table 3.2)

Table 2: Molecular stretches of active principles isolated from the hot water extracts of *Padina tetrastromatica* through FTIR Spectroscopic analysis

S.no	Peak	Bond type	Specific context	V_1 cm^{-1}
1	804.11	-	-	-
2	893.04	Alkenes	R2C=CHR	790 – 840
		C-X	C-Cl	800 – 850
3	1074.36	C-N	C-N	1030 – 1230
		C-O	C-O	1020 – 1275
		C-X	C-F	1000 – 1350
4	1261.46	N-O	$RONO_2$	1270 – 1285
5	1361.00	N-O	RNO_2	1350 – 1560
			R-S(=O)$_2$-OR’	1330 – 1420
6	1614.42	N-O	$RONO_2$	1620 – 1640
		N-O	RO-N=O	1610 – 1680 (Two)
7	1739.79	C-O	C=O	1650 – 1800
8	2852.82	C – H	C_{sp}3-H	2800 – 3000
9	2929.87	C – H	C_{sp}3-H	2800 – 3000
10	3330.07	-	-	3200 – 3400 (H-bonded)
11	3330.30	3200 – 3400	(H-bonded)	3200 – 3400 (H-bonded)

3. Cumulative Mortality after 30 days culture

The cumulative mortality of the *C. carpio* fed the *P.tetrastromatica* extract d diets were given in the Fig 3.3. There is only 15 % mortality were observed in the control. After 30 days of culture, 100% of the C. carpio groups that were fed a control diet died within 9 days. Additionally, in the diet E1 fed C. carpio, the cumulative mortality rate was prolonged by 15 days. The 200 mg coated meals used by the E2 group contribute to a reduction in overall mortality. After 30 days, this group dramatically improved (P 0.05) the ability to survive and withstand the resistance.

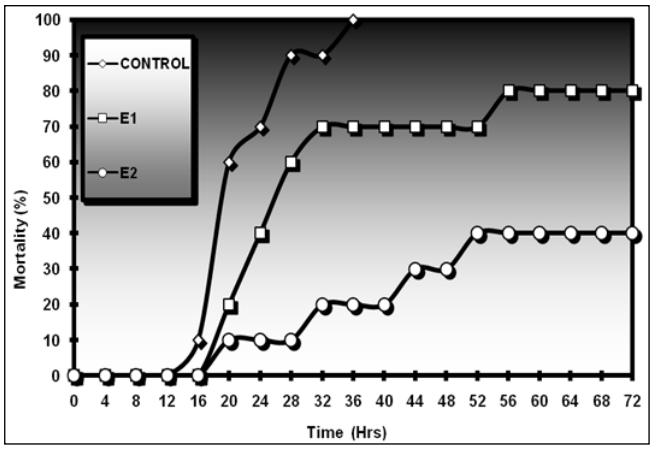

Fig 2: Cumulative mortality of the *C. carpio* fed the *P.tetrastromatica* extract incorporated diets fed and challenged with *A. hydrophila* after 30 days

4. Growth characteristics and survival of after 30 dpv

The weight gain (g) and specific growth rate (%) of 30th days were given in the Fig.3.4. The weight gain is recorded in the *P.tetrastromatica* extract coated 1.6g of the total period. This was significantly increased to (P< 0.05) 2.4 and 2.6 g of the total experimental period of 200mg diet fed groups. The specific growth rate (%) was observed also reflected the same manner in the *P.tetrastromatica* extract, 200mg diet fed groups. The SGR was observed in the control groups are 0.2 g. This was significantly increased to 0.31 and 0.34 % respectively in the E1 and E2 groups.

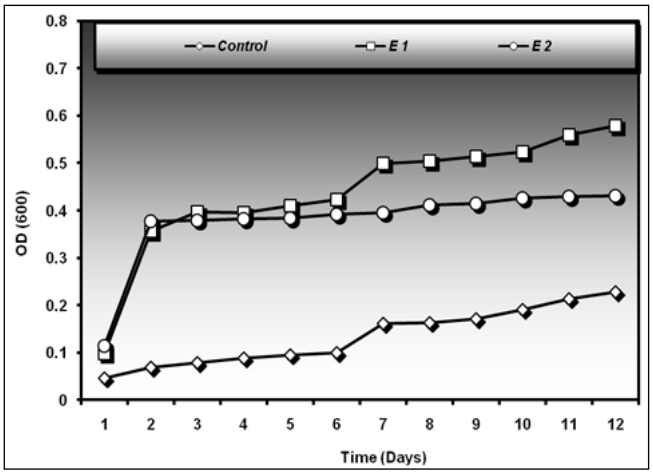

Fig 3: Growth rate of the *C. carpio* from the *P.tetrastromatica* extract incorporated diet treated at diffrent days

Growth inhibitory effect of *Padina tetrastromatica* extract

A hydrophila has lag phase (0–2 hours of incubation time), exponential (2–6 hours of incubation time), and stationary phase growth curves. For the purpose of the growth incubation assay, *A. hydrophila*, control and *P.tetrastromatica* extract were incubated for 6 hours. Samples were taken every 2 hours during this time. After 4

hours of incubation, the growth of *A.hydrophila* exposed to *P.tetrastromatica* extract was significantly reduced. The lag phase and exponential phase of bacterial growth, which were maintained from 0–2 hours and 2–6 hours of incubation, respectively, were little affected by control. Effective doses of *P.tetrastromatica* extract were observed in the prevention of bacterial growth, although the effect was minimal in the control. (Fig 3.5)

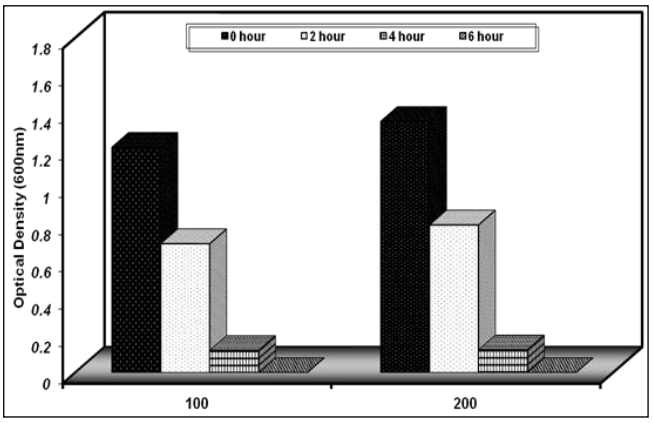

Fig 4: Growth inhibition assay performed between *A. hydrophila* and *P.tetrastromatica* extract

Discussion

A group of plants known as seaweeds or benthic marine algae inhabit either brackish or saltwater environments. Similar to land plants, seaweeds have pigments that enable them to make food through photosynthetic processes with the help of sunlight and nutrients found in seawater. Seaweeds have been investigated as possible biocidal and medicinal agents since they represent a rich and diverse source of bioactive natural compounds. Numerous compounds synthesised from macro algae, including those with antibacterial, antifungal, antiviral, antineoplastic, antifouling, anti-inflammatory, antitumoric, cytotoxic, and antimitotic activities, have seen an improvement in their biological activity in recent years. Seaweeds are currently an economically significant marine renewable resource that is offering key insights for the creation of novel medications to treat cancer, microbial infections, and inflammation.

The goal of the current study is to highlight the possible uses for crude algal extracts and find creative ways to use these under utilised resources for the good of humanity. There is a growing demand for novel antibiotics due to the rising resistance of microbes to existing antibiotics. Since seaweeds are a particularly rich source of bioactive compounds, the current study was done to look into the marine algae *Padina* spp potential's for fighting microbes.

Seaweed extracts in different solvents exhibited different antimicrobial activities. A wealth of structurally varied secondary metabolites can be found in seaweeds. Tuney *et al*. (2006) [16] were the first to notice the antibacterial potentials of seaweeds. These secondary metabolites give defence against herbivores, fouling organisms, and infections. They also have a function in reproduction, UV radiation protection, and as allelopathic agents. Numerous algae species have been shown to contain bacteriostatic or bactericidal compounds. Among the bactericidal compounds found in algae are aminoacids, terpenoids, phlorotannins, acrylic acid, phenolic compounds, steroids, halogenated ketones and alkanes, cyclic polysulphides, and fatty acids.

The results of the current investigation suggest that the broad peak in the *Padina tetrastromatica* extract about 2922 cm^{-1} may be caused by -OH stretching or -NH stretching, while the peak at 1041 cm^{-1} may be caused by C-O stretching. One at 673 cm^{-1} might be caused by C-C-H. One at 1624 cm^{-1} might result from $RONO_2$. Similar results reported Lisette D'Souza, (2008) [7] the spectrum for *Padina tetrastromatica* spp presence of the intense bands in the region 599 cm-1 that is very characteristic of Phosphate group. The intense bands are also observed at about 1648 cm^{-1} which are due to the presence of proteins that are assigned to the amide I vibrations. The bands at 3433 and 1054 cm^{-1} shows the presence of O-H functional group and polysaccharides respectively.

There is a growing demand for novel antibiotics due to the rising resistance of microbes to existing antibiotics. As a particularly rich source of bioactive compounds against the microorganisms that cause diseases and problems in people, animals, and plants. seaweeds. The antibacterial efficacy of crude extracts against the investigated pathogens was extremely diverse. Various solvents used to extract seaweed showed different antibacterial properties. The combined antimicrobial activity determined from the results above shows the presence of active compounds in seaweed extractions, which can be used to produce lead compounds for the pharmaceutical industry.

Conclusion

In all treatments, this study found that adding *P. tetrastromatica* extract to the common carp's diet improved growth performance. The highest value on growth rate was found in diets supplemented with 200 mg of E2, which also provided protection by raising the survival rate. When added to the diet of common carp, the control diet had no beneficial influence on growth performance, but it did have a favourable impact on survival rates. The addition of *P.tetrastromatica* extract enhances common carp growth performance and survival rate. The aquaculture of fish might benefit from this knowledge.

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