Original Research Article **Microbiology**

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

IN- VITRO EFFICACY OF PHYTOCHEMICALS AGAINST TRICHODINID CILIATES INFECTING ORNAMENTAL GOLDFISH, *CARASSIUS AURATUS*

MANDIRA SAHA, SUBHASH MONDAL, AND P.K.BANDYOPADHYAY*

Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani 741235, West Bengal, India

ABSTRACT

The ornamental fish trade plays an important role for socioeconomic upliftment of the backward people in our country with little investment of money. The business has been found to be a very profitable economic activity and deserves the scientific study and development. In spite of this lucrative business, India is suffering from loss due to the invasion of different ectoparasites. Trichodinids are known to be a major problem in fish farms causing serious damage, particularly under cultured condition. The wide varieties of antibiotics used for treating trichodiniasis not only make the environment unfavorable for aquaculture but the pathogen also becomes resistant towards the antibiotics over time. With the reminiscence in the use of herbal products, various botanicals have been heralded as crudes for particular pathogens, but the efficacy of these compounds for parasitic protozoa is questionable. Here, we tested a range of Basak (*Adhatoda vasica*) and Thankuni (*Centella asiatica*) plants extracts against a major aquarium pathogen, *Trichodina* sp. infecting the goldfish (*Carassius auratus*). Both the plant extracts significantly ($P < 0.05$ and $P < 0.01$) reduced parasite, and increased the survival time of fish *in-vitro*, after 5-10 days treatment only by using 4g and 8g food doses respectively. In a fully randomized experiment, the number of parasite was significantly reduced from infected fishes which were exposed to the mentioned phytochemical.The present study revealed that the use of these two phytochemical opened promising perspectives in terms of an alternative parasite treatment of chemical in aquaculture.

KEY WORDS: **Trichodiniasis, Phytochemical, Basak (*Adhatoda vasica*), Thankuni (*Centella asiatica*), antiprotozoan activity, ornamental goldfish.**

P.K.BANDYOPADHYAY*
Parasitology Laboratory, Department of Zoology, University of Kalyani,
Kalyani 741235, West Bengal, India

***Corresponding author**

Received on : 22-08-2016
Revised and Accepted on : 18-11-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b196-201

INTRODUCTION

India is considered as one of the gold mines for ornamental fishes. The ornamental fish culture accounted for US$238 and $283 million in global exports and imports, respectively, in 2005 alone and the trade is increasing. [1] But as the dark lies beneath the lamp, all novel projects are hampered by various hindrances, as with food fish, ornamental aquaculture particularly goldfish, *Carassius auratus* is greatly affected by infectious disease, the containment of which relies heavily on broad spectrum anti- parasitic compounds.[2, 3] Trichodinids are known to be a major problem in fish farms causing serious damage, particularly under cultured condition of goldfish and these are leading cause of diseases that result in severe economic impact. These parasite attacks goldfish, causing massive destruction of skin and gill epithelium and also cause hemorrhage those results in massive mortality. Due to the toxic effect of chemical antibiotics, use of these anti-parasitic compounds is being restricted. Malachite green was banned in the UK for use on food fish in 2002, as it is a known carcinogen.[2] Legal application of formalin is expected to be reduced in the near future, too, for both environmental and human health reasons.[3-5] Moreover, the use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and other therapeutic drugs and not being used in developing countries as well as using extended to developed countries.[6] The control of *Trichodiniasis* with freshly prepared Potassium permanganate[7]has been very expensive and it has no significant effect to control the disease. In correspond; there is a fast growing interest in screening antiparasitic and antibacterial substances from plants to replace antiparasitic and disinfectant alternatives.[8-10]Since India is a poor country, huge amount of money invested in fish farming and in chemotherapy is not economically sustainable. An alternative approach to overcome this problem leads to the use of medicinal herbs in curing diseases. Plant extracts decrease the selective pressure for developing antibiotic resistance.[11]The screening of plant extracts and natural products for anti-protozoan activity has shown that higher plants represented a potential source of new anti-infective agents.[12] India is a country, rich in medicinal plants like Thankuni (*Centella asiatica*) and Basak (*Adhatoda vasica*) which are most popular edible plants that had a strong interest to scientists and researchers, recognized as an economically important medicinal plant having wide variety of secondary metabolites such as tannins, alkaloids, flavonoids, triterpinoids, saponins, fatty acids and essential oils which possesses substances with anti-protozoan properties that can be used as potential alternative medicine[13,14] and has a sufficient proof of efficacy in microbes inducing pathogenesis. It has also been suggested that ethanolic extracts of these plants, used in allopathic medicine, are potential sources of anti protozoan, antiviral, antitumoral and antimicrobial agents.[15, 16]Hence, this study was designed to compare the efficacy of Basak and Thankuni components as an alternative treatment against trichodinid infections on ornamental goldfish, *Carassius auratus*, with the aim of determining whether any have the potential to be used commercially.

MATERIALS AND METHODS

Collection of plants

The leaves of Basak (*Adhatoda vasica*) and Thankuni (*Centella asiatica*) was collected from the cultivated land as well as local market**.** The plants was identified and authenticated by the Prof. Shankar Naryan Sinha, Profeesor of Botany, Dept. of Botany, University of Kalyani, Kalyani,741235, West Bengal, India. It was dried for about 15-20 days, and powdered mechanically with the help of the commercial stainless steel grinder. The dried powder were then sieved with the help of fine sieved cloth and prepared for extraction through Soxhlet apparatus.

Preparation of plant extract

30 gm of powder was used in 1:10 ratio with ethanol, methanol and water as extracting solvent in the Soxhlet apparatus. The extract was filtered after a run of six hours maintaining 40-50°C in a Buchner funnel with Whatman number1 filter paper. The filtrate was then processed further to concentrate the extract using Rota evaporator in 50- 55°C, which was followed by drying in air or incubator. A stock solution of 1% was prepared by dissolving 1 gm of crude extract in 100 ml of solvent respectively, and preserved at 4° C for further use.

Preparation food material for doses

Three doses (1gm, 4 gm, and 8 gm) were prepared. The solutions were mixed with the dry foods of fishes. The fish dry food was soaked in the prepared extracts and dried for 3 days. Then, doses were applied to fishes.

Preparation of experiment:

Seven aquaria of (3.0 × 1.5) ft sized were taken, cleaned and labelled properly. Three aquaria for each plant extract (*Adhatoda vasica* and *Centella asiatica*) were taken and each type contained three different (1gm, 4gm and 8gm) doses and a control (without any plant extracts**)** experiment was also carried out simultaneously. In each aquarium 20 goldfish were kept and water temperature was maintained at 32°C. Plant extract soaked food was supplied to the fish (twice daily) in each aquarium according to the labelling and doses. In control experiment normal food were supplied for 15 days. Parasite infection was observed after 5 days, 10 days and 15 days from each aquarium.

Parasite Examination

Mucus was scraped from the total surface of skin and gills for all examined goldfish groups after 5, 10 and 15 days of treatment for parasitic infections in the next two and four weeks. The degree of infestation of *Trichodina* sp. (+) = Slight, (++) = Medium, (+++) =Heavy from mucus and gills was subsequently counted under a microscope.

Statistical analysis

Treatments were compared by One Way Analysis of Variance (ANOVA) and in completely randomize design comparisons, of means were performed with post-hoc Tukey HSD test using SPSS (Version 20.0). The LC50

was determined using the probit procedure of SPSS. The significant level was set at $P < 0.05(*)$ and $P < 0.01(**)$.

RESULTS

The plant extracts, Basak and Thankuni had anti-parasitic activity affecting against *Trichodina sp*. showed antiprotozoan activity against *Trichodina sp* as seen in Table 1 and 2.After the clinical examination, it was noted that, most fish aggregated on the surface of the aquarium along with swollen appearance on gills followed by thick mucus covering. The scale of the external body surface become sloughing.

Table I
The antiprotozoan activity of Basak (Adhatoda vasica) extracts against Trichodinid on Gold Fish (C. auratus)

Dose (gm/kg)	Post treatment activity		
	5 Days	10 Days	15 Days
Control	++	++	+++
1 g Basak	++	++	+
4 g Basak	Nil	Nil	Nil
8 g Basak	Nil	Nil	Toxic

**Note: LC_{50} -10.34 g*

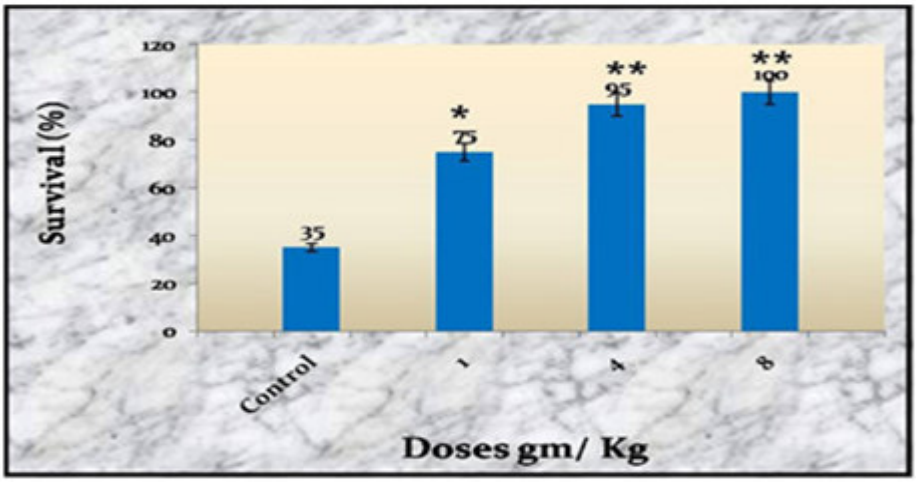
Table II
The antiprotozoan activity of Thankuni (Centella asiatica) extracts against Trichodinid on Gold Fish (C. auratus)

Dose (gm/kg)	Post treatment activity		
	5 Days	10 Days	15 Days
Control	++	++	+++
1 g Thankuni	++	++	++
4 g Thankuni	+	++	+
8 g Thankuni	+	Nil	Nil

**Note: LC_{50} -14.88 g*

Survival rate decreased in the control group up to 15 days after completion of the challenge infection. However, this was increased in the both Basak and Thankuni treatment group to up to 100 % with the doses of 1, 4 and 8 g / kg diet, respectively which was significantly different ($P < 0.05$ and $P < 0.01$) from their control groups (Fig.1). For determining the acute toxic level, LC_{50} value has been recovered for the both plant extracts and kept the working doses much lower than their LC_{50} value. The best suitable and effective dose of Basak plants is 4gm, in which all the parasitic burden has been removed by 5 days only, whereas by 8gm dose for 15 days, some solid adherence in the gill of infected fish has been found with little respiratory problem (Table-1). Thankuni at 8 gm was able to remove all Trichodina sp. from goldfish after 10 day treatment (Table-2). All treatments were significantly different from their control groups.

Figure 1
Survivability analysis of the goldfish after treatment



The performance of infected goldfish for cumulative survivability (%) after treatment with selected phytochemical. Data given are mean numbers of both the plants. The significant level was set atP < 0.05(·) and P < 0.01(··) which indicate significantly increased the fish survivability.

DISCUSSION

The ectoparasitic disease of fish plays an important role in the economic losses in the fish farms through mortality and/or decrease growth rate during fish rearing in intensification systems. Hassan[17] reported 34.3% trichodinid infection in fish, while El-Khatib[18] detected that 30% and 66% of tilapia spp. were infecting with *Trichodina* in each season. Hyperirritability and flashing of fries were the main clinical signs of the fish diseased by trichodinosis. Fishes were also suffering from dull excessive mucus on the external body surface, anorexia, poor growth, skin darkening and weak escape reflex and ultimately death which leads to massive production loss in the ornamental fish industry.[19] Therefore, to inhibit this devastating damages, some necessary action has to be taken. The development of disinfection agents to treat protozoan infestation is one of the most fascinating stories in the history of aquatic animal health. In terms of recent trends for controlling trichodiniasis in intensified aquaculture some chemical component such as formalin and potassium permanganate are aimed at killing the parasite are widely used.[20, 21] In spite of these advantages of chemical treatment in aquatic systems have been discouraged and now appears to be inadequate to control this parasitic infestation.[21] though they have some toxic and carcinogenic effect on fish health.[22, 23] Thus, there is an urgent need to systematically evaluate the plants used in traditional medicine as an alternative control methods required for controlling trichodiniasis in aquaculture.[24] Plant is the most accessible source of medicine which can be effectively used in the aquaculture industry to treat fish disease and to reduce the effect of hazardous chemical materials. Considering all these aspects we have chosen economically important two plants Thankuni (*Centella asiatica*) and Basak (*Adhatoda vasica*) which can effectively eliminate those parasite and increase fish production. This will be economically sustainable which possesses substances with anti-protozoan properties that can be used as potential alternative medicine[13, 14] and has a sufficient proof of efficacy in microbes inducing pathogenesis. The present study showed that in terms of this beneficial effect, to avoid overdosing the acute toxicity of both Basak and Thankuni to healthy goldfish was determined by performing an LC_{50} bioassay of 96 hrs. The 96 hrs median lethal concentration (96 hrs LC50) of Basak andThankuni was 10.34 g and 14.88 g, respectively while the therapeutic dose was 8 gm highest in case of both the plants and this indicated the a wide safety margin of Plant extracts as a medication for trichodinosis for goldfish. This finding is augmented by the recommendation of US Environmental Protection Agency.[25] After determining the acute doses for treating the protozoan parasites of ornamental fishes, the present study has shown that the treatments with 4 gm/Kg food diet, soaking with Basak extracts represent a significant reduction in the trichodinid burden of goldfish by 5 days only in comparison to their control group whereas of 8 gm dose for 15 days shows some toxic effect on fishes as such solid adherence in the gill of a fish. Therefore, from this 4 gm dose are considered to be the most effective doses of Basak for complete eliminations of *Trichodina* [26-28] Thankuni at 8 gm was able to remove all *Trichodina* sp. from goldfish after 10 day treatment [27, 28] which is so time consuming. All treated groups were significantly different from the control group.[29-33]Therefore, from this study it can be recommended that Basak plant has immediate effects on parasites. Ekanem et. al. [34] revealed the antiprotozoan activity of different medicinal plants which matches with the present study. Consequently, the parasite-induced mortality of the fish host was significantly reduced. These findings corroborated with the study done earlier by some previous authors by using different plants.[24, 35]

CONCLUSION

According to the present study, we can conclude that the use of these two plants extracts opened promising perspectives in terms of parasite treatment in aquaculture. This could be seen as a better alternative for aquaculture and the environment as the plant extract does not have a toxic effect on environment with a wide safe margin, and they eradicate *Trichodina* sp. completely. The results reported in this study have demonstrated that preparations from traditional medicinal plants are potent for the control of parasitic protozoa in cultured goldfish. However, the preparations still have to be tested under field conditions, and toxicity tests must be extended to other fish species. Further phytochemical studies, towards the isolation and characterization of the active compounds are recommended.

ACKNOWLEDGEMENT

One of the authors (MS) is thankful to the University Grants Commission, New Delhi for financial support under Special Assistance Program [No.F-3-11/2012 (SAP-II)].

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. European Commission, Background Information on the Consultation Process on Monitoring International Trade in Ornamental Fishhttp://www.ornamental-fishint.org/uploads/M6/Q6/M6Q6bR8R4fxaZ8fa0nJqjA/UNEP-WCMC-Background.pdf(accessed 18.07.2012), 2008
2. Srivastava S, Sinha R and Roy D. Toxicological effects of malachite green. Aquat Toxico,66: 319–329. 2004
3. Wooster GA, Martinez CM, Bowser PR. Human health risks associated with formalin treatments used in aquaculture: initial study. North Am J Aquacult,67: 111–113. 2005
4. Cabello FC Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environ Microbiol, 8: 1137–1144. 2006
5. Commission of the European Communities, Proposal for a Regulation of the European Parliament of the Council Concerning the Placing on the Market, Use of Biocidal Productshttp://eurlex.europa.eu/LxUriServ/LexUriServ.do?uri=COM:2009:0267:FIN:EN:PDF(accessed 18.07.12), 2009
6. Kruse H, Soram H.Tromster of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. Appl Environ Microbiol,60 (1):4015-4021, 1994.
7. Eissa IAM. Parasitic fish diseases in Egypt. Dar El- Nahda El- Arabia publishing, Abd El- Khalak Tharwat, 23 Edn; St. Cairo, Egypt 2002.
8. Singh SK, Patel JR, Dubey PK, Dangi A. Pharmacognostic study and phytochemical screening of leaf of *Adhatoda vasica* (Acanthaceae). J Med Plants Stud, 2(4): 29-31, 2014
9. Reverter M, Bontemps N, Lecchini D, Banaigs B,Sasal P. Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture, 433: 50–61, 2014
10. Noor El- Deen A I, Abd El Hady O K, Liala A M, Zaki SM. A trial for control of some external parasitic diseases cultured *Oreochromisniloticus* in Egypt. Life Sci J, 12(8): 25-29, 2015
11. K. Lewis, F.M. Ausubel. Prospects for plant-derived antibacterial *Nature Biotechnology* 24: 1504-1507SAS Institute. SAS/SAT User's guide-version 6. Cary, 1989, 2006
12. Press JB. Biodiversity. Exciting prospects for drug discovery and development melting report of the Monroe wall symposium, Chemtracts Org Chem. 9:286-289, 1996
13. Jain SK, Defilipps RA. Medicinal Plants of India, Vol. 1. Michigan, USA: Reference Publications Inc.: 92, 1991.
14. Srinivasan K, Sivasubramanian S, Kumaravel S. phytochemical profiling and gcms study of *Adhatoda vasica* leaves Int J Pharm Bio Sci, 5(1): 714 – 720 2014
15. Ghosh K, Indra N. Phytochemistry, *in vitro* Free Radical Scavenging, Chelating and Toxicity of *Centela asiatica* L. (Apiaceae) Ethanolic Leaf Extract, Int J Pharm Sci Rev Res, 29(1): 59: 328-334, 2014
16. Vinoth B, Manivasagaperumal R, Rajaravindran M. Phytochemical analysis and antibacterial activity of *Azadirachta indica* a juss. Intl J Res Plant Sc, 2(3): 50-552012
17. Hassan MAH. Trichodiniasis in farmed freshwater Tilapia in Eastern Saudi Arabia. JKAU: Eng. Sci. 11 (1): 1–3 (1419 A.H./1999 A.D.), 1999
18. El-Khatib NRH. Some Studies on Ectoparasitic Infestation in Fresh Water Fishes. M.V. Thesis, Cairo University, 1989
19. Mohamed A, Abd El-Galil, Aboelhadidb SM. Trials for the control of trichodinosis and gyrodactylosis in hatchery reared *Oreochromis niloticus* fries by using garlic. Vet Parasitol, 185: 57-63, 2012
20. Cross DG. A review of methods to control ichthyopthiriasis. Prog Fish Cult 34: 165-170 1972
21. Tieman DM, Goodwin AE. Treatments for Ich infestations in channel catfish evaluated under static and flow-through water conditions. N Am J Aquacult, 63: 293-299 2001
22. Alderman DJ. Malachite green: a review. J Fish Dis, 8: 289-2981985
23. Wahli T, Schmitt M, Meier W. Evaluation of alternatives to malachite green oxalate as a therapeutant for ichthyophthiriosis in rainbow-trout, *Oncorhynchus mykiss*, J Appl Ichthyol 9: 237-249, 1993
24. Noor El Deen AIE, Mohamed RA. Application of some medicinal Plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*). Report Opin J, 1 (6):1-5 (Cumulated 6). 2009
25. C.E. Boyd, Water Quality for Pond Aquaculture, Alabama Agricultural Experiment Station, Auburn University, AL. 1998
26. Ahmed S, Garg M, Ali M Sing M, Md Athar T, ansari SH. A phyto pharmacological overview on *Adhatoda zeylanica* Medic. Syn. *A. Vasica*. (Linn.) Nees. Nat Prod Radiance, 8 (5): 549-554, 2009
27. Balachandran C, Duraipandiyan V, Raj MK, Ignacimuthu S, Balakrishna K, Rajput VK, Kalia NP, Khan IA. Anti-mycobacterial and cytotoxic (A549) properties of Vasicinone isolated from *Adhatoda vasica* (L.) Nees. Asian Pac J Trop Biomed, 1: 1-4, 2012
28. Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatod vasica*. 2015 L DOI:10.4103/0973-8258.49381,
29. Zhang M, Hettiarachchy SN, Horax R, Kannan A, Praisoody MDA, Muhundan A, Mallangi CR. Phytochemicals, antioxidant and antimicrobial activity of *Hibiscus sabdariffa*, *Centella asiatica, Moringa oleifera* and *Murraya koenigii* leaves. J Med Plants Res, 5: 6672-80, 2011
30. Devi NN, Jeeva P, Femina W, Screening for phytochemical, antibacterial and cytotoxic activity of medicinal plant of Tamilnadu, Int J Pharma Res Bio Sci, (1):2012
31. Schelkle B, Snellgrove D, Cable J. In vitro and in vivo efficacy of garlic compounds against

Gyrodactylus turnbulli infecting the guppy (*Poecilia reticulata*). Vet Parasitol, 198: 96– 101, 2013

32. Fridman S, Sinai T, Zilberg D. Efficacy of garlic based treatments against monogenean parasites infecting the guppy (*Poecilia reticulata* (Peters) Vet Parasitol, 203: 51–58, 2014
33. Syed SA, Bari A. Qualitative analysis of various components of *Allium sativum* (fresh Garlic bulb), extracted in different organic solvents using gc-high Resolution mass spectrophotometer (jms-700). IntJ Curr Res Chem Pharml Sci 1(8): 122–126, 2014
34. Ekanem AP, Obiekezie A, Kloas W, Knopf K. Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. Parasitol Res, 92: 361–366, 2004
35. Chitmanat C, Tongdonmuan K, Nunsong W. The use of crude extracts from traditional medicinal plants to eliminate *Trichodina* sp.in tilapia (*Oreochromis niloticus*) fingerlings Songklanakarin. J Sci Technol, 27: 359–364, 2005.