QUALITATIVE ANALYSIS OF PHYTOCHEMICALS AND CAROTENOID PRODUCTION OF AEGLE MARMELOS SUPPLEMENTED FEED IN XIPHOPHORUS HELLERI (Heckel,1848)

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Abstract: Bael or Aegle marmelos is a spiritual, religious and medicinal plant, native of India, Bangaladesh and spread throughout South East Asia. An experiment conducted to assess the impact of dried Aegle marmelos leaf supplemented feed at the rate of 3% to Xiphophorus helleri for two months(1-02-2019 to 30-3-2019).Preliminary phytochemical tests carried out using aqueous extract revealed the presence of phytochemicals like alkaloids proteins, saponin, tannin,phenol, glycosides, coumarin and flavonoids.The study outcome revealed positive effect of A.marmelos feed which promoted colourin X. hellerias compared to control diet. The quantitative estimation of carotenoid obtained was11.92 µg/g wet weightfor CF and 13.56 µg/g wet weight for TD1 hence enlightened the efficacy of A.marmelos leaf. Thus the study conducted aim to emphasize the incorporation of herbal active principles as natural pigment enhancers.

Index Terms-Aegle marmelos, Xiphophorus helleri, carotenoid.

I Introduction

Ornamental fish keeping and rearing is now a commercial venture active only in four states of India viz. Tamilnadu, Kerala, Maharashtra and West Bengal with diversified hue pattern, comes under eight closely related families namely *Ananbantidae, Callichthyidae, Characidae,Cobitidae,Cichlidae, Cypridontidae, Cyprinidae* and *Poecillidae*. India is an abode of a variety of ornamental fishes[1,2]turned out into an indispensable part of interior decoration in the 21st century.

It is an ultimate fact that along with aesthetic pleasure ornamental fish keepingindustry generated income especially for the jobless rural and educated personality. Naturally colored fish often show fade coloration under intensive conditions. They play fundamental roles in the ecological complexity and stability of these systems [3] Hobbyists prefer it as a part time activity [4]as the production cost is cheaper with huge returns within a short interval of time. Ornamental fishes are also known as living jewels.

Prevailing drugs and vaccines used for curation being synthetic induces deleterious impacts on the biotic and abiotic elements of environment. Synthetic drug production is also expensive, labour intensive and their mode of action relies against microbes was also stated[5]. Plant ingredients which could enhance growth along with pigmentation need to supplement while preparing aqua feed, promote survival in ornamental fishes[6]. Globally, fish culture is expanding into new direction, intensifying and diversifying-several growth promoters and hormones have been tested for enhancing feed conversion efficiency and for increasing fish culture productivity. Several researches are carried out to find out new compounds in phytonutrients which increase the all over performance of aquaculture candidates [7]. The present work aims to emphasize the need for natural pigment enhancers as an alternative to synthetic carotenoids.

II MATERIALS AND METHODS

The leaves of *A.marmelos* procured from my residence was washed, dried thoroughly in shade, grinded mechanically to fine powder stored in air tight bottle for qualitative analysis of phytochemicals.The extractive value or extractive yield calculated by the formula. Extractive yield % = $(W_1/W_2) \times 100$ where, W_1 = net weight of powder in grams after extraction and, W_2 = total weight of powder in grams taken for extraction. 1000mg of extract was dissolved in 100ml of ethanol solvent subjected to preliminary qualitative phytochemical screening tests outlined [8].

2.1 Qualitative Tests

Test for Carbohydrates

Fehling's test:

Extracts were hydrolysed with dilute HCl and neutralised with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Test for Alkaloids

Hager's test: Extracts were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow coloured precipitate.

Test for Proteins

Xanthoproteic test: Extract was treated with few drops of conc.hno3.formation of yellow colour indicates the presence of proteins.

Test for Saponin

Extract was mixed with distilled water and shaken for 5 minutes continuously. This lead to the formation of white foam indicated the presence of saponin.

Test for Tanin

4ml extract was treated with 4ml $FeCl_3$. Formation of green colour indicated the presence of condensed tannin.

Test for Phenols

Ferric chloride test: Extracts were treated with 3 to 4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Test for Glycosides

Modified Born Tragers test: extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volume of benzene. The benzene layer was separated and treated with ammonium solution. Formation of rose pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

Test for Coumarin

3ml of 10% NaOH was added to 2ml of aqueous extract. Formation of yellow colour indicated Coumarin.

Test for Flavanoids

1ml of extract was mixed with 1ml of very dilute $FeCl_2$. Appearance of pale green colour indicates the presence of flavonoids.

2.2 Estimation of Carotenoids

The feeding experiments prolonged for a period of 60 days where fishes of approximate uniform weight of 1.5 g purchased from Achus aqua Jewels, Kollam, Kerala were acclimatised for a period of fourteen days. The indoor experiment conducted in glass aquaria with water carrying capacity of 30L. After supplementing 3% feed twice from 1st January 2020 to 28th February 2020. The fishes in control tank were provided commercial feed and those fishes in the test tank with *A.marmelos* incorporated feed.

The commercial feed taken was Taiyo noted as the control feed(CF) while the experimental diet prepared with the commonly available ingredients like rice bran, ground nut oilcake, wheat, prawn and medicinal plant as TD1. After an experimental period of 60days the total carotenoid concentration in the fish muscle was analysed by using pigment extraction method [9]. Total carotenoid concentration in the fish muscle was analyzed immediately after the recommended period of experiment by using pigment extraction method [10].

One-gram tissue of *X.helleri*was taken from headless and degutted fishwas stored in a 10 ml screw capped clear glass vials. To the sample 2.5 g of anhydrous Sodium sulphate added andmeshed with a glass rod gently along the sides of the vial, 5 ml of chloroform was added into it.The vials with the tissue samples and chemicals kept overnight in the refrigerator. The next day chloroform formed a clear layer of 1-2 cm above the caked residue. 0.3ml aliquots of chloroform taken from the vial dilutedto 3 ml with absolute ethanol. Similarly the blank also prepared for comparison. The optical density was read at 380, 450, 470 and 500 nm in a spectrophotometer.The wave length at which maximum absorption was recorded used for calculation using the formula

Total carotenoid concentration (µg/g wet wt.) = [Absorption at maximum wavelength/ (0.25 sample weight (g)] x 10] where, 10 = dilution factor and 0.25 = Extinction coefficient.

III RESULTS AND DISCUSSION

The extractive yield% obtainedin aqueous extract was 8.60. Phytochemical screening test performed revealed the presence of phytochemicals enlisted below (Table-1) accounted for their therapeutic values. Herbs are safe, widely available and play an important role in aquaculture[11], reduce the side effects that occur due to the application of synthetic compounds and made it eco- friendly [12].

Aegle marmelos commonly known as bael (or bael), belonging to the family Rutaceae, is a moderate sized, slender aromatic tree. Its medicinal properties have been described in the ancient medical treatise in Sanskrit[13]in CharakaSamhita. All the plant parts like leaves, roots, barks, seeds and fruits of Bael are important ingredients of several traditional formulations against various diseases due to bioactive compounds [14,15]. Biochemical compounds of bael leaves, have been used in several diseases like diabetes, cardiovascular and anti-inflammatory functions[16] due to alkaloids, terpenoids, steriods, phenols, glycoside, tannins and coumarins [17,18,19&20].

Table 1: Phytochemical screening result

Constituents	**Ethanol extract**
Alkaloids	+
Proteins	+
Carbohydrates	+
Flavanoids	+
Glycosides	+
Phenols	+
Saponins	+
Tannins	+
Coumarin	+

Key: - Trace, +Present

The extracts and fractions of *A. marmelos* leaves also inhibits the growth of clinical isolates of dermatophytic fungi like *Trichophyton mentagrophytes, T. rubrum, Microsporumcanis, M gypseum*and *Epidermophytonfloccosum*[21]. The leaf extract of *A. marmelos* had significant immunostimulant activity against *A. hydrophila* infecting freshwater *C. carpio* fish [22].Therefore it is very clear that they have the ability to stimulate the immunity of fish. Stressed fishes are highly prone to diseases due to the decreased pigmentation. Alarming situation can be tackled by everlasting eco-friendly application of natural products in its crude or extract form [23]. Biodegradable plant products elevate antistress improved growth with anti-pathogen property not only in edible but also in ornamental fishes [24].

Leaves and fruits of *A.marmelos* (Bael) are important sources of phytochemicals and nutrients. The fruits and leaves of the plant are of significant nutritive value and can play a great role as good sources of minerals needed for the maintenance of good health. Proximate analysis revealed that moisture content, ash, protein, crude fiber, carbohydrate, pH and acid value occurred in appreciable amounts in both the fruits and leaves samples [24].

The bioactive constituents present in plants such as alkaloids, flavonoids, and phenolic compounds have been reported to make them exhibit medicinal and physiological activities[25,26&27]. Fish food incorporated with *A.marmelos* is effective for supressing the growth of pathogens and it will also prevent environmental stess induced diseases [28].*A.marmelos* were effective in combating the toxic effect of heavy metals and more efficient in relieving the stress induced by heavy metals therefore aquatic species could be protected from the effect of heavy metals[29]have antimicrobial property against *A. hydrophila*impart healthiness without using antibiotics[30].Antibacterial products from seaweed and algae cured diseases reported[31].

Colour is one of the major factor which determine the price of the ornamental fish in the world market [32,33] depend primarily ant on chromatophores[34,35] incur through the diet [36] differed among species[37,38]. Therefore, to increase the skin and flesh colour in captivity, fish must obtain an optimum level of carotenoids in their diet [39]. *X.helleri* fed with experimental diet revealed better result than the commercially available feed. The highest carotenoid content expressed by experimental feed was 13.56 µg/g wet weight while for control feed, the value obtained from quantitative estimation was 11.92 µg/g wet weight with depleted level of carotenoid. From the experimental result it was observed that both the fishes of CF and TD show maximum absorbance at 380 nm. This work thus suggested that even though fishes are unable to synthesize carotenoids, the supplementation of carotenoid enhancers like Bael leaf in feed improved their colour.

Total carotenoid concentration in the body of fishes fed with control and experimental diet revealed better result for plant material incorporated concerned to the control feed hence approved and support the work done in Koi carps with 250mg of astaxanthin[40] with a saturation point in the accumulation of carotenoids in dwarf cichlids[41]. The conducted work thus suggests that even though fishes are unable to synthesise carotenoids the supplementation of carotenoid enhancers in feed augment their colour hue [6].

As the Aqua feed industry seeks a natural and environmental friendly source of material to improve growth and colouration in ornamental fishes, thereby enhancing commercial acceptability, this incorporated feed paves a new path for the development of Aqua feed industry. On conclusion, the phytochemicals associated with *A.marmelos*enhanced growth and colour hue of *X.helleri*. Moreover the application of plant ingredients in feeds is cost effective, and ecofriendly.The generated data thereby not only provided the basis for its wide uses as a therapeutic both in traditional medicine but also opened a gateway of sustainable ornamental fish production.

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