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EFFICACY OF *Gracilaria verrucosa* EXTRACT BATHS AGAINST *Trichodina* sp. IN COMET GOLDFISH *Carassius auratus*

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ABSTRACT

Trichodina sp. is a major ectoparasite affecting *Carassius auratus*, reducing its aesthetic and market value. This study evaluated the efficacy of *Gracilaria verrucosa* aqueous extract against *Trichodina* sp., its impact on fish survival, and the effective dosage. The experiment was conducted using a Completely Randomized Design (CRD) with four treatment groups: K (methylene blue, 0.6 mL/L), P1 (*G. verrucosa* extract, 10 mL/L), P2 (*G. verrucosa* extract, 15 mL/L), and P3 (*G. verrucosa* extract, 20 mL/L), each with three replications. Observations included clinical signs, behavior, parasite prevalence and intensity, time to parasite death, fish survival, and water quality. Infected fish showed excess mucus, scale loss, pale gills, and behavioral changes. Treatment P3 significantly reduced parasite intensity to 2.93 individuals/fish, caused parasite death within approximately 8.5 minutes, and resulted in 100% survival. Parasite lysis was observed in treatments K, P2, and P3. Water quality remained within suitable ranges, with temperature at 26.5–27.9°C, pH 7–8.1, and dissolved oxygen 4.8–5.8 mg/L. These findings suggest that *G. verrucosa* aqueous extract at 20 mL/L is effective against *Trichodina* sp. and safe for comet fish.

How to Cite

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INTRODUCTION

Ornamental fish possess high aesthetic value and have become an important export commodity, with Indonesia ranking fifth globally (Khoironi and Saskara, 2017). More recent market analyses still position Indonesia among important exporters within the ornamental fish value chain, highlighting the continuing economic relevance of freshwater ornamental aquaculture (Tarihoran et al., 2023). One of the most popular ornamental freshwater species is comet fish *Carassius auratus*, valued for its attractive coloration and adaptability to various environments (Hafiz et al., 2020). However, ornamental fish farming often faces disease challenges, particularly infections caused by ectoparasites such as *Trichodina* sp., which induce trichodiniasis characterized by excessive mucus production, lesions, and reduced immune response (Afifah et al., 2014; Agustina et al., 2018). Trichodinids are widely recognized as common ciliate parasites on fish gills and skin and can reduce fitness by impairing respiration and predisposing fish to secondary problems under stressful culture conditions (Lom and Dyková, 1992). Physical damage to ornamental fish reduces their aesthetic appearance and decreases their market value (Musdian et al., 2016; Kuri et al., 2022).

Ectoparasite control commonly relies on chemical treatments such as methylene blue, which is effective but may result in residual toxicity and environmental impacts when overused. In addition to residue concerns, recent ecotoxicological evidence indicates that methylene blue release can pose risks to non-target aquatic organisms in receiving waters, supporting the need to minimize unnecessary discharge and seek safer options (Li et al., 2023). Therefore, natural compounds such as seaweeds are considered safer and more eco-friendly alternatives (Hutson et al., 2012; Siddik et al., 2023). Seaweeds (marine macroalgae) are also widely documented sources of valuable nutrients and diverse secondary metabolites (e.g. phenolics, terpenoids, polysaccharides) that can contribute antimicrobial and antiparasitic effects, making them promising candidates for aquaculture health management (Holdt and Kraan, 2011; Harwanto et al., 2020; Windarto et al., 2024a; Windarto et al., 2025a; Cheng et al., 2025). Among seaweeds, red algae (Rhodophyta) are particularly important because they contain various bioactive constituents, including polysaccharides, proteins, peptides, and phenolic compounds, which have been associated with antioxidant, antibacterial, antihypertensive, and other biological activities (Windarto et al., 2024b; Windarto et al., 2025b). Therefore, Rhodophyta species such as *Gracilaria verrucosa* are promising candidates for further exploration as natural sources of bioactive compounds in aquaculture health management.

Gracilaria verrucosa, a red seaweed abundantly found in Indonesia, contains bioactive compounds such as flavonoids, saponins, tannins, phenols, and quercetin, which exhibit antiparasitic, antibacterial, and antioxidant properties (Matfuch et al., 2016; Febrianto et al., 2019; Olasehinde et al., 2019; Ouahabi et al., 2023; Windarto et al., 2025c). Research on the use of *G. verrucosa* extract for controlling *Trichodina* sp. remains limited. Specifically, compared with the broader literature on seaweed-derived antiparasitic activity and natural-product approaches to parasite management (Hutson et al., 2012; Cheng et al., 2025), there is still insufficient dose-response evidence for an aqueous *G. verrucosa* immersion treatment targeting *Trichodina* sp. in freshwater ornamental culture, including how treatment concentration relates to parasite suppression dynamics and host survival under short-term bath exposure. Therefore, research on treating *Trichodina* sp. with *G. verrucosa* extract is necessary to determine the effect of the extract on *Trichodina* sp. and the survival of goldfish.

Accordingly, this study aimed to (1) evaluate the efficacy of *G. verrucosa* aqueous extract immersion at different concentrations for suppressing *Trichodina* sp. infection in comet fish, (2) compare the antiparasitic performance of the extract with a conventional chemical treatment, and (3) identify an effective concentration that maintains high host survival after treatment. We hypothesized that increasing concentrations of *G. verrucosa* aqueous extract would produce stronger and faster reductions in *Trichodina* sp. infection metrics, and that an optimized extract concentration would achieve parasite control comparable to chemical treatment while maintaining equal or better post-treatment fish survival.

MATERIALS AND METHODS

Experimental design and fish

The experiment used a completely randomized design consisting of four treatment groups: K (methylene blue, 0.6 mL/L), P1 (*G. verrucosa* extract, 10 mL/L), P2 (*G. verrucosa* extract, 15 mL/L), and P3 (*G. verrucosa* extract, 20 mL/L). Each treatment was replicated in three aquaria, with five fish per aquarium. Therefore, a total of twelve glass aquaria and sixty comet fish were used in this study. Twelve glass aquaria (30 × 40 × 30 cm) were prepared, disinfected, rinsed thoroughly, and air-dried prior to use. Each aquarium was filled with 5 L of freshwater and provided with gentle aeration. Fish were obtained purposively from a local ornamental fish seller and acclimated under laboratory conditions before the experiment. Mean fish length was 8.87 ± 0.46 cm and mean body weight was 7.18 ± 0.91 g. Only fish with confirmed *Trichodina* sp. infection based on microscopic examination of wet mounts from skin and gills were included. Stocking density was five fish per aquarium. Fish were not fed during the 4 h immersion period to minimize deterioration of water quality.

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Ectoparasite confirmation and enumeration

Ectoparasite examination was conducted on skin and gills using wet-mount preparations. For each examined fish, a gentle skin scrape from the lateral body surface and a gill scrape/clip were placed on a microscope slide with a drop of aquarium water, covered with a coverslip, and observed immediately under a light microscope. The presence of *Trichodina* sp. was confirmed based on its circular body shape, adhesive disc, denticles, and ciliary movement. Parasites were enumerated using a hand counter.

Morphological and morphometric observations were performed to support taxonomic identification. The observed structures included body diameter, adhesive disc diameter, denticle ring diameter, membrane, blade, ray, denticle, and radial pins. These characteristics were compared with previous descriptions of *Trichodina* species, particularly Chen (1963) and Martins et al. (2012). No silver nitrate impregnation or staining method was applied in this study; therefore, the parasite is referred to as *Trichodina* sp., with morphological characteristics consistent with *Trichodina nobilis*.

Preparation of Gracilaria verrucosa aqueous extract

Fresh *G. verrucosa* thalli were collected from Jepara, Indonesia. The seaweed was identified based on morphological characteristics by BBPBAP Jepara. A voucher specimen was deposited under voucher number: GV-SW25. After collection, the seaweed was washed thoroughly with clean freshwater to remove sand, epiphytes, and other debris, then air-dried or shade-dried at 28-30°C until a constant weight was obtained. The dried material was ground into powder using a grinder and sieved to obtain a uniform powder.

The aqueous extract was prepared by macerating *G. verrucosa* powder in distilled water at a powder-to-solvent ratio of 1:10 [w/v] for 4 h at room temperature with periodic stirring. The mixture was filtered through filter paper to remove solid residues and centrifuged at 5,300 rpm for 5 min to clarify the extract. The supernatant was collected as the stock extract. The extract was stored at 4°C until use (Hutson et al., 2012; Windarto et al., 2026).

Immersion (bath) treatment

Fish were immersed in their respective treatment solutions (K, P1–P3) for 4 h under continuous aeration. After the 4 h immersion period, fish were transferred to clean freshwater for post-treatment maintenance.

Post-treatment maintenance

Following immersion, fish were maintained for 7 days. Fish were fed twice daily to apparent satiation, waste was siphoned, partial water exchange was performed as needed, and water quality was monitored daily (temperature, pH, dissolved oxygen). Fish behavior and survival were observed daily throughout the 7-day period.

Observation parameters

Clinical signs and behavioral symptoms

Clinical signs (e.g. mucus production, external lesions) and behavioral symptoms (e.g. reduced appetite, rubbing/flashing, sluggish movement) were recorded prior to treatment and monitored during the post-treatment maintenance period (Afifah et al., 2014).

Time to death of Trichodina sp.

For each treatment, parasite mortality observations were conducted in triplicate using actively motile *Trichodina* sp. individuals isolated from infected fish. Time to death was recorded from initial exposure until complete cessation of movement.

Intensity

The intensity of parasitic infection was calculated using the formula proposed by Cameron (2002). For clarity and consistency with common parasitological usage, mean intensity was interpreted as the number of parasites per infected host (Bush et al., 1997). Intensity was assessed from wet-mount counts and summarized per treatment group.

Prevalence

The prevalence of parasitic infection was calculated using the formula described by Cameron (2002) as follows:

$$\text{Prevalence (\%)} = (\text{Total infected fish} / \text{Total fish}) \times 100$$

This definition is consistent with standard parasite ecology terminology (Bush et al., 1997).

Survival rate (SR)

Survival rate (SR) during the 7-day post-treatment period was calculated following Effendie (1997):

$$SR = (N_t / N_0) \times 100\%$$

where:

- SR = Survival rate (%)
- N_t = Number of live fish at the end of the experiment (individuals)
- N_0 = Number of fish at the beginning of the experiment (individuals)

Phytochemical screening

The saponin test is indicated by the formation of foam that persists for 30 seconds. The tannin test is marked by a color change to dark green, steroids by a red coloration, and the triterpenoid test results in a bluish-green color (Hidayah et al., 2016). The alkaloid test is characterized by the formation of a precipitate. Meanwhile, the flavonoid test is indicated by a color change, in which flavanones and flavonols turn red, and glycogen or glucose change from green to blue (Marliana et al., 2005).

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Water quality

The observed water quality parameters included dissolved oxygen, temperature, and pH. Measurements were recorded during the immersion period and daily during the 7-day post-treatment maintenance to ensure that basic environmental conditions remained within acceptable ranges for *C. auratus* and did not confound treatment effects.

Data analysis

Data were expressed as mean ± SD (n = 3). Normality and homogeneity of variance were assessed prior to analysis. When assumptions were met, differences among treatments were tested using one-way ANOVA (α = 0.05), followed by Tukey's HSD post hoc test for all pairwise comparisons. When assumptions were not met, the Kruskal–Wallis test was applied, followed by Dunn's multiple comparisons test with adjusted p-values. Analyses were conducted in SPSS 27.0.

RESULTS

Comet fish *C. auratus* suspected of ectoparasite infection exhibited clinical symptoms such as excessive mucus production, scale loss, body redness, pale gills, and gill swelling (Fig. 1). Clinical symptoms before and after treatment are presented in Table 1.

Table 1. Clinical symptoms before and after treatment

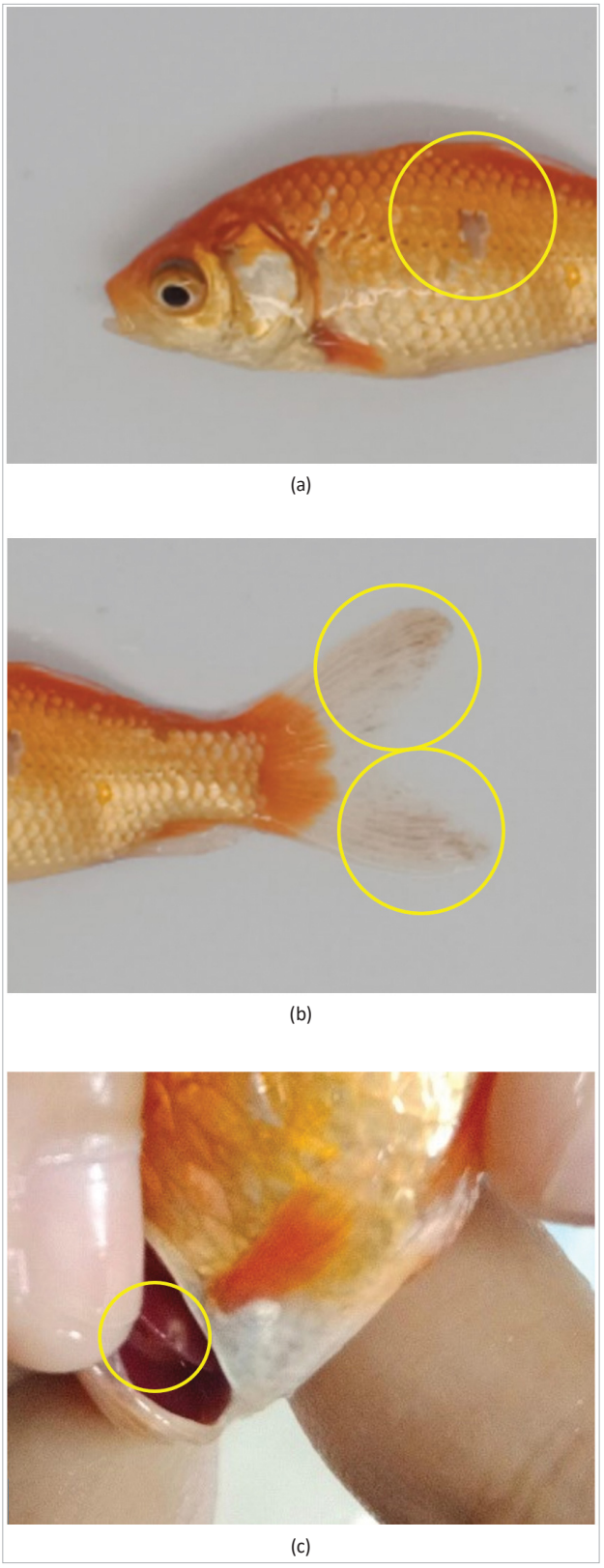
No	Treatment	Before treatment	After treatment
1	K (0.6 mL/L)	+++	+
2	P1 (*G. verrucosa* extract 10 mL/L)	+++	++
3	P2 (*G. verrucosa* extract 15 mL/L)	+++	+
4	P3 (*G. verrucosa* extract 20 mL/L)	+++	+

Note: +: loose scales, pale gills, swollen gills, ++: slight excess mucus, loose scales, redness, pale and swollen gills, +++: excess mucus, loose scales, redness, pale and swollen gills (Hardi, 2015)

Immersion with *G. verrucosa* extract effectively reduced mucus production and accelerated the recovery of redness. The fish also showed abnormal behaviors, including reduced feeding response, body rubbing against the aquarium surface, staying near the aeration area, and remaining at the bottom, as shown in Table 2.

Effect of G. verrucosa extract on the morphology of Trichodina sp.

The morphology of *Trichodina* sp. on comet fish *C. auratus* is circular, with distinct structures such as an adhesive disk, denticles, and cilia (Fig. 2). The average body diameter is 68.87 µm, with 25–27 denticles observed (Table 3).



(a)

(b)

(c)

Fig 1. Clinical symptoms of comet fish. (a) loose scales, (b) redness of the tail, (c) swollen gills.

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Table 2. Behavior of comet fish *C. auratus*

Treatment	K			P1			P2			P3		
Replication	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Phase	Before immersion											
	+	+++	+++	+++	+	+	+	+++	+	++	+	+++
	During 4-hour immersion											
	++	++	+++	+++	++	+	++	+++	+++	+++	+++	+++
	After immersion											
	+	+	+++	++	+	+	+	+	++	++	++	++
	+	+	+++	++	+	+	+	+	++	+	+	++
	+	+	+++	+++	++	+	+	++	+	+	+	+
	+	+	+++	+	++	++	++	+++	+	+	+	+
	+	+	+	+	+++	+++	++	+++	+	+	+	+
	+	+	+	+	+	+++	+++	+	+	+	++	+
	+	++	+	+	+	+	+	+	+	+	+++	+

Note: +: responsive to feed, ++: good response to feed, stays at the bottom/rubs body against the aquarium/approaches aeration, +++: poor response to feed, stays at the bottom/rubs body against the aquarium/approaches aeration (Afifah et al., 2014)

Table 3. Morphometric and meristic characteristics of *Trichodina* sp.

Characteistics (µm)	Observation result	Chen 1963 di Basson dan Van As (1994)	Martins et al. (2012)
Diameter			
Body	67.86 − 69.89 µm (68.87 ± 1.02) µm	63.0 − 76.0 µm (69.2 ± 4.3) µm	77.7 ± 5.7 µm (67.8 − 86.8) µm
Adhesive disk	60.80 − 63.10 µm (61.61 ± 1.29) µm	505 − 63.0 µm (573 − 4.0) µm	65.1 ± 7.9 µm (50.0 − 76.5) µm
Denticle ring	52.31 − 55.56 µm (53.52 ± 1.77) µm	300 − 40.0 µm (35.9 ± 2.9) µm	44.8 ± 5.9 µm (37.1 − 56.8) µm
Width			
Membrane	3.66 − 4.56 µm (4.29 ± 0.54) µm	5.0 − 7.0 µm (5.9 ± 0.7) µm	-
Blade	5.40 − 7.30 µm (6.68 ± 1.12) µm	6.5 − 9.0 µm (7.5 ± 0.6) µm	7.7 ± 0.5 µm (6.3 − 8.6) µm
Ray	6.53 − 7.50 µm (7.24 ± 0.62) µm	9.0 − 11.5 µm (9.9 ± 0.6) µm	10.3 ± 1.3 µm (7.8 − 12.6) µm
Denticle	13.19 − 15.48 µm (14.34 ± 1.15) µm	8.0 − 11.0 µm (9.6 ± 1.0.15) µm	13.19 µm
Quantity			
Denticle	25 − 27 (25.67 ± 1.15)	21 − 27	25 − 28
Radinal pin	11 − 12 (11.67 ± 0.58)	11 − 13	11 − 13 (12 ± 0.7)

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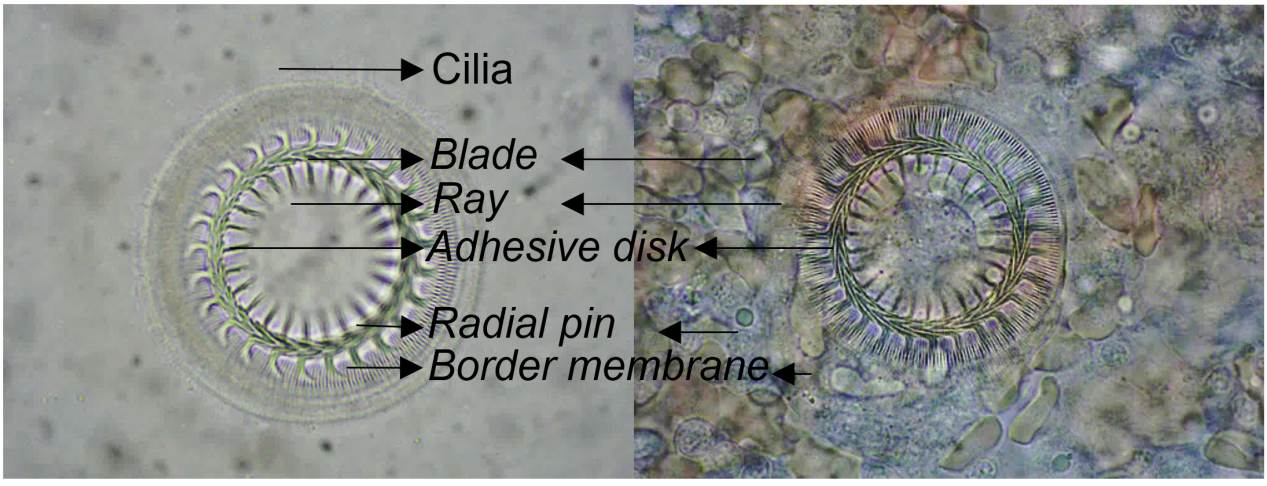


Fig 2. *Trichodina* sp. at 1000x magnification. (A) skin surface, (B) gill.

The morphometric values are consistent with the species description of *Trichodina* reported by Chen (1963) and Martins et al. (2012). Immersion in *G. verrucosa* extract caused lysis in the adhesive disk region (Fig. 3).

Trichodina sp. in the treatment groups showed an increase in body diameter, adhesive disk, and denticle ring compared to the control (Table 4).

Table 4. Morphometric and meristic characteristics of *Trichodina* sp. among treatments

Characteistics (µm)	K	P1	P2	P3
Diameter				
Body	67.86 − 69.89 µm (68.87 ± 1.02) µm	74.20 µm	68.61 µm	74.10 µm
Adhesive disk	60.80 − 63.10 µm (61.61 ± 1.29) µm	65.86 µm	63.83 µm	66.31 µm
Denticle ring	52.31 − 55.56 µm (53.52 ± 1.77) µm	60.47 µm	56.90 µm	55.54 µm
Width				
Membrane	3.66 − 4.56 µm (4.29 ± 0.54) µm	4.26 µm	3.59 µm	4.12 µm
Blade	5.40 − 7.30 µm (6.68 ± 1.12) µm	7.39 µm	6.78 µm	6.27 µm
Ray	6.53 − 7.50 µm (7.24 ± 0.62) µm	7.77 µm	6.27 µm	7.27 µm
Denticle	13.19 − 15.48 µm (14.34 ± 1.15) µm	13.21 µm	12.42 µm	11.18 µm
Quantity				
Denticle	25 − 27 (25.67 ± 1.15)	27	28	25
Radinal pin	11 − 12 (11.67 ± 0.58)	12	11	11

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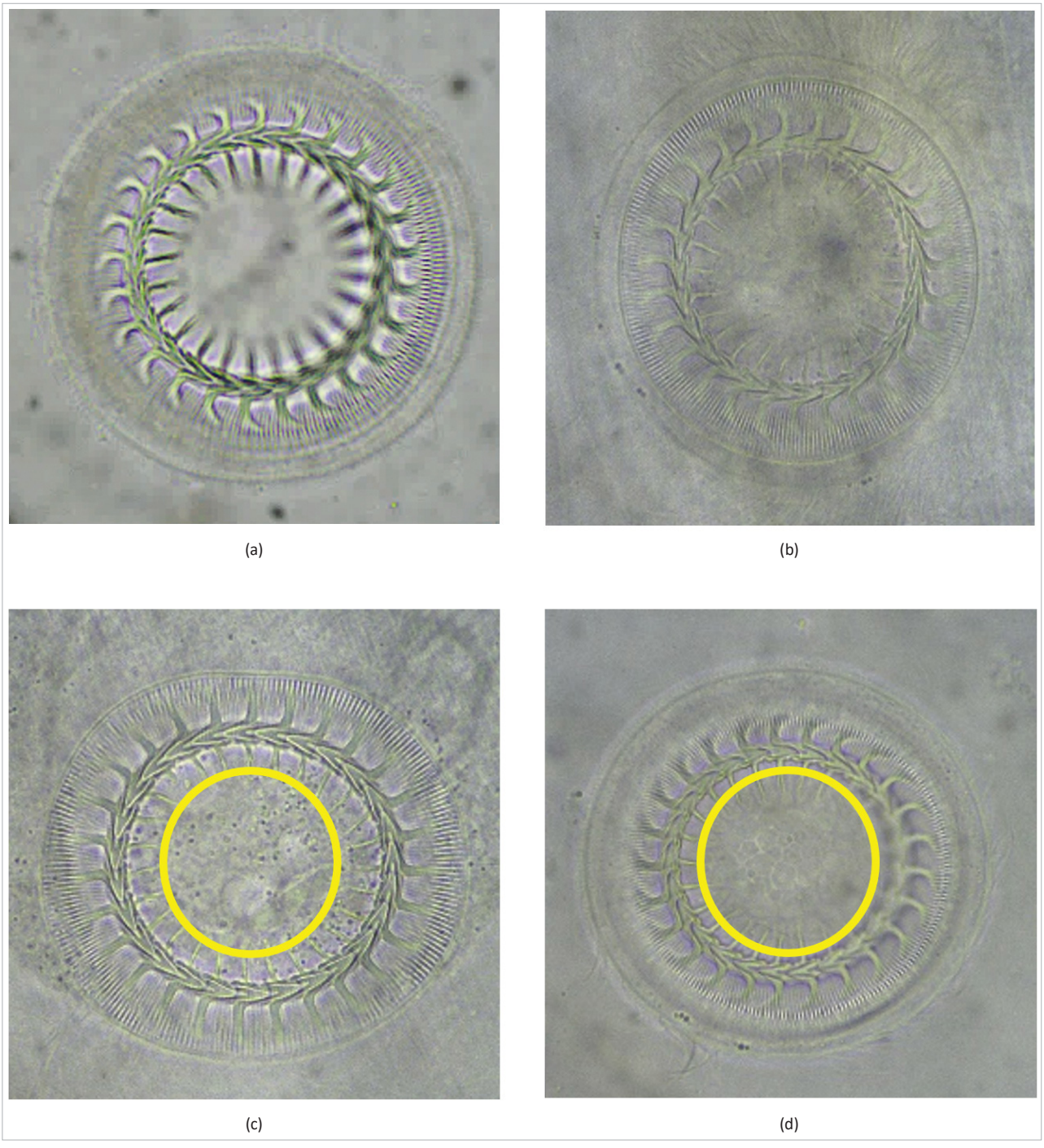


Fig 3. *Trichodina* sp. post-treatment at 1000x magnification. (A) K (0.6 mL/L), (B) P1 (*G. verrucosa* extract 10 mL/L), (C) P2 (*G. verrucosa* extract 15 mL/L), (D) P3 (*G. verrucosa* extract 20 mL/L); yellow circles indicate lysis of the adhesive disk.

Effect of G. verrucosa extract administration on the prevalence and intensity of Trichodina sp.

Before treatment, the prevalence of *Trichodina* sp. was 100% with an intensity of 24.88 ind/fish (moderate category) (Table 5).

After immersion treatment with *G. verrucosa* extract, prevalence in groups K and P1 remained at 100%, while in P2 and P3 it decreased to 86.67% (Fig. 4). Post-treatment intensity showed the lowest value in P3 (2.93 ind/fish; low category) and the highest in K (39.33 ind/fish; moderate category) (Fig. 5). ANOVA indicated a significant treatment effect ($p < 0.05$), and Tukey's HSD test showed that P3 (*G. verrucosa* 20 mL/L) differed significantly from K, P1, and P2. The administration of *G. verrucosa* extract at 20 mL/L (P3) resulted in the lowest infection intensity (2.93 ind/fish), indicating greater efficacy in reducing *Trichodina* sp. intensity compared with methylene blue treatment (K, 39.33 ind/fish).

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Table 5. Intensity and prevalence of *Trichodina* sp. in comet fish *C. auratus* before treatment

Mucus (ind)	Gills (ind)	Number of ectoparasites (ind)	Number of infected fish (ind)	Number of observed fish (ind)	Prevalence (%)	Intensity (ind/fish)
1,249	244	1,493	60	60	100	24.88

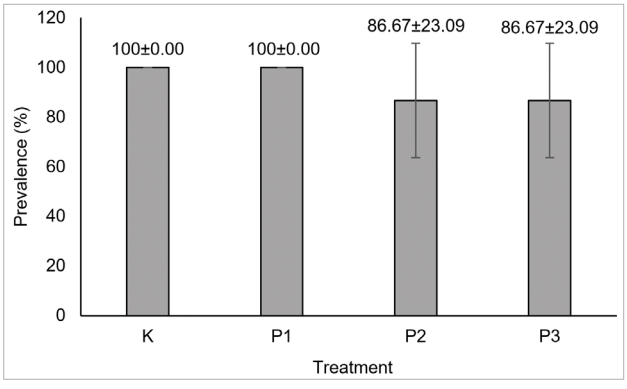


Fig 4. Prevalence of *Trichodina* sp. post-treatment

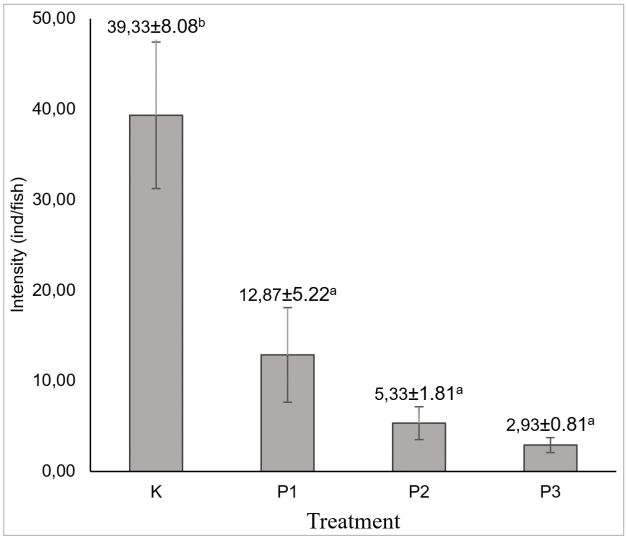


Fig 5. Intensity of *Trichodina* sp. after treatment. Values with different superscript letters between treatments indicate a significant difference (P < 0.05)

Effect of G. verrucosa extract on the time to death of Trichodina sp.

Immersion with *G. verrucosa* extract significantly affected the mortality time of *Trichodina* sp., which was 510 ± 109 seconds in P3, 780 ± 356 seconds in P2, and 1980 ± 442 seconds in P1 (Table 6). These results indicate that the higher the dose, the shorter the time to mortality.

Table 6. Time to death of *Trichodina* sp. after immersion in *G. verrucosa* extract

No	Treatments	Mean time to death (s) ± SD
1.	P1 (*G. verrucosa* extract 10 mL/L)	1980 ± 442.94
2.	P2 (*G. verrucosa* extract 15 mL/L)	780 ± 356.48
3.	P3 (*G. verrucosa* extract 20 mL/L)	510 ± 109.00

Effect of G. verrucosa extract on the survival rate of comet fish C. auratus

The survival rate of comet fish *C. auratus* after 7 days of maintenance showed differences among treatments (Fig. 6). Treatment P3 (*G. verrucosa* extract 20 mL/L) had the highest survival rate at 100%, while the control group (K, 0.6 mL/L) showed the lowest survival rate at 60%.

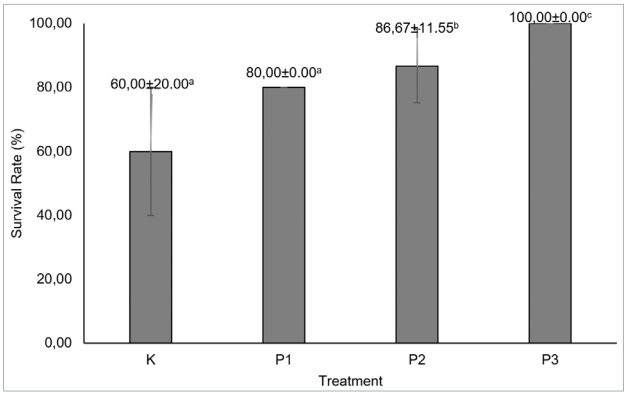


Fig 6. Fish survival rate. Values with different superscript letters between treatments indicate a significant difference (P < 0.05)

Phytochemical screening

The results (Table 7) revealed the presence of alkaloids and flavonoids, while saponins, tannins, steroids, and triterpenoids were not detected.

Water quality

Based on Table 8, the water temperature in all treatments ranged from 26.5 to 28°C, the pH ranged from 7 to 8.1, and the dissolved oxygen (DO) ranged from 4.8 to 5.8 mg/L.

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Table 7. Phytochemical analysis of aqueous extract of *Gracilaria verrucosa*

No	Secondary metabolites	Test result
1	Saponin	-
2	Tanin	-
3	Steroid	-
4	Triterpenoid	-
5	Alkaloid	+
6	Flavonoid	+

Description: + : contains secondary metabolite compounds, – : does not contain secondary metabolite compounds

Table 8. Water quality

No	Treatment	Water quality parameter range		
		Temperature (°C)	Potential of hydrogen	Dissolved oxygen (mg/L)
1	K (0.6 mL/L)	26.7 − 27.9	7 − 7.9	4.8 − 5.8
2	P1 (*G. verrucosa* extract 10 mL/L)	26.5 − 27.8	7.1 − 8.1	5.1 − 5.7
3	P2 (*G. verrucosa* extract 15 mL/L)	26.9 − 28	7 − 8	4.9 − 5.6
4	P3 (*G. verrucosa* extract 20 mL/L)	26.9 − 27.8	7.1 − 8.1	4.8 − 5.8
	Standard	26 − 30*	6.5 − 8.5*	>5*

Note: (*) According to BSN (2015)

Based on Table 8, water temperature in all treatments ranged from 26.5 to 28°C, pH ranged from 7.0 to 8.1, and dissolved oxygen ranged from 4.8 to 5.8 mg/L. Temperature and pH remained within the recommended range for comet fish culture. However, dissolved oxygen in some observations was slightly below the recommended standard of >5 mg/L. Therefore, although water quality was generally suitable for fish maintenance, dissolved oxygen was close to the lower acceptable limit and may require careful monitoring during immersion and post-treatment maintenance.

DISCUSSION

Comet goldfish infected with *Trichodina* sp. exhibited clinical symptoms such as excessive mucus production, scale loss, reddening, pallor, and gill swelling, as well as abnormal behaviors including reduced feeding response, body rubbing against the aquarium surface, and remaining at the bottom (Afifah et al., 2014; Haryono et al., 2016; Kasiati et al., 2016). *Trichodina* sp. infection causes irritation on the fish's surface due to the movement of the adhesive disc and cilia, which damage the epithelium and trigger excessive mucus production as a defense mechanism (Islami et al., 2017; Agustina et al., 2017). Immersion in *G. verrucosa* extract at concentrations of 15–20 mL/L was able to reduce mucus production, alleviate reddening, and improve fish activity levels. This improvement is presumably due to the bioactive compound quercetin present in *G. verrucosa*, which acts as an antiparasitic and anti-inflammatory agent by inhibiting lipoxygenase (LOX) and cyclooxygenase (COX) enzymes, thereby suppressing the formation of inflammatory mediators such as leukotrienes and prostaglandins (Matfuch et al., 2016; Yao et al., 2016; Olasehinde et al., 2019; Ouahabi et al., 2023; Aggarwal et al., 2025).

Trichodina sp. on comet fish exhibits a disc-shaped body with denticles and cilia, which function as attachment and locomotory organs (Azmi et al., 2013; Fatikasari et al., 2024; Islami et al., 2017). Based on identification results, the species observed was *Trichodina nobilis* as described by Chen (1963) and Martins et al. (2012). Immersion in *G. verrucosa* extract at concentrations of 15–20 mL/L (P2 and P3) induced morphological alterations such as membrane damage and lysis of *Trichodina* sp. The treatment affected the morphology of *Trichodina* sp., particularly by increasing body dimensions, including

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body diameter, adhesive disc, and denticle ring. This suggests a physiological response or morphological adaptation to the treatment. Moreover, the antiparasitic activity is likely associated with flavonoid compounds present in *G. verrucosa* extract, including compounds previously reported in this species, such as quercetin. Quercetin is known to induce apoptosis by disrupting intracellular ion balance and promoting the generation of reactive oxygen species (ROS), which damage the membranes and mitochondria of parasitic cells (Silva et al., 2013; Cataneo et al., 2019; Salehi et al., 2020). The accumulation of ROS ultimately leads to cellular content leakage and parasite cell death.

The prevalence and intensity of *Trichodina* sp. prior to treatment were 100% and 24.88 ind/fish, respectively. The high level of infestation is attributed to the parasite's rapid reproduction by binary fission and its ability to survive without a host for more than two days (FAO et al., 1985; Islami et al., 2017). Infections typically occur on the skin and gills, which are nutrient-rich tissues, and are exacerbated by stress from high stocking densities (Afifah et al., 2014; Arbie et al., 2014; Kuri et al., 2022).

Treatment with *G. verrucosa* extract at a dose of 20 mL/L reduced prevalence to 86.67% and intensity to 2.93 ind/fish. This effect is associated with the presence of quercetin, a bioactive flavonoid with antiparasitic properties, which induces the production of reactive oxygen species (ROS), leading to membrane and mitochondrial damage in the parasite (Silva et al., 2013; Cataneo et al., 2019; Salehi et al., 2020). Post hoc pairwise comparisons (Tukey HSD) indicated that P3 differed significantly from methylene blue treatment, suggesting that *G. verrucosa* extract at 20 mL/L was more effective in reducing *Trichodina* sp. intensity. These findings highlight the potential of *G. verrucosa* extract as a natural alternative for ectoparasite control in aquaculture systems.

Immersion in *G. verrucosa* extract accelerated the mortality of *Trichodina* sp., with the 20 mL/L dose inducing death within 510 ± 109 seconds. This antiparasitic effect is presumed to be mediated by the presence of bioactive compounds. Previous studies have shown that flavonoids such as quercetin may exhibit dual roles depending on the biological context, acting as pro-oxidants against parasites while exerting antioxidant effects in host tissues (Matfuch et al., 2016; Olasehinde et al., 2019; Silva et al., 2013; Xu et al., 2019; Ouahabi et al., 2023; Salehi et al., 2020).

Administration of *G. verrucosa* extract was effective in improving the survival rate of comet fish, with the highest survival observed at doses of 20 mL/L (100%), 15 mL/L (86.67%), and 10 mL/L (80%). This improvement is possibly associated with flavonoid compounds present in the extract, which act as both antiparasitic and antioxidant agents, suppressing the growth of *Trichodina* sp. and minimizing secondary infections by enhancing the fish's immune response (Matfuch et al., 2016; Olasehinde et al., 2019; Ouahabi et al., 2023). Flavonoid compounds, such as quercetin, induce apoptosis and regulate reactive oxygen species (ROS), disrupting intracellular ion balance and damaging mitochondrial cells, thereby impairing the parasite's energy production (Silva et al., 2013; Cataneo et al., 2019; Salehi et al., 2020).

Phytochemical analysis of the aqueous extract of *G. verrucosa* revealed the presence of bioactive compounds, namely alkaloids and flavonoids, while saponins, tannins, steroids, and triterpenoids were not detected. The absence of these compounds is attributed to the use of a polar solvent (water), which is ineffective in extracting non-polar compounds such as steroids and triterpenoids, as well as the low levels of saponins and tannins (Leksono et al., 2018). Alkaloids are likely present in salt form, making them soluble in polar solvents, and are known for their toxic properties and pharmacological activities, including antiparasitic, anti-inflammatory, and antifungal effects (Prayoga et al., 2019; Maisarah et al., 2023; Souza et al., 2020). The flavonoids detected are presumed to be quercetin and its derivatives, which possess antioxidant, anti-inflammatory, and antiparasitic properties (Matfuch et al., 2016; Silva et al., 2013; Salehi et al., 2020; Hasnat et al., 2024).

Water quality during the 7-day maintenance of comet fish *C. auratus* remained within the optimal range as recommended by BSN (2015). Water temperature ranged from 26.5–28°C, pH from 7–8, and DO from 4.8–5.8 mg/L, which is still within the tolerance limits for fish survival, although close to the minimum threshold (>5 mg/L). Low dissolved oxygen levels can reduce respiratory activity, decrease feed response, and increase disease susceptibility (Nurcahyo, 2015).

CONCLUSION

Administration of *G. verrucosa* extract had a significant effect on reducing the infection intensity of *Trichodina* sp. in comet fish *C. auratus* ($P < 0.05$), with the control group (K) showing a statistically significant difference compared to treatments P1, P2, and P3. Immersion of comet fish in *G. verrucosa* extract for 4 hours showed that treatment P3 (20 mL/L) was the most effective dose, reducing the intensity of *Trichodina* sp. to 2.93 individuals/fish, which falls within the low category, while maintaining a 100% survival rate.

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UČINKOVITOST KUPKI S EKSTRAKTOM *Gracilaria verrucosa* PROTIV *Trichodina* sp. KOD ZLATNE RIBICE (*Carassius auratus*)

SAŽETAK

Trichodina sp. je glavni ektoparazit koji utječe na *Carassius auratus*, smanjujući njezinu estetsku i tržišnu vrijednost. U ovoj studiji procijenjena je učinkovitost vodenog ekstrakta *Gracilaria verrucosa* protiv *Trichodina* sp., njegov utjecaj na preživljavanje riba i učinkovitu dozu. Pokus je proveden korištenjem potpuno randomiziranog dizajna (CRD) s četiri skupine tretmana: K (metilensko plavo, 0,6 mL/L), P1 (ekstrakt *G. verrucosa*, 10 mL/L), P2 (ekstrakt *G. verrucosa*, 15 mL/L) i P3 (ekstrakt *G. verrucosa*, 20 mL/L), svaka s tri ponavljanja. Promatranja su uključivala kliničke znakove, ponašanje, prevalenciju i intenzitet parazita, vrijeme do uginuća parazita, preživljavanje riba i kvalitetu vode. Zaražene ribe pokazale su višak sluzi, gubitak ljuski, blijede škrge i promjene u ponašanju. Tretman P3 značajno je smanjio intenzitet parazita na 2,93 jedinke/ribi, uzrokovao je smrt parazita unutar otprilike 8,5 minuta i rezultirao 100%-tnim preživljavanjem. Liza parazita uočena je u tretmanima K, P2 i P3. Kvaliteta vode ostala je unutar prikladnih raspona, s temperaturom 26,5–27,9°C, pH 7–8,1 i otopljenim kisikom 4,8–5,8 mg/L. Ovi nalazi upućuju na to da je vodeni ekstrakt *G. verrucosa* u koncentraciji od 20 mL/L učinkovit protiv *Trichodina* sp. i siguran za ribe komete.

Ključne riječi: cilijati, upravljanje ektoparazitima, terapija uranjanjem, prirodni antiparazitik, uzgoj ukrasnih riba, Rhodophyta

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DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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The ethics statement has been revised to ensure full transparency regarding ethical approval, consent procedures, and compliance with relevant guidelines and regulations.

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