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Immunomodulatory effect of *Cassia alata petals* in *Garra rufa* (Doctor Fish)

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ABSTRACT

Cassia alata, a well-known medicinal and ornamental plant have several biological activities such as antimicrobial, purgative, anti-inflammatory, analgesic, hypoglycemic and antitumor activities. Considering the growing demands of herbal medicine and the need for natural sources for immune stimulants the immuno stimulant potency of the common ethno-medicinally important plant *Cassia alata* was investigated. Petals of *Cassia alata* was used for this study and feed was prepared from it. Phytochemical screenings were also performed using the petroleum ether extract of the petals. Freshwater fish *Garra rufa* was used as a specimen model in the study. After the feeding period, the blood samples were collected from the fishes of each group and haematological parameters were analysed. The finding suggests that the feed of *Cassia alata* is a good immuno stimulant.

KEY WORDS: *Cassia alata, Garra rufa,* haematological parameters, immunostimulant.

1. INTRODUCTION

Cassia alata Linn belongs to the class Fabaceae. It is identified as an important medicinal and ornamental plant. *Cassia alata* is a native of tropical America. It is now widespread in warm countries. It includes several medicinal values such as antimicrobial, antifungal anti – inflammatory, purgative and antitumor. It is commonly called as candlestick plant (England), ringworm, wild senna (England), semaiagathi (Tamil). All the parts of this plant have medicinal properties. It is rich in important phytochemical constituents such as polysaccharides, glycosides, alkaloids, phenols, flavonoids, cardiac glycosides. *Garra rufa* also called as doctor fish, nibble fish, kangal fish and bone fish. They are native of northern and central Middle East. *Garra rufa* are known to remove dead skin and producing diathanol. Diathanol is a chemical associated with the regeneration of skin. The fish have no teeth and they nibble the dead or unhealthy skin. This allows the process of regeneration of cells to occur. Although they are hardy and capable of tolerating cooler waters, *Garra rufa* prefer tropical conditions .Ichthyotherapy, is a method of treating skin diseases using two different types of fish, *Cyprinion macrostomus* and *Garra rufa*. Ichthyotheraphy was applied for first time in therapeutic purpose only in kangal spa in the central Anatolia region of Turkey. *Garra rufa* can tolerate wide variety of water conditions and temperatures. pH should be maintained at 7.0 (neutral) and temperature should be between 65 and 85 degrees Fahrenheit (18-30 degree centigrade). They are omnivores and require a balanced diet. Mortality rate of therapeutic fish *Garra rufa* by *Aeromonas sobria* were prevalent among ichthyotheraphy treatments. Among the variety of health issues in fishes, infections caused by bacteria are highly pathogenic to warm water fishes. The severity of infection caused by Bacterial sp in *Garra rufa* were reported in several studies. Antibiotics used against these infections have been identified to increase the risk of developing antibiotic resistant strains. Vaccines were also developed but they are not available in market. Ancient histories the medicinal plants sources were used as the immune modulating agents in traditional medicines. The use of medicinal plants sources as immune modulators may show a strong antibacterial effect in preventing bacterial infections. Medicinal plants are used not only to treat diseases but also as growth promoters, stress resistance boosters, preventatives of infections and they also act as immuno stimulants. Thus, the present study was aimed to evaluate the effect of *Cassia alata* petals as an immune stimulant and to increase the resistance of the fishes against the infection of microbes.

Figure.1. *Cassia alata*

2. MATERIALS AND METHODS

2.1. Fish rearing and conditioning: The experimental fish *Garra rufa* were purchased from Jeevan Aqua Palace, Chennai. The fishes of uniform length (10 cm) and weight (25±5g) were segregated from stock and acclimatized for 10 days to lab condition, temperature (30±2°C), pH (7.5- 7.8) and for a photoperiod (12:12-h L/D).

2.2. Flower collection and processing: The medicinal plant *Cassia alata* was collected from local places of Kanchipuram, Tamil Nadu and was scientifically authenticated. These flowers were shade dried and crushed into powder using ball mill and the powder was used for the experimental study.

2.3. Sample preparation: The finely powdered petals were extracted with petroleum ether using soxhlet's apparatus.The extract obtained was used for the phytochemical screening.

2.4. Phytochemical screening of *cassia alata* petal extract:

2.5. Test for flavonoids: To 1ml of extract, 2ml of distilled water and few drops of 10% aqueous $Fecl_3$ were added. Formation of brown colour indicates the presence of flavonoids.

2.6. Test for alkaloids: Extract were treated with Mayer's reagent (potassium Mercuric Iodide). Formation of yellow coloured precipitate indicates the presence of alkaloids.

2.7. Test for phenols: To 1ml of extract, 2ml of distilled water and few drops of 10% aqueous $Fecl_3$ were added. Formation of blue or green colour indicates the presence of phenols.

2.8. Test for cardiac glycosides: 5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was layered with 1ml concentrated sulphuric acid. Formation of brown ring indicates the presence of cardiac glycosides.

2.9. Test for steroids: To the 2 ml of the extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of blue of green ring formed indicates the presence of steroids.

2.10. Test for terpenoids: 5ml of extract were mixed with 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added along the sides of the test tube. A reddish brown colour at the interface indicates the presence of terpenoids.

2.11. Test for glycosides: 1ml of the extract was dissolved in 1ml of glacial acetic acid and cooled, after cooling, 2-3 drops of ferric chloride was added. To this 2ml of concentrated H_2SO_4 was added carefully along the walls of the test tube. Appearance of reddish brown ring at the junction of two layers indicates the presence of glycosides.

2.12. Test for anthraquinones: To 1ml of extract, 2N Hydrochloric acid was added and the mixture was heated in water bath for 15 minutes, cooled and filtered. The filtrate was mixed with layer of chloroform and was separated with it. These mixtures was treated with 10% KOH solution, the aqueous layer becomes pink-red, which confirms the presence of anthraquinones.

2.13. Test for quinones: To 1ml of extract 1ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of quinones.

2.14. Feed preparation: The feed was prepared by mixing the flower powder along with the commercial feed; with the addition of required quantity of water. The pellets were prepared by hand pelletizer having 1.8mm diameter in size. Finally the pellets were air dried, packed in airtight polythene bags and labelled.

2.15. Growth performance: The growth performance was assessed in terms of percentage weight gain.

Weight gain % = [(Final weight- Initial weight) / initial weight] 100

2.16. Experimental design: Fishes were divided into four groups of seven fishes each. Control fishes received normal diet throughout the experimental period. Group 2 fishes received 5 grams of commercial feed and 5 grams of *Cassia alata* powder. Group 3 fishes received 5 grams of commercial feed and 10 grams of *Cassia alata* powder. Group 4 fishes received 5 grams of commercial feed and 15 grams of *Cassia alata* powder. Experimental feeding was done for 45 days.

2.17. Blood collection: After the feeding period, the blood samples were collected from each group. The blood from caudal fin was collected and stored in 1.5ml heparinized eppendorf tubes.

2.18. Giemsa staining of blood smears: Place a drop of blood on a sterile slide and streak a thin smear by means of a second slide or cover glass. Air- dry it quickly. Place 1ml of wright-Giemsa stain upon the smear, in sufficient quantity to cover the entire surface for 3-4 minutes. Add 2ml of distilled water or phosphate buffer and it is allowed to stand for some time. The stained smear was rinsed with water until the edges show faintly pinkish red. The film is allowed to dry in air.

2.19. Phenoloxidase preparation: For the preparation of Phenol oxidase, the haemolypmh were collected from the test samples[14.] The haemolymph were diluted and centrifuged for 5 mins, and then the supernatant was removed and the pellet washed with phosphate buffer. The pellet was homogenised after adding fewmicrolitre of cold phosphate buffer, and then centrifuged for 15 mins. Then supernatant were dripped into the microtubes. Then the PO activity was measured using spectrophotometer at 490nm.

3. RESULTS AND DISCUSSIONS

3.1. Phytochemical constituents of Cassia *alata* petals: The presence of glycosides, cardiac glycosides, phenols, quinones, anthraquinones, alkaloids, flavonoids were observed in petroleum ether extract of *Cassia alata* petals. These compounds are responsible for the immune stimulating potency present in petals of *Cassia alata*.

Table.1.Phytochemical screening of *Cassia alata*

Phytochemicals	Result
Glycosides	+
Cardiac glycosides	+
Phenols	+
Terpenoids	-
Anthraquinones	+
Flavonoids	+
Alkaloids	+
Steroids	-
Quinones	+

+/- Presence or absence of phytochemicals

It has been reported that *cassia alata* is rich in many phytochemical constituents that are responsible for the immune stimulating property. In the present study, the petals of *cassia alata* was used as a feed to the ornamental fishes to develop resistance against infections.

During the feeding period, the treated fish (Group 2, Group 3 and Group 4) showed active movements when compared to the control (Group 1). The morphological characters of the fish were also observed after the experimental period. The initial and the final weight of the fish under each group was noted, which was then used to calculate the net weight gain. The results proved that, there is a good increase in weight of the fish fed with the extracted product than the fish fed with the commercial feed.

Table.2.Net gain in weight of the experimental groups

Parameters	Group 1	Group 2	Group 3	Group 4	P value
Initial weight (g)	24.33±0.577	24.66±0.577	26 ± 2.64	26 ± 1	0.0045
Final weight (g)	26 ± 1	28 ± 1.73	30.33 ± 1.52	27 ± 1	0.0045
Weight gain (%)	5.49 ± 2.45	14.88 ± 6.19	17.15 ± 7.88	3.84 ± 0.15	0.0093

After the feeding period, the blood samples were collected from each group of fishes. The giemsa stain helped us study the adherence of pathogenic bacteria. The haematological parameters was studied in the group. The total WBC cell count increases except group 4 when compared to control, which has proved the immune stimulant property of the formulated feed. The increase in white blood cell count will result in the increase of immune responses.

The primary function of fish lymphocytes seems to be to act as the cells of specific immune system via antibody production. The results from the present study showed an increase in the lymphocytes count in the blood samples collected from the treated fishes (Group 2, Group 3 and Group 4) when compared to the control (Group 1). Though, the group 4 didn't show a regular increase in the lymphocytes counts, the other two groups showed a competent result.

Table.3.Haematological parameters in control and experimental fishes

Cells	Group 1	Group 2	Group 3	Group 4	P value
Lympocytes	48.66±0.577	50.66±0.577	51.66±0.577	46± 1	0.0000
Neutrophils	42.66±0.577	38.33±0.577	35± 1	39.33±0.577	0.0000
Eosinophils	5.33± 0.577	3.33± 0.577	3.66 ±0.577	4.6± 0.577	0.0100
Monocytes	3.66± 0.577	3.7± 1	3.9 ±1	3.4 ±1	0.0000
Basophil	3.66± 0.577	2.33 ±0.577	1.33± 0.577	2.33± 0.577	0.0078
Total WBC count	11,200±264.57	17,150±132.28	23,372±118.09	16,000± 100	0.0000

***P <0.05 - Statistically significant**

Fish monocytes are involved in the phagocytic property of the immune system. The present study proved a gradual increase in the monocytes count in the treated fish groups (Group 2, Group 3, Group 4) when compared to the control (Group 1). This enhances the probability of the groups to be more resistant to infections. The Prophenoloxidase activity measured in the haemolypmh showed increase in immunostimulant. Similarly, the other cells like eosinophils, neutrophils and basophils are observed to have increased amount when the fishes are subjected to any infections or inflammations. Such type of work has been conducted in the Indian carp *Labeo rohita* and an identical results were observed.

4. CONCLUSION

The phytochemical constituents like glycosides, cardiac glycosides, quinones, phenols, anthraquinones, flavonoids and alkaloids were found in the petroleum ether extract of *Cassia alata* petals. These secondary metabolites are responsible for the immune stimulating activity in the doctor fish *Garra rufa*. The increase in white blood cell count illustrates the immune stimulating potency of *Cassia alata.* The decrease in neutrophils, eosinophils, basophil cells states that there were no inflammatory effects or allergic response of the given feed *Cassia alata.* The increase in lymphocytes demonstrates a specific immune response due to the potential feed *Cassia alata*. The present investigation from the above staining and enzyme activity has confirmed the immune stimulant potency of *Cassia alata* petals.

REFERENCES

Ali S, Kasoju N, Luthura A, Singh A, Sharanabasava H, Sahuand A, Indian medicinal herbs as source of antioxidants, Food. Res. Int., 41, 2008, 1-15.

Anderson D P, Immuno stimulants, adjuvants and vaccine carriers in fish: applications to aquaculture, Annual Review of Fish Diseases, 2, 1992, 281-307.

Barnali P, Prasenjit M, Tanaya G, Ravineranath S, Takhelmayum A, Amit C, Sumanta G, Basudeb B, Prasanta K. M, Isolation and structural determination of an Antibacterial Constituent from leaves of *Cassia alata* Linn, Journal of Pharmacognosy and Phytochemistry, 2, 2013, 326-333.

Biresh S, Suraj K, Praveen P, Mayank M, HPLC analysis and antioxidant potential of plant extract of *Cassia alata*, Asian J. pharmaceutical science & technology, 4, 2014, 4-7.

Cavit Kum and Selim Sekkin, The Immune System Drugs in Fish : Immune Function, Immunoassay, Drugs Recent Advances in Fish Farms, 2012, 169-202.

El-Mahmood A.M, and Doughari J.H, Phytochemical screening and antibacterial evaluation of the leave and the root extracts of *Cassia alata* Linn, African journal of pharmacy and Pharmacology, 2 (7), 2008, 124-129.

Fuzellier M.C, Mortier F, Leetard P, Antifungal activity of *Cassia alata* L., Ann. Pharm. Fr., 40, 1982, 357-363.

Ibrahim D, Osman H, Antimicrobial activity of *Cassia alata* from Malaysia, J. Ethnopharmacol., 45, 1995, 151-156.

Jan Raa, The use of immune-stimulants in fish and shellfish feeds, Avancesen Nutricion Acuícola V. Memorias del V Simposium Internacional de Nutricion Acuícola, 2000, 19-22.

Mohsen Taleh, Mohammas Saadati, Partial characterization of phenoloxidsae enzyme in the haemophytes of *Helicoverpa armigera* Hubner, Journal of King Saud University, 26, 2014, 285-289.

Neil M.R, Evelyn M.C, Michelle G, David S, Cathy H, Fiona G, Isolation of *Streptococcus agalactia*and an aquatic binavirus from doctor fish *Garra rufa* Linn, Irish Veterinary, 66 (16), 2013.

Palanichamy S, Nagarajan S, Analgesic activity of *cassia alata* leaf extract and kaempferol 3-o-sophoroside, Journal of Ethnopharmacology, 29 (1), 1990, 73-8.

Pulok K Mukherjee, Neelsh K Nema, Santanu Bhadra, Immunomodualtory leads from medicinal plants, Indian journal of Traditional knowledge, 13(2), 2014, 235-256.

Saheli C, Sabyasachi C, Dey K.K and Sikha D, Study of antioxidant activity and immune stimulating potency of the ethnomedicinal plant, *Cassia alata* (L) Roxb., Med Aromat plants, 2, 2013, 1-6.

Sakai M, Current research status of fish immunostimulants, Aquaculture, 172, 1999, 63-92.

Satheeshkumar P, Ananthan G and Senthil Kumar D, Haematology and biochemical parameters of different feeding behavior of teleost fishes from Vellar estuary, India, Comp Clin Pathol., 2011, 275-300.

Somchit M.N, Reezal I, Elysha N, Mutalib A.R, *In vit*ro antimicrobial activity of ethanol and water extracts of *Cassia alata*, J. Ethnopharmacol., 84, 2003.

Uribe C, Folch H, Enriquez R, and Moran G, Innate and adaptive immunity in teleost fish: a review, Veterinarni Medicina, 56 (10), 2011, 486–503.

Wijendra and Pathiratne, Evalution of immune responses in an Indian carp, labe orohita (Hamilton) fed with levamisole incorporated diet, J. Sci. Univ. Kelaniya, 3, 2007, 17-28.

William H.W, A review of fish welfare and public health concerns about the use of *Garra rufa* in foot spas, Fish veterinary journal, 13, 2012, 3-16.