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ANTIPROTOZOAN ACTIVITY OF HERBAL EXTRACTS AGAINST *Trichodina modesta* LOM, 1970 (Ciliophora: Trichodinidae) IN GOLD FISH *Carassius auratus*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Trichodiniasis is a severe disease of aquaculture that kills a large number of fish. The discovery of new antiprotozoal drugs to treat *Trichodina* infections is gaining popularity. The purpose of the study was to see how efficient two medicinal plants were against *Trichodina* infection. The methanol extracts of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* seemed to have antiprotozoal action against *Trichodina modesta* Lom, 1970, with 5 day LC50 values of 2.80 and 4.43 g/ kg, respectively. Concentrations of *Piper kadsura* (Choisy) Ohwi extracts of 2.5, 5.0, and 8.0 g/kg resulted in mortality of *Trichodina modesta* Lom, 1970 of 44.7, 88.3, and 100%. These results suggested that the methanol extracts of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* have the potential to be employed to control *Trichodina* infection in an environmentally acceptable manner.

Keywords: *Trichodina*; ectoparasite; fish disease; control; medicinal plant extract.

1. INTRODUCTION

Disease outbreaks caused by infection with protozoan parasites are a significant limiting factor in the development of effective aquaculture. We have identified ciliophoran parasites infesting the ornamental fish *Carassius auratus* on the fish's body surface, tail fin, and gill area. The use of a variety of medications and pesticides not only makes the environment unsuitable for aquaculture, but also allows illnesses to become resistant to them over time. Plant extracts were found to be a successful alternative therapy for parasitic ciliates in this study, which was previously regarded to be impossible.

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Trichodinids are a group of ciliate ectoparasites that belong to the Trichodinidae family, which was discovered by Claus in 1874. The genus *Trichodina* Ehrenberg, 1831 is one of the ten genera that make up the Trichodinidae family, which Claus 1874 described as having a low frequency of occurrence. Trichodinid ciliophorans are causing a fish disease known as trichodiniasis [1-4]. The genus *Trichodina* was the subject of the majority of studies on the variety of trichodinid ciliophorans conducted in India [5-15].

The parasite epidemic can be a significant limiting factor for aquaculture operations. The outbreak of *Trichodina modesta* Infection in small fish, which has been particularly severe, has resulted in massive financial losses. Infected fish become lethargic, produce copious mucus, and finally become off-feed, resulting in many fatalities in the population. There are numerous studies accessible on the usage of medicinal plant extracts to manage trichodiniasis that can be found worldwide.

Formalin used to be an effective treatment for trichodiniasis, but it currently appears to be ineffective in controlling this parasitic illness [16]. The use of chemical agents leads to an increase in unneeded costs and the likelihood of hazardous residues in fish flesh and the environment [17].

Food safety is a significant problem worldwide, so food manufacturers must work hard to create safe food items. Plant antiparasitic compounds are being investigated as a potential replacement for chemical and antibiotic alternatives, rising in popularity.

The study's goal was to determine the efficacy of two medicinal herbs in treating *Trichodina* infection in *Carassius auratus*. It appeared that the methanol extracts of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* were effective against *Trichodina modesta* Lom, 1970, with 4-hour 2.80 and 4.43 g/ kg, respectively, against *Trichodina modesta* Lom, 1970.

2. MATERIALS AND METHODS

2.1 Collection of Host Fishes

The fish were collected from a variety of different fish farms. Aerated glass aquaria (20 L) were used to house the collected fishes, kept at the following temperatures, pH values, and dissolved oxygen concentrations: 18-20 degrees Celcius, 6.6 ± 0.1, and 5.8–7.2 mg/L of dissolved oxygen. The length and weight of the specimens (mean weight 20 ± 0.21 g, mean length 10.5± 0.22 cm) were measured and documented.

2.2 Identification of Trichodinid Ciliophorans with Light Microscopy

To make gill and skin smears, no grease slides were utilized. Klein's [18] dry silver impregnation method was employed to impregnate slides containing trichodinid ciliophorans. At a magnification of 1000X, prepared slides were examined with an Olympus research microscope (Model CH 20i) fitted with an oil immersion lens, and photographs were captured with an Olympus digital camera. Unless otherwise stated, all measurements are in micrometers and are following the uniform specific characteristics proposed by Lom [19], Wellborn [20], and Arthur and Lom [21]. In each example, the minimum and maximum values are given, followed by the arithmetic mean and standard deviation, enclosed in parenthesis. In the case of denticles and radial pins, the mode is supplied instead of the arithmetic mean. It is possible to measure the length of the denticle by measuring it from the tip of the blade to the end of the ray. The adhesive disc plus the border membrane are added together to determine the body diameter. The description of denticle elements follows the guidelines of Van As and Basson [22]. Sequence and method of the description of denticle elements follow the recommendations of Van As and Basson [23].

2.3 Collection of medicinal plants and preparation of plant extracts

Fresh plant materials were obtained from reputable medicinal plant dealers in Kolkata. The washed plants were dried under shade and physically powdered in a commercial electrical stainless-steel blender (30–40 mesh) using a commercial stainless-steel blender. The powdered samples were freeze-dried at a - 45 °C to guarantee that all water had been removed. The dry powder (100 g) of each species was extracted with methanol (1,000 mL x three times) for 48 hours. After being filtered separately, the methanol filtrates were separated and evaporated at lower pressure in a vacuum rotary evaporator.

2.4 Acute Toxicity Examination

To determine the acute toxicity of both plant extracts to healthy goldfish, the extracts were subjected to a four-day bioassay. Preliminary research was conducted on goldfish in several 20-liter aquariums (each aquarium had 15 fish), with the goldfish exposed to several quantities of plant extracts (2.5, 5.0, 8.0, and 20.0 g/kg) to produce a survivorship range ranging from 0% to 100%. The two extracts were submitted to separate experimental protocols. In addition to the experimental fishes, a control group of fishes who were not exposed to the plant extract was included in the study. The fish were observed for four days, during which the number of dead fish was counted. According to Behrens and Kerber [24], the LC50 test results were statistically analyzed, and the working dose was determined.

2.5 Preparation of Fish Feeding Materials

The aquaria contained goldfish fed with four different concentrations of plant extracts (2.5, 5.0, 10.0, and 20.0 g/kg) during the experiment. When treating the ciliophoran parasites solely in the present study, the doses were significantly lower than the LC50 value and were almost as close to or less than their sublethal dose. The mixture was allowed to air dry for three days before being stored in a cold, dry location to enable optimal mixing of the dry food with the prepared extracts. Then, different doses of the extract were added to other foods and tested.

2.6 Experiment set up

Two sets of five 20-liter aquariums were cleaned and labeled appropriately in preparation for the goldfish experiment, which was conducted over a 15-day period. The two plant extracts took up four aquaria, each containing four distinct (0.625, 1.25, 2.5, 5.0, and 8.0 g/kg) doses, and a controlled aquarium was also included in the study (without any plant extracts). 15 fish were kept in each tank, and the water temperature was controlled between 22 and 28 degrees Celsius. Food soaked in plant extracts had been provided twice daily to the fish in each tank, following the labeling and dosage instructions. During the control tests, normal food was provided for a total of 15 days. After 5, 10, and 15 days, parasite infections were discovered in each aquarium, with the longest being 15 days. With each tank containing 15 goldfish, the treatments were carried out in three duplicates (repeats were done following each set of experiments or one after the other).

2.7 Examining the Infestation with Parasitic Infestation

To detect a parasitic infestation, a sample of mucus was scraped from the whole surface of the skin and gills of all of the goldfish groups studied after 5, 10, and 15 days of therapy. The level of trichodinid infection was defined as mild, medium, or heavy based on the amount of mucus taken from the entire body and gills, which was then used to count parasites under a microscope to establish the level of infestation.

2.8 Statistical Analysis

ANOVA was used to analyze the differences in efficacy between different doses. A completely randomized design comparison of means was performed using a post hoc Tukey's HSD test using SPSS to compare the differences in efficiency between different doses (trial version 20.0). The significant level was set at $P<0.05$(*) and $P<0.01$(**).

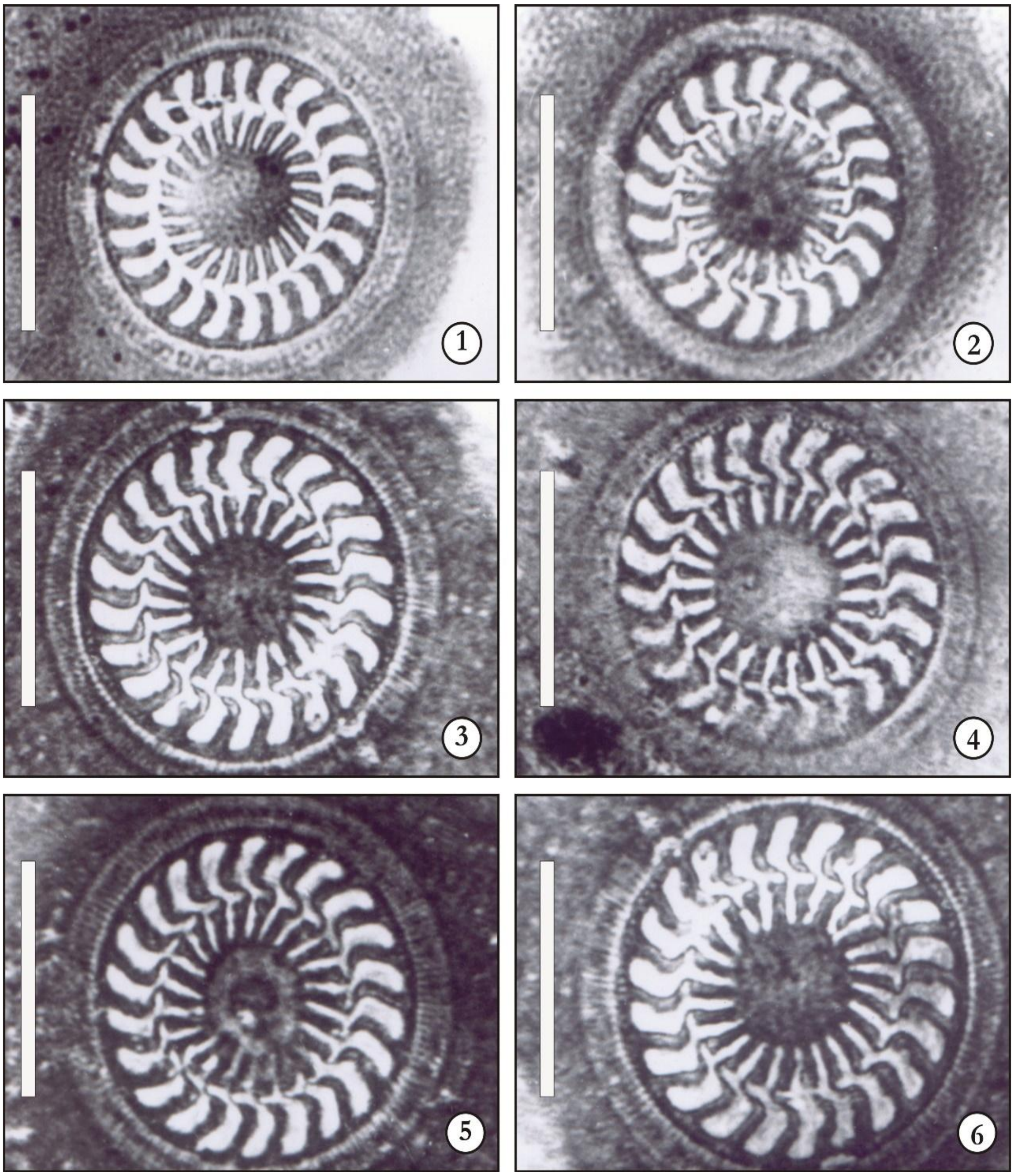
3. RESULTS AND DISCUSSION

During the study, protozoan parasites of the genus *Trichodina* were isolated from *Carassius auratus* and identified based on their morphological and morphometrical characteristics, as proposed by Lom [19], Wellborn [20], Arthur and Lom [21], Van As and Basson [22], and Van As and Basson [23] and others.

3.1 *Trichodina modesta* Lom, 1970 (Figs. 1-6)

3.1.1 Description of the body

Disc-shaped trichodinids with the following characters were found among them: body diameter 28.5 - 45.3 (38.7 – 1.2); border membrane width: 2.4 - 4.0 (3.3 - 0.5); denticulate ring diameter: 13.4 - 22.4 (18.5 - 2.0); number of denticles: 18.0 - 23.0 (23.0 - 1.0); denticle lengths: 3.4 – 5.8 (4.1 - 0.7); blade length: 3.4 - 5.1 (Unlike the adhesive disc, the core of the silver nitrate impregnated material is identical in appearance to the sticky disc. With a somewhat flat tangent point that is not completely parallel to the y+1 axis, the blade is sickle-shaped and has a sharp point. At the tip of the blade, it comes close to the y-axis but does not quite contact it. The blade apophysis is only seldom encountered however some examples do have a distinct apophysis that may be distinguished, located between the blade and the center section. There is a triangular and rounded shape in the center, which fills half of the area between the Y and y-1 axis. Some specimens have an indentation in the middle region of the specimen that is below the X-axis. A small connection connects the central area of the image to the ray of light. In this case, the ray is thin and straight, with a rounded point, and it runs parallel to the X and Y axes. There is evidence of ray apophysis. A few specimens exhibited discretely anterior or posterior-directed rays, with points that exceeded the Y-axis in one or both directions. The nuclear apparatus comprises a horseshoe-shaped macronucleus and an oval micronucleus, both of which are located in the y+1 position of the cell.



Figs. 1-6. Silver impregnated adhesive discs of *Trichodina modesta* Lom, 1970. Obtained from gills, scales and fins of *Carassius auratus*. (Scale bars 10 μm)

3.1.2 Efficacy of antiprotozoal agents against *Trichodina modesta* Lom, 1970

Table 1 shows the findings of an in vitro investigation on the acute toxicity of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* extracts to *Trichodina modesta*. The dose of extract employed and the length of time the extract was exposed to were highly associated with an increase in mortality. Over the course of four days, 0.625 g/kg *Piper kadsura* (Choisy) Ohwi and 1.25 g/kg *Zingiber officinale* failed to kill *Trichodina modesta* at the quantities tested. Experiments using 8 g/kg extracts of *M. officinalis* and *Zingiber officinale* resulted in the death of *Trichodina modesta* after 10 and 15 days, respectively. As the death rate increased, many *Trichodina modesta* had a shape transformation, which was assumed to be a precursor to cell lysis in many cases. These *Trichodina* cells were no longer alive at the end of the following observation period.

Table 1. Mortality of *Trichodina modesta* Lom, 1970 after being exposed to the methanol extracts of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale*

Final concentration (g/kg extract)	Mortality of *Trichodina modesta* Lom, 1970							
	Piper kadsura (Choisy) Ohwi				*Zingiber officinale*			
	1d	5d	10d	15d	1d	5d	10d	15d
0 (control)	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
0.625	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
1.25	0.0 ±0.0	0.0 ±0.0	8.0±2.0	20.7±2.5	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0
2.5	11.6±2.1	36.3±2.5	42.7±2.5	61.3±3.1	0.0 ±0.0	0.0 ±0.0	11.0±2.0	33.0±2.6
5.0	19.1±2.5	66.0±3.0	77.1±2.1	92.3±2.1	9.3±1.5	24.3±2.1	52.7±3.5	85.3±3.1
8.0	45.3±3.1	72.0±3.0	100.0±0.0	100.0±0.0	32.7±2.1	55.0±2.6	82.3±3.5	100.0±0.0

*(*Each value is expressed as mean ±standard deviation of three replicates)*

The use of ecofriendly and biofriendly plant-based products for parasite infestation control has recently received a great deal of attention, primarily because they have the potential to replace chemical parasiticides, which can pose a serious threat to the environment, leave toxic residues, and/or cause resurgence in target parasite populations, among other things. In recent years, researchers have focused their attention on the use of plants or plant extracts to manage protozoa infections Fournet et al. [25]; Camacho et al. [26]; Grabensteiner et al. [27]. In fish, several plant species have shown promising efficacy against parasites such as *Macleaya microcarpa* (Yao and colleagues [28], *Allium sativum* Buchmann and colleagues [29], *Macleaya pruriens*, and *C. papaya* Ekanem et al. [30], among others.

The goal of this study was to find new natural chemicals that may be used to control *Trichodina* infection in goldfish. According to El Deen and Mohamed [31], Militz et al. [32], there is an urgent need to conduct a comprehensive review of historically used medicinal herbs to propose an alternative way for managing trichodiniasis in aquaculture.

We investigated the antiprotozoal activity of two medicinal plants against *Trichodina* infection, namely *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale*, and found that they were both effective. The infection rate in the control condition gradually grew. In contrast, the infection rate in the aquarium fish treated with *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* slowly dropped and was eventually eliminated after days of treatment. Parasite counts were performed by examining the collected body and gill smears of the treated and untreated fish in a microscopically controlled environment. LC50 values for both plant extracts were retrieved, and working dosages were kept significantly lower than the LC50 values for both. 96-hour median lethal concentration (96-hour LC50) of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* was 2.80 and 4.43 g/ kg, respectively. Using the acute doses for treating ciliophoran parasites in ornamental fish that were determined, the survival rate of trichodinid-infected fish was reduced.

The LC50 values for both plant extracts have been obtained, and the working doses have been kept significantly lower than their LC50 values. The median fatal concentration (96 h LC50) of *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* were 11.12 and 14.05 g, respectively, whereas the therapeutic dose was 8 g, indicating that plant extracts have a significant safety margin when used as a medicine for trichodiniasis in goldfish (Trichodiniasis of goldfish). Using the acute doses for treating ciliophoran parasites in ornamental fish determined, the survival rate of trichodinid-infected fish reduced, and the parasitic load rose in the control group for the first 15 days of the trial.

Survival rates were increased by 65 percent, 85 percent, and finally, 100 percent in both groups treated with *Piper kadsura* (Choisy) Ohwi and *Zingiber officinale* with 1.25, 5, and 8 g/kg diet respectively, which were also significantly different ($P < 0.05$ and $P > 0.01$) from their control groups. The survival rates reached by both phytocomponents were the same, even when administered at varying doses; nevertheless, the parasite numbers were discovered to be different in each phytocomponent's treatment group.

The most suitable and effective dose of *Piper kadsura* (Choisy) Ohwi, in which all parasitic burdens have been removed, was 8 mg/kg doses in 10 days. In contrast, by using *Zingiber officinale* at 8 g/kg, all trichodinids were removed from goldfish after only 15 days treatment. All treatments were statistically significant compared to their respective control groups (Table 1). The findings of the current investigation corroborated those of Madsen et al. [33], Chitmanat et al. [34], and Noor El Deen and Mohamed [31].

4. CONCLUSION

The current study intends to characterize the parasite fauna of ornamental fish and how medicinal plant extracts regulate them. As a result, an attempt has been made, with some success, to manage these protozoan parasites by the use of herbal medicine. In this respect, the current research has thrown some light on the elements that contribute to ornamental aquaculture production losses, as well as relevant management solutions. The current findings demonstrate that mahogany leaf extracts can be employed in the production of antiprotozoan medicines, which is a significant finding. Meanwhile, more research is required for field evaluations in the practical system, and the structure–activity relationship of these active compounds must be elucidated even more.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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