

Methanolic extract of *Cassia tora* Seeds Effects on Growth Performance of Culturable Fishes, Rohu (*Labeo rohita*) and Catla (*Catla catla*)

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ABSTRACT

Herbal ingredients have attracted the scientific study since ages and their rich bioactive compounds are known to be loaded with nutrients essential for growth and also have repairing capacity to detoxify. The aim of the study was to evaluate the effects of Herbal diet *Cassia tora* seeds mixed with basal diet supplementation on the growth performance on Indian common carps *Labeo rohita* and *Catla catla*. *Cassia tora* seeds are medicinal plant species widely used as natural antioxidants and hepatic-nephric protector. Rohu and Catla is a commercially valuable and common edible fish species. The present study was undertaken to assess the effects of dietary *Cassia tora* seeds on the growth performance and antioxidant status of rohu and Catla. Fingerlings were fed on diets containing 2%, 5% and 10% *Cassia tora* seed for 28 days. The results showed that rohu and catla fed on *Cassia tora* seed with basal diet has increased growth rate. Moreover, *Cassia tora* seed supplementation improved the muscles protein contents, fibres, crude fiber, carbohydrates and crude fats and antioxidant status.

The results of the study concluded that the Herbal Diet fed to *Labeo rohita* and *Catla catla* showed significantly improved growth performance of fishes. The potential for improving growth parameters in fishes may be due to the nutritional and its antioxidant property of *Cassia tora* seeds. So, the present study recommends the use of herbal diet of *Cassia tora* seeds in fish feed in appropriate amount improved the growth performance in fishes.

Keywords: *Labeo rohita*, *Catla catla*, Herbal Diet, and *Cassia tora*.

Introduction

India is one of the few countries where almost all known medicinal plants can be cultivated in some part of the country or the other. Among the various plants in great demand in the country and abroad are Opium poppy, tropane, alkaloid-bearing plants, sapogenin-bearing Yam, Senna, Psyllium husk and seeds, cinchona, and Cassia. These herbal drugs symbolize safety in contrast to synthetic drugs, which are considered unsafe for both human beings and the environment.

Some important herbs include:

Flaxseeds: Flaxseeds are the dried ripe seeds of *Linum usitatissimum*, from the family Linaceae. While flaxseed is primarily cultivated for its oil, it also possesses many medicinal properties.

Spirulina: Spirulina is a blue-green algae obtained from *Spirulina platensis* or *S. maxima*, belonging to the family Oscillatoriaceae. This simplest form of photosynthetic algae grows in freshwater in planktonic form.

Garlic organosulfur compounds: Garlic consists of the fresh or dried bulbs of *Allium sativum*, from the family Lilaceae. The bulbs contain concentric bulblets with a distinctive strong odour and acidic taste.

Cassia tora: *Cassia tora* is used in folk and traditional medicines. It belongs to the family Leguminosae and is well-known and widely distributed in India and other tropical countries. The entire plant is of medicinal importance. Its leaves function as a laxative and purgative, useful for habitual constipation. The seeds possess antiparasitic properties, and their paste is applied topically to treat ringworms and scabies. The plant's root is used as a bitter tonic and stomachic, while the leaves serve as an antiperiodic aperient and anthelmintic. *Cassia tora* is also consumed as a leafy vegetable, and its roasted seeds are

used as a coffee substitute. Boiled seeds are administered to animals to treat hypogalactia.

1. Collection and Authentication of *Cassia tora*

Cassia tora seeds were collected during the month of July from the herbal garden of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, Madhya Pradesh. The freshly collected seeds (250 gms) were washed, dried, weighed, ground into a fine powder, and methanol (3 litre) was added for 73 hours. Maceration and defatting were done using 2 litre of petroleum ether solvent, and the extract was then recovered in an incubator at a low temperature of 40°C for 2 days. The crude extract was scraped, resulting in 310 gms of dried crude extract, which was mixed with different proportion of maize bran.

Phytochemical Evaluation of *Cassia tora*

Phytochemical Screening:

Preliminary screening of phytochemicals is a valuable step in detecting the bioactive principles present in medicinal plants, which may subsequently lead to drug discovery and development. For phytochemical screening, 50 gm of the methanolic extract was subjected to chemical tests to establish its phytochemical profile, and observations were recorded.

Requirements: Test tubes, test tube stand, spatula, filter paper, funnel, tripod stand, and different solvents.

Phytochemical and Biological Evaluation of *Cassia tora* (Fathalla *et al.*, 2015)

(A) Test for Alkaloids:

Mayer's Reagent: A portion of the extract was treated with a few drops of diluted hydrochloric acid and 0.5 ml Mayer's reagent. The formation of a white precipitate indicates the presence of alkaloids.

Wagner's Reagent: Few drops of dilute HCL and 0.5 ml Wagner's reagent were added to a portion of the extract. A brown flocculent precipitate indicates the presence of alkaloids.

(B) Test for Glycosides:

Fehling's Test: The extract was hydrolysed with HCl solution and neutralized with NaOH solution. A few drops of Fehling's solution were added. A red precipitate indicates the presence of glycosides.

(C) Test for Tannins:

Ferric Chloride Solution: A small quantity of extract (50 mg) was mixed with water and heated on a water bath. The mixture was filtered, and ferric chloride was added to the filtrate. A dark green solution indicates the presence of tannins.

(D) Test for Flavonoids:

Lead Acetate: To a small quantity of extract (50 mg), add lead acetate solution. A yellow-coloured precipitate is formed.

Alkaline Test: 2 ml of extract with the addition of increasing amounts of NaOH to the residue shows yellow coloration, which decolorizes after the addition of HCl.

(E) Test for Saponins:

Foam Test: 1 ml of extract was dissolved in 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable, persistent froth with a honeycomb structure, indicating the presence of saponins.

(F) Test for Terpenoids:

Salkowski Test: Five millilitres of extract was mixed with 2 ml of chloroform, and then 3 ml of concentrated H₂SO₄ was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

(G) Test for Anthraquinone:

In 2 ml of extract, 3 ml of benzene and 5 ml of 10% ammonia were added. The appearance of pink, red, or violet coloration in the ammonical layer indicates the presence of anthraquinones.

(H) Test for Emodins:

In 2 ml of extract, 2 ml of ammonium hydroxide and 3 ml of benzene were added. A red coloration indicates the presence of emodins.

(I) Test for Proteins:

In 2 ml of extract, a few drops of concentrated sulfuric acid were added. A white precipitate indicates the presence of protein.

Feed preparation: The commercially available maize flour was purchased online from Big Basket, Bhopal, Madhya Pradesh, and used as a basal diet for the present study. Three herbal diets (H) containing *Cassia tora* extract were mixed into the basal diet and formed into pellets. Each diet contained a different ratio of protein:

H-1 (2% *Cassia tora* extract and 98% maize)

H-2 (5% *Cassia tora* extract and 95% maize)

H-3 (10% *Cassia tora* extract and 90% maize)

H-0 (control diet i.e. regular diet).

After forming into pellets, the complete feed was dried at 40°C in an incubator. After 3 to 4 hours, pellets were removed and stored at room temperature. The size of the pellets was measured by a digital vernier calliper from the Fisher scientific. This feed size was suitable for both the fishes Rohu and Catla's mouth. The physical appearance of H-1 pellets was brown, H-2 was chocolate brown, and H-3 was blackish brown. The water stability of these feeds was measured by taking 5 gms of pellet in 250 ml of water in a borosil glass beaker and tested in triplicate. The changes that occurred were observed until the whole pellets were dissolved. Feeding responses were classified into four: Orientation, Approach, Capture, Rejection, and Ingestion. Shape influenced food capture, whereas taste and texture influenced ingestion. Longer pellets were preferred over round ones, and softer pellets were preferred over harder ones. (L. Strameyer, 1989).

Fish Feeding Trial: A total of 16 Rohu and Catla fishes, weighing 28gms to 30gms and measuring 10-15cm in size, were used as experimental animals for 28 days. They were acclimatized for 7 days in four aquaria, and fishes were starved for 24 hours before being given the experimental diets. The fishes in group H-0 were fed with the regular diet and treated as the control group, while H-1, H-2, and H-3 were the test groups. The fishes were fed twice a day at the rate of 5% of their body weight, and their size was adjusted on a weekly basis. The water in the four aquaria was siphoned daily and replaced with clean, well-aerated water. Water quality parameters like temperature, dissolved oxygen (DO), and pH were measured daily using a DO meter (Orion 1230) and pH meter (Hanna - HI-96107) respectively. Sampling was done every week. (Kafle *et al.*, 2021)

Fish growth measurement: After completion of the 28th day, sampling of weights using an electronic balance (Autotech Anex Digital) from each aquarium was done, and the length of the fishes was measured by a measuring scale. These fishes were then released back into their respective aquarium. Growth parameters like initial weight (gm/fish), final weight (gm/fish), total weight gain (gm/fish), daily weight gain (gm/fish), survival rate (%), and sizes were calculated at the end of the experiment. (Kafle *et al.*, 2021)

**Table :1 Fish Feeding and Growth Performance of
Rohu of HMDD for 28 days Trial**

Parameters	Group-H0	Group-H1	Group-H2	Group-H3
Initial mean weight(g/fish)	26.89±0.81	26.98±1.08	27.04±1.04	27.16±0.97
Final weight(g/fish)	36.19±1.00	40.89±1.85	53.78±0.96	103.78±1.154
% weight gain	9.3±0.17	13.91±0.35	26.74±0.76	76.62±0.32
Specific growth rate	1.060±0.05	1.484±0.08	2.455±0.20	3.703±0.23
Average daily weight gain	0.332±0.07	0.496±0.55	0.955±0.02	2.736±0.28
% Survival rate	100	100	100	100

Mean values in the same row are significantaly different
(p < 0.05)

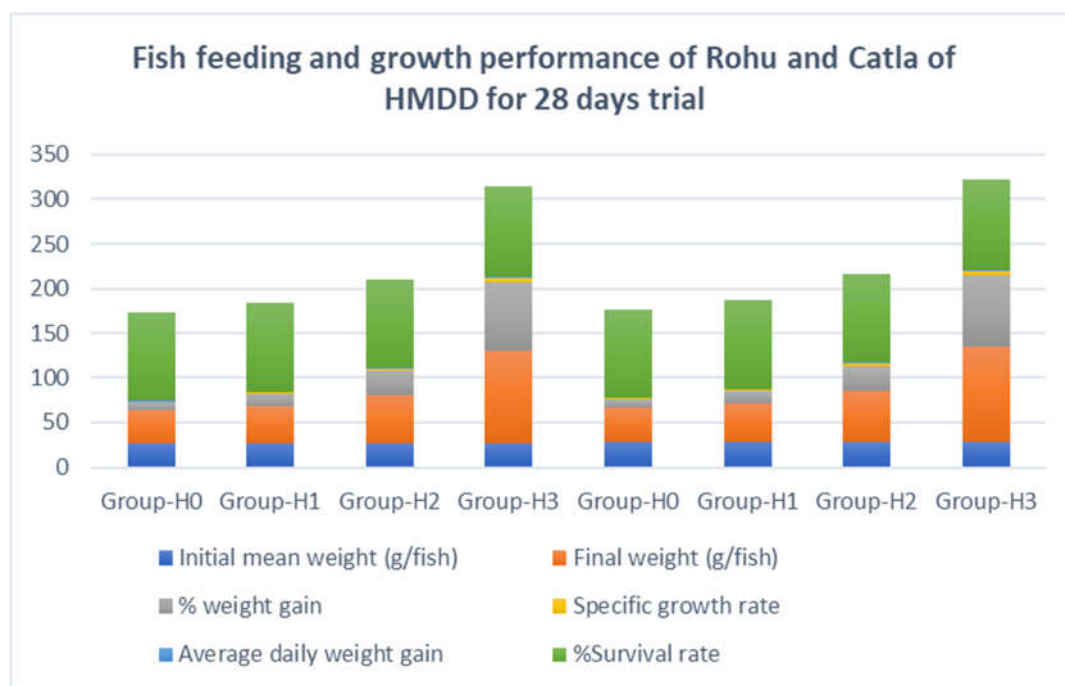
**Table :1 Fish Feeding and Growth Performance of
Catla of HMDD for 28 days Trial**

Parameters	Group-H0	Group-H1	Group-H2	Group-H3
Initial mean weight(g/fish)	28.60±0.94	28.81±0.86	28.99±0.72	29.07±0.60
Final weight(g/fish)	37.90±1.98	42.60±1.57	56.60±0.87	107.12±1.16
% weight gain	9.3±0.17	13.79±0.30	27.61±0.70	78.05±0.45
Specific growth rate	1.005±0.02	1.396±0.06	2.389±0.10	4.648±0.34
Average daily weight gain	0.332±0.022	0.492±0.04	0.986±0.34	2.78±0.31
% Survival rate	100	100	100	100

(p < 0.05)Mean values in the same row are significantly different

Result

The seed of *Cassia tora* is a good source of protein, crude fiber, carbohydrates and crude fats. The crude protein, crude fibers, carbohydrates and crude fats content were found to be 13.79%, 7.78%, 33.47%, 16.08% in the raw seeds of *Cassia tora* respectively. The effects of *Cassia tora* seeds diet on the growth performance of Rohu and Catla fishes indicated that the dietary inclusion of different levels of *Cassia tora* seeds with basal diet maize has positive effects on fishes growth H-3 diet. A significant increase in weight in %WG (percentage weight gain) SGR (Specific growth rate) of diet H-3 in comparison with control and H-1 and H-2 diets. The results also indicated that H-3 diet has statistically higher % of average daily Weight.gain. The fishes survival rate were 100% for all the experimental groups. The diet H-0, H-1, H-2, H-3 results are shown in **Table -1**.



Statistical Analysis:The values shown are Mean $\pm$ SEM

Significance: $p < 0.05$, compared to control (one way ANOVA followed by Tukey Kramer/ Tukey HSD multiple comparison tests).

Discussion and Conclusion

Phytochemical Investigation of *Cassia tora* Seeds:

A phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides in the crude extract of *Cassia tora* seeds. These compounds are responsible for potential bioactivities, (Assam et al., 2019).

Cassia tora seeds are used as an appetizer, growth promoter, hepato-nephric protector, immunomodulator, antistress agent, and antioxidant in aquaculture. The aim is to provide safe and eco-friendly compounds to replace antibiotics and chemical compounds, as well as to enhance immune status and control the toxicity of heavy metals in fish. The seed extract mixed with the basal diet is used as an immunostimulant, resulting in a significant enhancement of the fish immune system to prevent heavy metal toxicity. *Cassia tora* acts as a growth promoter and is used as an inexpensive source of protein in fish meal, increasing the protein content and thus improving its nutritional value. Therefore, *Cassia tora* seeds with the basal diet serve as plant-based antioxidants and growth promoters that minimize the effects of toxic elements in fish. They replace synthetic antioxidants in fish feed and are used as an additive in aquatic ecosystems. (Elham Awad et al., 2017).

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