

5. SUMMARY AND CONCLUSION

Plants have been kindred with tremendous ability and potentiality for the management of various activities in all organisms. It contains many phytochemical components which are useful in the discovery of therapeutic agents. The importance of phytotherapeutics in aquaculture was innumerable. The herbs acts as growth promoter, appetite stimulator, immunostimulant, as anti-bacterial agent, antifungal agent, anti- viral agent, anti-stressors, aphrodisiac and so on. Hence the present study was under taken to analyse the presence of phytochemical components and to characterize the bioactive compounds in the leaf extracts of *C.aromaticus* and *O.basilicum* and to compare the efficiency of these leaf extracts on growth characteristics, survival rate, feed utilization efficiencies, haematological and biochemical parameters, physiological and immunological parameters in *O.mossambicus*.

The first phase of the study aimed at complete exploration of phytochemical constituents and characterization of bioactive compounds in the leaf extracts of *C.aromaticus* and *O.basilicum*. The phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, steroids and tannins in both the leaf extracts. UV- visible spectroscopy indicated the presence of phenol, phenolic acids, flavonoids and anthrocyanides in the leaf extract of *C.aromaticus* and the presence of phenol in *O.basilicum*. FTIR analysis showed characteristic absorption band and stretching for alcohols, phenols, alkanes, alkynes and nitro compounds in the leaf extract of *C.aromaticus* and alcohols, phenols, amines, amides, alkanes, aldehydes, ketones, carboxylic acids and esters in the leaf extract of *O.basilicum*. Hence the phytochemical constituents in the leaf extracts were responsible for various biological activities.

The second phase of the study involves the growth characteristics, feed utilization efficiencies and proximate composition of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) for a period of 45 days. On 15th, 30th and 45th day after treatment growth parameters such as final body weight, weight gain, growth rate, relative growth rate and specific growth rate were observed. *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed exhibited higher growth at all levels of supplementation, compared to those fishes fed with control feed.

The results showed that the growth parameters of *O.mossambicus* fed with T2 feed showed significantly high ($P<0.05$) than other treatments and control. An overall, growth parameters has significantly increased ($P<0.05$) in *O.mossambicus* fed with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) when compared to control group after 15 ,30 and 45 days of treatment.

Feed utilization efficiencies such as FI, FER, FCR,PI,PER,LI and LER were observed in *O.mossambicus* on 15th ,30th and 45th day after treatment. FI, FER, FCR,PI,PER,LI and LER were significantly high ($P<0.05$) in treatments when compared to control. On the other hand, feed conversion ratio was significantly high ($P<0.05$) in control fishes than treatments. *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed showed increased feed utilization efficiency at all levels of supplementation, compared to those fishes fed with control feed.

The proximate composition such as moisture, protein, carbohydrate, fat and ash content were analyzed initially before the experiment and after 45 days of treatment with with of *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed). *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at all levels of supplementation showed a significant increase ($P<0.05$) in the proximate composition while compared to control. Moisture content ($72.55\pm 0.45\%$),protein content ($21.02\pm 0.54\%$),carbohydrate content ($7.71\pm 0.20\%$), fat content ($5.82\pm 0.37\%$) and ash content ($3.49\pm 0.43\%$) was high in T2 fishes and low (moisture- $70.5\pm 0.5\%$,protein – $12.76\pm 0.41\%$, carbohydrate – $4.94\pm 0.21\%$,fat – $3.49\pm 0.53\%$,ash – $2.32\pm 0.11\%$) in control fishes.

The third phase of study deals with haematological and biochemical parameters of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) for a period of 45 days.

The haematological parameters such as erythrocyte count, leucocyte count, Hb, PCV, MCH and MCHC were analyzed initially before the experiment and after 45 days of treatment. The haematological parameters were significantly high ($P<0.05$) in *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at all levels of supplementation when compared to control. Among the treatments T2 fishes showed high values for erythrocyte count ($1.92 \pm 0.06 \times 10^6$ million cells/cu.mm),leucocyte count($9800 \pm 0.05 \times 10^3$ thousand cells/cu.mm), haemoglobin content (8.86 ± 0.20 g/dl), PCV(12.39 ± 0.35 %), MCH(47.42 ± 0.38 gm/dl) and

MCHC(71.76±1.13 gm/dl) whereas control showed a minimum of (erythrocyte count- 0.89± 0.08 x10⁶ million cells/cu.mm., leucocyte count - 1200± 0.03 x10³ thousand cells/cu.mm, haemoglobin content - 2.75±0.24 g/dl, PCV - 5.24±0.04 %, MCH - 28.40±0.11 gm/dl and MCHC - 49.13±1.07 gm/dl).

The serum biochemical parameters such as protein, glucose and cholesterol content were analyzed initially before the experiment and after 45 days of treatment. After 45 days of treatment, the serum protein content was high (52.84±1.11 mg/dl) in T2 fishes when compared to control (23.51±0.06 mg/dl). On the other hand, serum glucose (35.09±1.02 mg/dl) and cholesterol content (57.86±1.12 mg/dl) were high in control fishes and low in T2 fishes (serum protein -21.25±0.80 mg/dl and serum cholesterol -43.94 mg/dl).

The fourth phase of the study involves with the study of antibacterial activity of *C.aromaticus* and *O.basilicum* leaf extracts against *A.hydrophila* and physiological and immunological parameters of *O.mossambicus* fed with *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) for a period of 30 days and challenged with *A.hydrophila* .

C.aromaticus inhibited the growth of *A.hydrophila* by producing maximum zone of inhibition of 20 mm, followed by *O.basilicum* (18mm) whereas the positive control chloramphenicol showed 22mm of inhibition.

To observe the changes in morphology of bacterial cells , the bacterial samples treated with *C.aromaticus* and *O.basilicum* leaf extracts and untreated bacterial samples were subjected SEM analysis. There was significant difference in the morphology of *A.hydrophila* treated with *C.aromaticus* and *O.basilicum* leaf extracts when compared to control. The cells treated with *C.aromaticus* and *O.basilicum* leaf extracts was showed shrinkage, cell membrane damage and partial disappearance of cytoplasm.

The leaf extracts of *C.aromaticus* and *O.basilicum* at three different concentrations (2.5, 5 and 10 gm/kg of feed) were given as feed supplement for 30 days in *O.mossambicus*. After 30 days, the fishes were fed with only control feed. Fishes were injected intramuscularly with 0.1ml of 10⁵ cfu/ml of *A.hydrophila* on 30th and 37th day. The fishes were monitored regularly for changes in behavior and mortality. On 37th day T2, T3, T5 and T6 have 0% mortality, 100% survival and 100% relative level of protection whereas control caused 13.4% mortality,86.6% survival and 0% relative level of protection. On 45th day , T2,T3,T5 and T6 showed 0% mortality, 100%

survival and 100% whereas the control caused 26.67% mortality and 73.33% survival.

Antibody response to *A. hydrophila* by various experimental groups fed with *C.aromaticus* and *O.basilicum* at different concentrations (2.5, 5 and 10 gm/kg of feed)) were found out by using antibody titre. The antibody titre (log 2 values) was higher (1.041) in T2,T3, T5 and T6 followed by T1 (0.954), T4(0.845) whereas control had a low value of 0.698 on both 37th day. On 45th day the antibody titre (log 2 values) was high (1.041) in T2,T3,T5 and T6 followed by T1 and T4 as 1.000 whereas control showed a minimum value of 0.698. On overall view, the antibody titer value has significantly increased ($P<0.05$) in *O.mossambicus* supplemented with leaf extracts at different concentration when compared to control on 37th and 45th day of treatment. A comparison of antibody response of fish supplemented with leaf extracts at higher concentrations on different days of treatment was significantly higher ($P<0.05$).

Hence it is concluded that *C.aromaticus* and *O.basilicum* supplemented feed at three different concentration (2.5, 5 and 10 gm/kg of feed) for 45 days influenced the growth rate, feed utilization, proximate composition, haematological, biochemical, physiological and immunological parameters of *O.mossambicus*. Balanced feed with leaf extract (*C.aromaticus* and *O.basilicum*) as feed supplement is optimum for enhancing growth and immune response against the pathogen *A.hydrophila*. Therefore this study is very useful in the field of aquaculture for the production of less expensive and ecofriendly aqua feeds.