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RESEARCH

Astrocaryum aculeatum fixed oil is effective against dactylogyrideans without compromising gill structure in *Colossoma macropomum*

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Abstract

Dactylogyrideans infect the gills of *Colossoma macropomum* (tambaqui), causing parasitosis that requires therapeutic intervention. Phytotherapeutics may provide an alternative to chemotherapeutants, which have limited efficacy.This study evaluated the anti-dactylogyridean efficacy of *Astrocaryum aculeatum* fixed oil on tambaqui, as well as the effects of bath treatments on hematological parameters and gill histopathology. The fixed oil, composed predominantly of palmitic and oleic acids (95.5%), exhibited in vitro efficacy against the dactylogyrideans *Anacanthorus spathulatus*, *Notozothecium janauachensis*, and *Mymarothecium boegeri*, with efficacy depending on the dose. The tegument of these dactylogyrideans exhibited few structural alterations that could account for the observed efficacy. Fish tolerated baths containing 20–160 mg/L of *A. aculeatum* fixed oil, as no fish died and similar behavioral changes were observed across all concentrations. The highest concentration of *A. aculeatum* fixed oil (160 mg/L) was then used in 2-h therapeutic baths administered over four consecutive days to tambaqui, alongside one group exposed to culture tank water (negative control) and another exposed to absolute ethyl alcohol (positive control). These bath treatments achieved high efficacy (74.4%) against dactylogyrideans infecting the gills of tambaqui. Treatment with *A. aculeatum* fixed oil increased plasma protein and glucose levels, as well as mean corpuscular volume, while decreasing mean corpuscular hemoglobin concentration. Fish exposed to ethyl alcohol developed macrocytic hypochromic anemia, and no severe structural alterations of the gills were observed in any of the three groups. Therefore, baths with *A. aculeatum* fixed oil were effective against dactylogyrideans infecting tambaqui without compromising gill structure, as they induced mild stress in the fish and should therefore be administered with caution.

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Introduction

Dactylogyrideans are parasitic helminths that primarily infect the body surface, fins, and gills of fish, particularly freshwater species. They reproduce quickly due to their short, direct life cycle. Several dactylogyridean species are pathogenic to farmed fish, adversely affecting their health, reducing their growth rates, and causing mortality associated with dactylogyriasis (Li et al. 2022; Ávila-Castillo et al. 2025; Brito et al. 2026a). This disease is difficult to control with typical chemotherapeutants (Li et al. 2022; Yılmaz & Yildiz, 2023; Malheiros et al. 2023; Queiroz et al. 2025). This negatively impacts the productivity and production of aquaculture and presents a significant challenge to the food production industry, which requires effective solutions to ensure continued growth. Additionally, fish farmers must reduce their dependence on traditional chemotherapeutants (Queiroz et al. 2025).

In the Amazon region, the growing demand for *Colossoma macropomum* (tambaqui), a major source of high-quality animal protein, vitamins, and omega-3 fatty acids for many traditional populations and an important species in aquaculture, has led to the intensification of production systems. This intensification has increased the occurrence of dactylogyriasis, particularly infections caused by *Anacanthorus spathulatus* Kritsky, Thatcher & Kayton 1979; *Notozothecium janauachensis* Belmont-Jégui, Domingues & Martins 2004, and *Mymarothecium boegeri* Kritsky, Thatcher & Kayton 1979 (Luz et al. 2021; Malheiros et al. 2023; Queiroz et al. 2025; Brito et al. 2026a). As *C. macropomum* is the most widely cultured fish in the Amazon region, its intensive cultivation depends on technological innovations and therapeutic alternatives to ensure sustainable and continuous production (Luz et al. 2021; Queiroz et al. 2025; Brito et al. 2026a). The intensive production system modifies the host-parasite-environment relationship, frequently resulting in stressful conditions that can lead to immunosuppression in fish. Although there are few records of *C. macropomum* mortality caused by dactylogyrideans (e.g., Mangas et al. 2020), these ectoparasites can severely damage the gills of hosts, even when there are no apparent signs of dactylogyriasis. This damage includes epithelium displacement, focal hyperplasia of epithelial cells, congestion, shortening of the secondary lamellae, and complete fusion of the secondary lamellae (Tavares-Dias et al. 2021). This damage may compromise the intensive production of *C. macropomum* because it causes respiratory disruption, which harms growth. It may also easily provoke secondary bacterial infections, leading to grave morbidity and massive mortality.

Currently, aquaculture requires effective antiparasitic control methods that are also safe and environmentally friendly (Hashimoto et al. 2016; Zhou et al. 2022; Malheiros et al. 2023; Jawaji et al. 2024; Queiroz et al. 2025; Brito et al. 2026a, b a,b). Conventional chemotherapeutic agents have been used for a long time; however, they present limitations regarding environmental sustainability, fish welfare, parasitic resistance development, toxicity to non-target organisms, and withdrawal periods for fish consumption (Zhou et al. 2022; Yilmaz & Yildiz 2023; Malheiros et al. 2023; Jawaji et al. 2024; Queiroz et al. 2025; Brito et al. 2026b). Such limitations regarding chemotherapeutants have led to the need for new treatments and the discovery of alternative methods, such as phytotherapeutics with bioactive properties for controlling and treating ectoparasitic infections (Hashimoto et al.

2016; Zhou et al. 2022; Yilmaz & Yildiz 2023; Malheiros et al. 2023; Queiroz et al. 2025; Brito et al. 2026a), including dactylogyrideans.

Fixed oil extracted from the fruits of *Astrocaryum aculeatum* Meyer (tucumã), a tropical palm tree from the Arecaceae family, widely distributed throughout the Amazon region and parts of Central America, has a chemical composition that includes mono- and polyunsaturated fatty acids, carotenoids (e.g., β-carotene), phenolic compounds, and phytosterols (Linhares et al. 2017; Ibiapina et al. 2021; Machado et al. 2022; Daleaste et al. 2025). These bioactive compounds in *A. aculeatum* oil exhibit antibacterial and antifungal activities (Jobim et al. 2014; Machado et al. 2022). However, the antiparasitic potential of fatty acids in fish aquaculture remains poorly explored. Treatments using solid lipid nanoparticles (SLNs) loaded with *A. aculeatum* fixed oil have been ineffective against dactylogyrideans in the gills of *C. macropomum* (Brito et al. 2026a). On the other hand, studies have reported the anthelmintic efficacy of plant-derived fatty acids in controlling *Heterobothrium okamotoi* (Hirazawa et al. 2000), dactylogyrideans (Malheiros et al. 2023), and *Gyrodactylus turnbulli* (Jawaji et al. 2024). These findings indicate the therapeutic potential of different phytotherapeutics. Thus, this study aimed to evaluate the anti-dactylogyridean efficacy of bath treatments with *A. aculeatum* fixed oil against these parasites on *C. macropomum* gills, as well as the effects of treatment on hematological parameters and gill histopathology.

Material and methods

Obtaining the oil and chemical composition of *A. aculeatum* fixed oil

The fixed oil of *A. aculeatum* used in all trials was obtained as previously described by Brito et al. (2026a), who characterized its chemical composition as containing linoleic acid (2%), stearic acid (3.5%), palmitic acid (26.5%), and oleic acid (68.0%). In addition, they determined the following values: acidity index (2.00 ± 0.15 mg/NaOH/g), saponification index (109.69 ± 1.00 mg/KOH/g), density (0.989 ± 0.01 g/mL), and peroxide value (0.04 ± 0.01 meq/kg).

Fish, acclimation and parasitic dactylogyrideans

Three hundred fingerlings of *C. macropomum* (5–8 g) were acquired from a commercial fish farm in Macapá and transported to the Aquaculture and Fisheries Laboratory at Embrapa Amapá in Macapá, State of Amapá, Brazil. The fish were acclimated in one water tank with a capacity of 500 L, with constant aeration and continual water renewal (1.1 L/min) for 25 days. They were fed four times a day with commercial feed containing 36% crude protein (Guabi, São Paulo, Brazil). Previously, 20 apparently healthy fish (for a total of 160 branchial arches) were examined for the presence of dactylogyrideans (mean intensity of 40.1 ± 21.2 per fish), and all were found to have infected gills. These naturally infected fish were used in the in vitro and in vivo trials.

During the acclimation period, the water parameters were monitored every two days to evaluate the temperature (30.3 ± 0.1 °C), dissolved oxygen (5.6 ± 0.2 mg/L), pH (5.4 ± 0.2), total ammonia (0.05 ± 0.2 mg/L), alkalinity (10.0 ± 0.001 mg/L), and hardness (10.0 ± 0.01 mg/L), with the aid of a multiparametric probe (Hanna, USA, model HI 9829). The tank was siphoned weekly to remove accumulated organic material from the bottom.

The methodology used in the experiment was approved by the Ethics Committee for the Use of Animals (CEUA) of Embrapa Amapá (Protocol No. 026-CEUA/2023).

In vitro trials with *A. aculeatum* fixed oil and scanning electron microscopy of parasitic dactylogyrideans

Specimens of *C. macropomum* (12.6 ± 7.8 g and 7.9 ± 1.8 g) were euthanized by medulla section for the collection of branchial arches. To evaluate the in vitro efficacy of *A. aculeatum* fixed oil concentrations against parasitic dactylogyrideans, the removed branchial arches were immediately placed individually in Petri dishes (5 cm) containing different concentrations of oil (10, 15, 20, and 25 mg/L). For each concentration of oil, three replicates were used. In each Petri dish, a field of view containing ± 20 parasites was selected in each replicate of the different *A. aculeatum* fixed oil concentrations tested (Table 1). The parasites were observed every 15 min to count the number of live and dead parasites. Parasites were considered dead if they detached from the gill tissue or completely lost mobility while still attached (Hashimoto et al. 2016). The same observation and replicates were used in the two control groups: one with water from the culture tank and the other with ethyl alcohol. The in vitro assays were performed at room temperature (18 °C) and using cold light stereomicroscopes. The highest concentration tested was 25 mg/L because the visualization of the parasites was impaired by the cloudy appearance of the oil in contact with the water in the Petri dish at higher concentrations.

Solutions of *A. aculeatum* fixed oil were prepared at different concentrations (10, 15, 20, and 25 mg/L), using absolute ethyl alcohol (5% v/v) and distilled water as solvents. For each concentration tested, the weight of the oil was calculated based on a final volume of 25 mL. For all concentrations of *A. aculeatum* fixed oil, 1.25 mL of absolute ethyl alcohol (5% v/v) was added as the primary solvent, and the remaining volume was made up with distilled water, as shown in Table 1. All concentrations of solutions were homogenized using a magnetic stirrer at 10 g and heated to 55 °C for 1 h to ensure their complete solubilization. As a negative control, a solution containing only absolute ethyl alcohol (0.04 L: 5% v/v) and distilled water (9.96 L: 95% v/v) was prepared and subjected to the same agitation and heating protocols to rule out solvent interference in the results. This control solution was prepared considering the maximum concentration of absolute ethyl alcohol used in diluting the *A. aculeatum* fixed oil. After the in vitro trials ended, the antiparasitic efficacy of each treatment was determined following the recommendations of Wang et al. (2008).

Table 1 Preparation method of solutions containing *Astrocaryum aculeatum* fixed oil

Concentration (mg/L)	Weight of oil (mg)	Ethyl alcohol (L)	Distilled water (L)
10	100	0.04	9.96
15	150	0.04	9.96
20	200	0.04	9.96
25	250	0.04	9.96
40	400	0.04	9.96
80	800	0.04	9.96
160	1,600	0.04	9.96

At the end of the in vitro assays, all the gill arches used were gently collected and fixed in 2.5% glutaraldehyde prepared in a 0.1 M phosphate buffer at pH 7.2 for scanning electron microscopy analysis of dactylogyrideans exposed to each essential oil. Then, the gills underwent three washes in a 0.1 M phosphate buffer at 15-min intervals. The samples were dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, 96%, and 100%) for 10 min at each concentration (Luz et al. 2021). The samples were analyzed and photomicrographed using a scanning electron microscope (Hitachi, Tokyo, Japan, Model TM3030Plus) in the Pharmaceutical Research Laboratory at the Federal University of Amapá in Macapá, Brazil.

Tolerance trials, treatments of baths with *A. aculeatum* fixed oil and parasitological analysis procedures

After determining the efficacy of the concentrations in the in vitro *test*, tolerance trials were conducted using *A. aculeatum* fixed oil at 20, 40, 80, and 160 mg/L for 4 h. A total of 60 *C. macropomum* (12.9 ± 8.7 g and 8.2 ± 1.1 cm) were randomly distributed in 12 tanks of 100 L with five fish per replicate, and a total of 15 fish per treatment. During the tolerance tests, the tanks were maintained with constant aeration, and the water flow was interrupted to add the fixed oil. All alcoholic solutions of *A. aculeatum* fixed oil were prepared using absolute ethanol (5% v/v) and distilled water as solvents, as previously described (Table 1). Each solution was prepared 10–12 h in advance, homogenized with a magnetic stirrer at 10 g, and heated to 55 °C for 1 h to ensure complete solubilization. For the negative control, a solution containing only absolute ethyl alcohol (0.04 L: 5% v/v) and distilled water (9.96 L: 95% v/v) was used and subjected to the same agitation and heating protocols. This control solution was based on the highest concentration of absolute ethyl alcohol used in the dilution of *A. aculeatum* fixed oil. The time of mortality and the behavior of the fish during the treatments were observed and recorded. Changes in fish behavior (e.g., opercular movement, caudal slapping, and response to mechanical stimuli) were considered in the analysis. Fish were considered dead when they ceased opercular movement and caudal slapping and no longer responded to mechanical stimuli. At the end of the tolerance trials, since the fish exposed to 160 mg/L tolerated this higher concentration, it was selected for subsequent treatment baths.

An independent experiment was conducted with *A. aculeatum* fixed oil. A total of 117 *C. macropomum* (15.2 ± 3.6 g and 12.1 ± 1.0 cm) that were naturally infected with dactylogyridean parasites in their gills and showed no other signs of disease were randomly distributed into nine 100-L tanks. Three treatments, each with three replicates consisting of 10 fish per replicate, were evaluated. One group of fish was subjected to a therapeutic bath with 160 mg/L of *A. aculeatum* fixed oil for 2 h daily for four consecutive days. This *A. aculeatum* fixed oil solution was prepared daily with an advance of 10–12 h using absolute ethanol (5% v/v) and distilled water, as previously described (Table 1), homogenized using a magnetic stirrer at 10 g, and heated to 55 °C for 1 h to ensure complete solubilization. For the negative control, a solution of absolute ethyl alcohol (0.04 L, 5% v/v) and distilled water (9.96 L, 95% v/v) was prepared and subjected to the same agitation and heating protocols and was used for exposure of a control fish group. A control group of fish was exposed only to water from the culture tank.

During the therapeutic baths, constant aeration was maintained, and the water flow remained closed throughout these treatments. After the exposure period, the running water flow was turned on. Four days after treatment with *A. aculeatum* fixed oil began, 90 fish

(30 fish per treatment and 10 fish per replicate) were randomly collected. Fish were euthanized using medullary section, and the gills were collected and preserved in 5% formalin for subsequent parasite counting, following the procedures described by Eiras et al. (2006). The identification of parasite dactylogyrideans was according to Cohen et al. (2013). After quantifying and identifying the parasites, the parasitic prevalence and mean abundance were calculated (Bush et al. 1997), as well as the antiparasitic efficacy of the treatments (Wang et al. 2008).

Blood parameters of *C. macropomum* gills treatments of baths with *A. aculeatum* fixed oil

After the end of the four baths with *A. aculeatum* fixed oil, and before gill removal of 90 fish, five fish per replicate (15 fish per treatment, 14.2 ± 4.6 g and 11.1 ± 1.0 cm) were randomly selected to have blood samples taken from their caudal vessels using syringes containing EDTA (10%). The blood was used to determine the total red blood cell count using a hemocytometer, the hematocrit using the microhematocrit method, and the hemoglobin concentration using the cyanmethemoglobin method. Later, these data were used to calculate hematimetric indices: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) (Ranzani-Paiva et al. 2013). The remaining blood was centrifuged at 75 G (MCD-2000 centrifuge, Brazil) for 7 min to obtain plasma, which was used to determine glucose and total protein levels. Glucose concentration was determined using the colorimetric-enzymatic glucose oxidase method with a commercial kit (Biotécnica, MG, Brazil). Plasma total protein concentration was determined using the Biuret method with a commercial kit (Biotécnica, MG, Brazil). Each biochemical parameter was measured using a digital spectrophotometer (KASVI Model 2022/2025) at the recommended wavelength.

Histopathology of *C. macropomum* gills after treatments of baths with *A. aculeatum* fixed oil

After the end of the four baths with *A. aculeatum* fixed oil, 27 fish (13.5 ± 4.1 g and 10.5 ± 1.1 cm) randomly collected were euthanized by medullary section, and the gill arches of three fish from each replicate (9 fish per treatment) of each treatment (with oil, ethyl alcohol, and water from the culture tank) were collected for histopathological analysis. The gill arches were fixed in Davison's solution for at least 48 h and then transferred to 70% ethyl alcohol. Then, the gill arches were dehydrated in an ascending order of alcohol concentration (70, 80, 90, and 100% I, 100% II, and 100% III), diaphanized in absolute xylene, impregnated, and embedded in paraffin to obtain blocks. The paraffin blocks were sectioned to a thickness of 5 µm using a microtome (Leica DM 1000). After preparing duplicate slides, the gill arches were stained with hematoxylin and eosin (HE). Images were captured with a digital camera (Moticam 2300 3.0 M Pixel) attached to an optical microscope connected to a computer.

Histopathological alterations were evaluated semiquantitatively by ranking the severity of gill lesions and calculating the mean assessment value (MAV) of gill lesions for each treatment group (Schwaiger et al. 1997). Additionally, the histopathological alteration index (HAI) was calculated using a method previously described by Poleksić & Mitrović-Tutundžić (1994).

Statistical analysis

The abundance of parasites, blood parameters, and histopathological data were previously evaluated for normality and homoscedasticity using the Shapiro–Wilk and Bartlett tests, respectively. Since the data did not follow normal distribution pattern, the Kruskal-Walli's test was used. The Dunn test was applied to compare the medians (Zar 2010) using IBM SPSS Statistics 24.0 software. A statistical difference was accepted at $p < 0.05$. The survival period (in hours) was determined from the beginning of the experiments until the parasites died. This factor was used in the Kaplan–Meier survival analysis. The survival curves were obtained for each treatment. Results were statistically compared using the log-rank test and were considered significant at $p < 0.01$. The statistical analyses were performed using Jamovi software (version 2.7.17).

Results

In vitro efficacy of *A. aculeatum* fixed oil and scanning electron microscopy of dactylogyrideans

After 45 min of exposure to 25 mg/L of *A. aculeatum* oil, 100% of dactylogyrideans *A. spathulatus*, *N. janauachensis,* and *M. boegeri* died; however, *A. spathulatus* was the predominant parasite. At 20 mg/L, the efficacy was 73.9%, increasing to 100% after 1 h. However, exposure of the parasites to 10 and 15 mg/L reached 100% efficacy only after 2 h. In the control group exposed to ethyl alcohol, parasite death began after 45 min, reaching 100% after 9 h. In the control group exposed only to culture tank water, the maximum survival time of the parasites was 9 h (Figs. 1 and 2).

Parasites exposed to higher concentrations of the oil contracted immediately and were detached from the gills. Figure 3A-D shows morphological alterations to the tegument of dactylogyrideans exposed to *A. aculeatum* fixed oil, as observed by SEM. Parasites in the control groups, which were exposed to cultivation tank water or ethyl alcohol, exhibited defined body shapes with shallow, visible wrinkles on their surfaces (Figs. 3A-B). In contrast, parasites exposed to 20 mg/L (Fig. 3C) or 25 mg/L (Fig. 3D) of *A. aculeatum* fixed oil exhibited shrinkage and a wilted appearance.

Tolerance of *C. macropomum*, and the anti-dactylogyridean efficacy of treatments with baths of *A. aculeatum* fixed oil

Tolerance tests revealed that the fish were resistant to all concentrations of *A. aculeatum* fixed oil, as no mortality occurred and the fish exhibited similar behavioral changes at all concentrations (Table 2). Thus, the highest concentration of *A. aculeatum* fixed oil was used in the fish bath treatments.

The parasites in the gills of *C. macropomum* were species of Dactylogyridae, i.e., *A. spathulatus*, *N. janauachensis,* and *M. boegeri*. The prevalence of these parasites was similar between treatments, while the abundance differed. Fixed oil of *A. aculeatum* was effective in controlling dactylogyridean parasites, reducing their number by 74.4% after four days of bath treatment (Table 3).

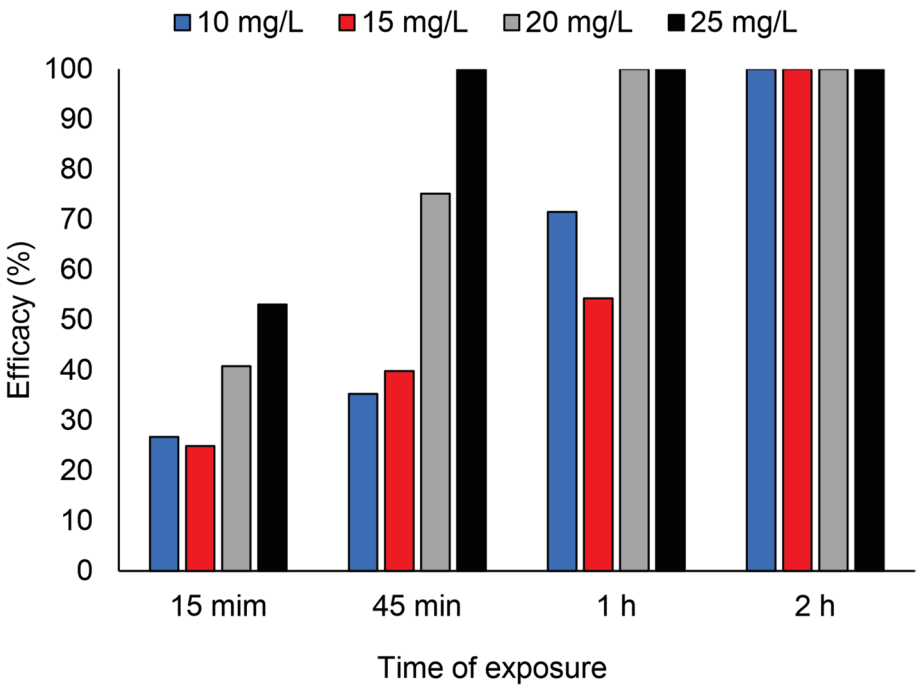

Fig. 1 In vitro efficacy of *A. aculeatum* fixed oil against parasitic dactylogyrideans at different concentrations and exposure times. Three replicates were used in each treatment

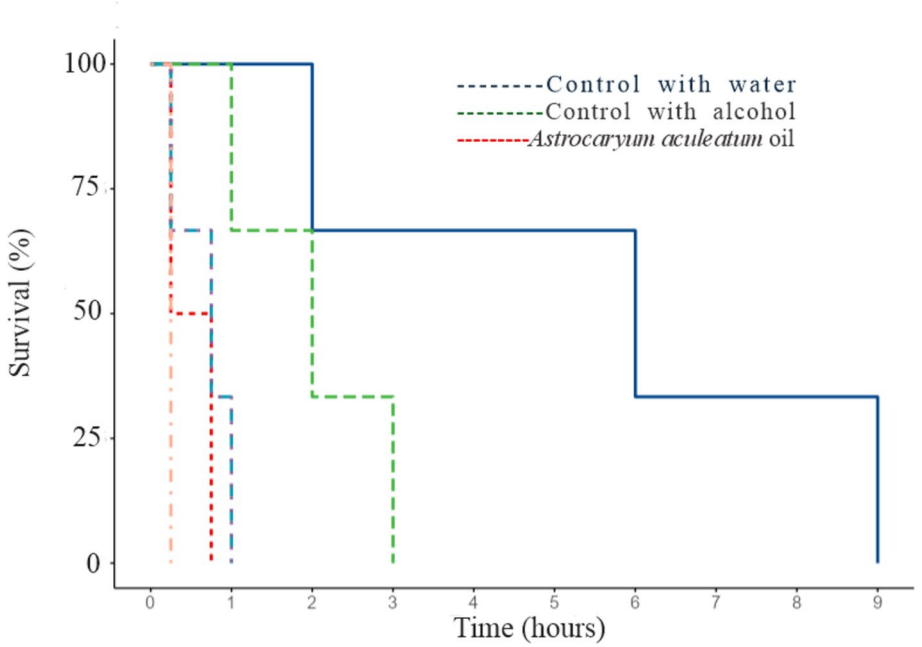

Fig. 2 Kaplan–Meier estimated survival curves of dactylogyrideans after in vitro exposure to control group (water or alcohol) and to different concentrations of *Astrocaryum aculeatum* fixed oil

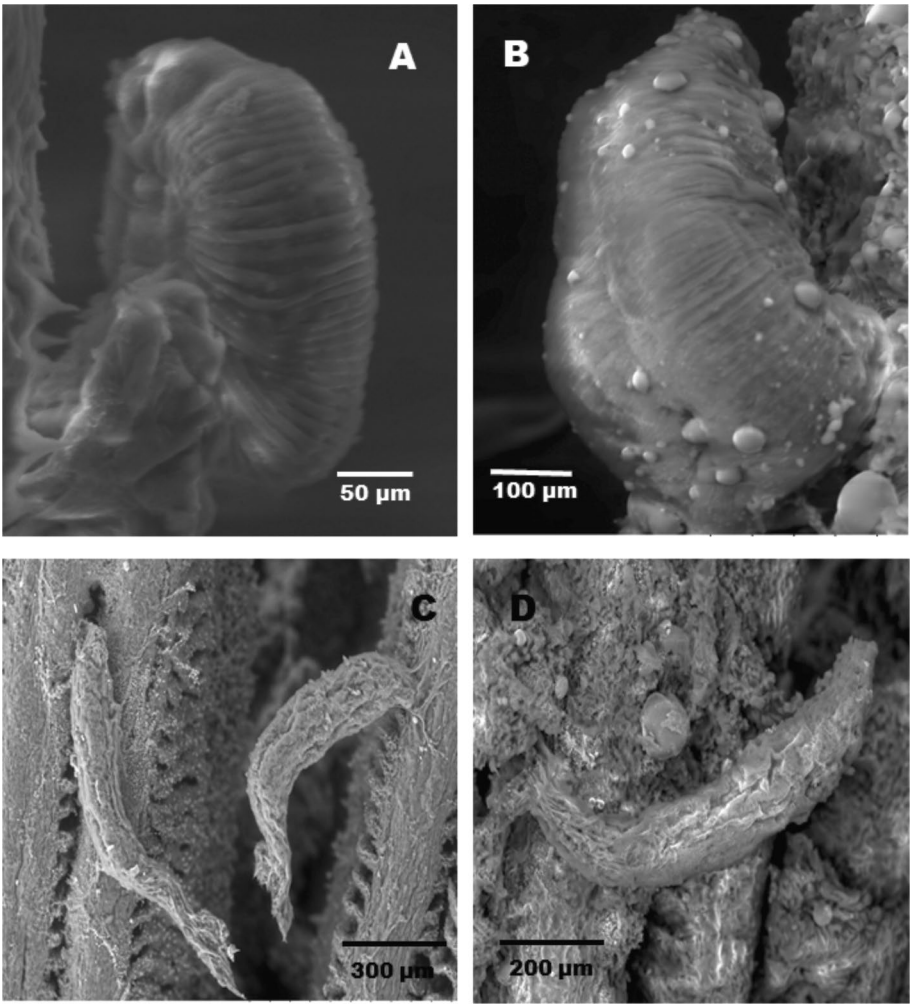

Fig. 3 Scanning electron microscopy of dactylogyrideans exposed to *Astrocaryum aculeatum* fixed oil. (**A**) Parasite after 9 h of exposure to cultivation tank water. Scale bar: 50 µm. (**B**) Parasite after 6 h of exposure to ethyl alcohol. Scale bar: 100 µm. (**C**) Parasites after 60 min of exposure to 20 mg/L of *Astrocaryum aculeatum* fixed oil. Scale bar: 300 µm. (**D**) Parasite after 45 min of exposure to 25 mg/L of *Astrocaryum aculeatum* fixed oil. Scale bar: 200 µm

Blood parameters of *C. macropomum* after treatments with baths of *A. aculeatum* fixed oil

Compared to both control groups, plasma glucose levels increased in fish treated with 160 mg/L of *A. aculeatum* fixed oil. Meanwhile, plasma protein levels decreased in control- fish exposed to ethyl alcohol compared to fish exposed only to culture tank water and those treated with 160 mg/L of *A. aculeatum* fixed oil. Control group fish exposed to ethyl alcohol exhibited a reduction in total red blood cell count and hemoglobin concentration compared to the control group exposed only to the culture tank

Table 2 Mortality and behavioral changes in *Colossoma macropomum* exposed to different concentrations of *Astrocaryum aculeatum* fixed oil for 4 h

Concentration (mg/L)	Mortality (%)	Behavioral changes
20 mg/L	0	Active swimming on the surface of the tank, normal opercular beats, and resting on the bottom of the tank
40 mg/L	0	Active swimming on the surface of the tank, normal opercular beats, and resting on the bottom of the tank
80 mg/L	0	Agitation, active swimming with upward and downward movements on the surface of the tank, accelerated opercular beats, and resting at the bottom of the tank
160 mg/L	0	Agitation, active swimming with upward and downward movements on the surface of the tank, accelerated opercular beats, temporary grouping, reduced tail movement, and resting on the bottom of the tank

Three replicates were used in each concentration

Table 3 Efficacy and parasitological indices of dactylogyrideans in the gills of *Colossoma macropomum* subjected to therapeutic baths with *Astrocaryum aculeatum* fixed oil

Treatments	Prevalence (%)	Mean abundance	Dunn test	Efficacy (%)
Control with cultivation tank water	100	98.1 ± 29.4a	60.1	-
Control with ethyl alcohol	100	75.9 ± 19.9a	77.4	22.6
160 mg/L of oil	100	19.2 ± 9.5[b]	55.5	74.4

Three replicates were used in each treatment. Data expressed as mean ± standard deviation. Different letters in the same column indicate differences by the Dunn test ($p < 0.05$)

water. Hematocrit remained unchanged in all treatment groups. The mean corpuscular volume (MCV) increased in fish exposed to ethyl alcohol (control) and in fish treated with 160 mg/L of *A. aculeatum* fixed oil. Mean corpuscular hemoglobin concentration (MCHC) decreased in both groups compared to the control group exposed only to the culture tank water (Table 4).

Histopathology of *C. macropomum* gills after treatments with baths of *A. aculeatum* fixed oil

The alterations observed in the gills of fish exposed to 160 mg/L of *A. aculeatum* fixed oil were detachment and hyperplasia of the epithelium. These alterations also occurred in fish exposed to culture tank water or ethyl alcohol (Fig. 4). However, partial fusion of the gill lamellae was occasionally found in fish of all groups, including the control groups, while aneurysms occurred only in the gills of a few fish exposed to 160 mg/L of *A. aculeatum* fixed oil. Fish exposed to 160 mg/L of *A. aculeatum* fixed oil or control exposed to ethyl alcohol had higher ($p < 0.05$) mean assessment values (MAV) than fish of the control group with culture tank water, while the histopathological alteration index (HAI) was similar ($p > 0.05$) between all groups (Table 5).

Table 4 Blood parameters of *Colossoma macropomum* after bath treatments with *Astrocaryum aculeatum* fixed oil

Parameters	Control with water	Control with alcohol	160 mg/L of oil
Glucose (g/dL)	67.2 ± 5.5[a]	69.8 ± 5.6[a]	79.8 ± 5.9[b]
Total protein (mg/dL)	2.4 ± 0.3[a]	2.1 ± 0.3[b]	2.7 ± 0.2[a]
RBC ($\times 10^6$/μL)	1.69 ± 0.06[a]	1.48 ± 0.004[b]	1.73 ± 0.24[a]
Hemoglobin (g/dL)	8.1 ± 1.3[a]	7.0 ± 1.3[b]	7.1 ± 0.6[a,b]
Hematocrit (%)	24.2 ± 3.7[a]	25.7 ± 2.6[a]	25.3 ± 4.1[a]
MCV (fL)	143.6 ± 22.6[a]	173.1 ± 17.3[b]	149.4 ± 30.1[b]
MCHC (g/dL)	34.1 ± 6.9[a]	27.5 ± 6.1[b]	29.0 ± 6.3[b]

Three replicates were used in each treatment. Data expressed as mean ± standard deviation. Different letters in the same column indicate significant differences between treatments ($p < 0.05$). RBC: Red blood cells; PAS-GL: Positive-PAS granular leukocytes; MCHC: Mean corpuscular hemoglobin concentration; MCV: Mean corpuscular volume

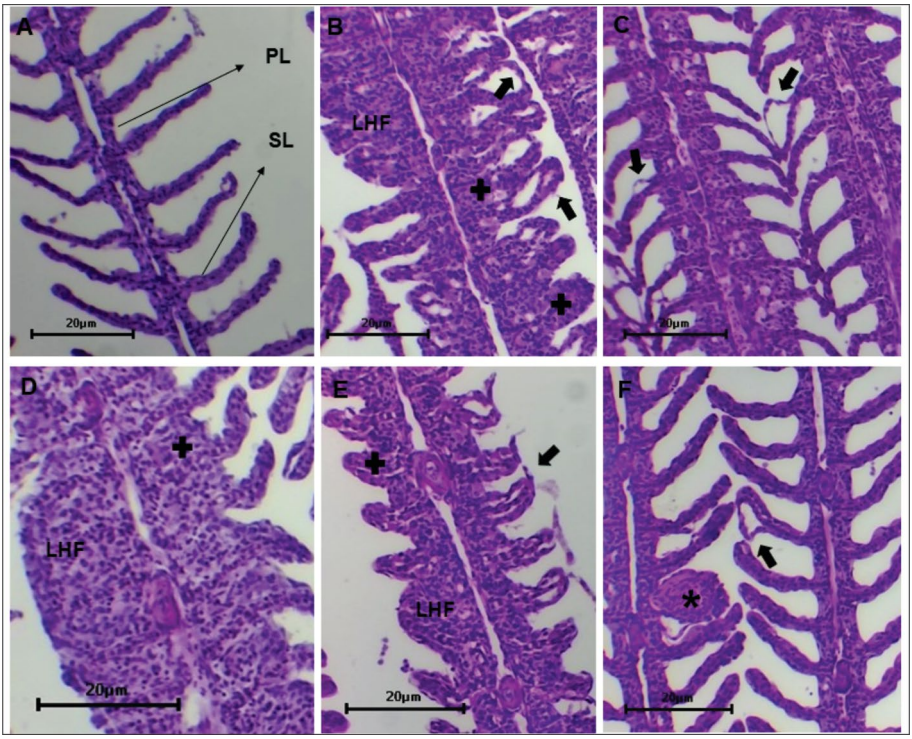

Fig. 4 Histopathology of the gills of *Colossoma macropomum* exposed to 160 mg/L of *Astrocaryum aculeatum* fixed oil. Gills of fish exposed to culture tank water showing primary (PL) and secondary (SL) lamellae (**A**), lamellar epithelial detachment (arrow), lamellar hyperplasia (cross), and lamellar hyperplasia with fusion (LFH) (**B**). Gills of control fish exposed to ethyl alcohol showing detachment of the lamellar epithelium (arrow) (**C**), lamellar hyperplasia (cross), and lamellar hyperplasia with fusion (LFH) (**D**). Gills of fish exposed to 160 mg/L of *Astrocayum Aculeatum* fixed oil showing lamellar hyperplasia in the filament (cross), lamellar hyperplasia with fusion (LFH), lamellar epithelial detachment (arrow) (**E**) and aneurysm (asterisk) (**F**). Three replicates were used in each treatment. Scale bar: 20 µm

Table 5 Histopathological alteration index (HAI) and mean assessment values (MAV) of *Colossoma macropomum* gills after bath treatments with *Astrocaryum aculeatum* fixed oil

Treatments	N	MAV	HAI	Severity of lesions according to HAI
Control the cultivation tank water	9	1.0 ± 0.9^{a}	0.9 ± 0.8^{a}	Normal functioning of the gills
Control with ethyl alcohol	9	$2.1 \pm 1.7^{a,b}$	2.2 ± 3.4^{a}	Normal functioning of the gills
160 mg/L of oil	9	3.1 ± 1.4^{b}	3.7 ± 4.2^{a}	Normal functioning of the gills

Three replicates were used in each treatment. Data expressed as mean ± standard deviation. Different letters in the same column indicate significant differences between treatments ($p < 0.05$)

Discussion

Oils extracted from oleaginous plants, including palm trees, as well as from other vegetable sources, depending on the extraction method (e.g., hydraulic press, steam distillation, or solvent extraction), are mostly made up of fatty acids with parasiticide activity

(Hirazawa et al. 2000; Jawaji et al. 2024). The fixed oil of *A. aculeatum* predominantly contains palmitic and oleic acids (95.5%) and shows high in vitro efficacy against dactylogyrideans obtained from *C. macropomum* gills; this efficacy is dependent on the dose. Similar results were reported by Malheiros et al. (2023) with fixed oil of *Carapa guianensis*, which is mostly composed of 82.1% oleic and palmitic acids, used against dactylogyrideans, and by Hirazawa et al. (2000) with caprylic acid against *H. okamotoi*. In the present study, the dactylogyrideans *A. spathulatus*, *N. janauachensis,* and *M. boegeri* contracted and detached rapidly from the gills when exposed to the highest concentrations of *A. aculeatum* fixed oil, although few ultrastructural alterations, such as shrinkage and a wilted appearance, were observed. Similar behavior was also observed in *Heterobothrium okamotoi* exposed to caprylic acid (Hirazawa et al. 2000) and in *Dactylogyrus* sp. exposed to essential oils from *Mentha piperita*, *Melaleuca alternifolia*, or *Citrus limon* (Yilmaz & Yildiz 2023). The tegument of dactylogyrideans plays essential roles in their parasitic lifestyle, including the absorption and secretion of substances, osmoregulation, mechanical support, and protection against harmful agents (Pimentel-Acosta et al. 2019; Brito et al. 2026a). Consequently, severe damage to the parasites' tegument after antiparasitic treatment can result in physiological compromise, leading to their death and detachment from fish gills (Pimentel-Acosta et al. 2019). Both outcomes are crucial for therapeutic effectiveness because antiparasitic treatments seem to induce osmoregulatory failure by lipophilic intercalation into the tegumental syncytium of these parasites. This destabilizes lipid rafts, driving the allosteric inhibition of Na^+/K^+-ATPase and the disruption of aquaporins. The resulting loss of membrane potential triggers a massive Ca^{+2} ion influx, activating intracellular proteases that degrade the cytoskeleton, causing tegumental blebbing, osmotic shock, and haptor paralysis, interrupting the supply of ATP to the parasites (Zhang et al. 2020; Brito et al. 2026b).

Developing new antiparasitic treatments requires an in-depth understanding of their toxicity to fish and the safe, appropriate management of therapeutics. *Colossoma macropomum* showed tolerance to 20–160 mg/L of *A. aculeatum* oil; no deaths were observed, and the fish exhibited similar behavioral changes at all tested concentrations. Bath treatments with 160 mg/L of *A. aculeatum* fixed oil were 74.4% effective against *A. spathulatus*, *N. janauachensis,* and *M. boegeri* in the gills of *C. macropomum*. Due to the effectiveness of treatments with *A. aculeatum* oil, studies using biomarkers to evaluate the possible toxicity of therapeutic baths for tambaqui are necessary. Similar studies have demonstrated the anthelmintic effectiveness of other phytotherapics, most of which are composed primarily of fatty acids. For example, *C. guianensis* oil was effective against *A. spathulatus*, *N. janauachensis*, and *M. boegeri* in *C. macropomum* (Malheiros et al., 2023), and *Phaeodactylum tricornutum* fatty acid ethyl esters were effective against *G. turnbulli* in *Poecilia reticulata* (Jawaji et al. 2024). Hirazawa et al. (2000) also demonstrated the efficacy of supplementing the diet of *Takifugu rubripes* with 2.5 g/kg of caprylic acid. However, bath treatments with *A. aculeatum* oil SLNs are ineffective against *A. spathulatus, N. janauachensis,* and *M. boegeri* in *C. macropomum* gills (Brito et al. 2026a).

Dactylogyrideans *A. spathulatus*, *N. janauachensis*, and *M. boegeri* are parasites that attach to the gills of *C. macropomum,* causing displacement of the gill epithelium, focal hyperplasia of epithelial cells, lamellar fusion, congestion, shortening of the secondary lamellae of the gills, and a complete fusion of the secondary lamellae (Tavares-Dias et al. 2021). However, the gills of *C. macropomum* treated with 160 mg/L of *A. aculeatum* fixed oil, as well as both control groups, exhibited detachment of the lamellar epithelium and hyperplasia of the epithelium, while only a few fish exposed to fixed oil had aneurysms in gills. These few changes do not affect the functioning of this organ.

Therefore, these results indicated that the bath treatments were not responsible for the lesions in the gills of *C. macropomum*. Similarly, Brito et al. (2026a) found that the detachment of the gill lamellar epithelium, lamellar epithelial hyperplasia, lamellar hyperplasia with fusion, and aneurysms in *C. macropomum* after baths with SLNs containing *A. aculeatum* fixed oil were lesions caused by *A. spathulatus*, *N. janauachensis*, and *M. boegeri*.

Blood parameters are one of the most commonly used methods to evaluate the health and physiological status of fish because they respond quickly to factors such as stress, parasitic infections, and the toxicity of antiparasitic treatments, all of which can affect the hematopoietic system of fish (Malheiros et al. 2023; Ranzani-Paiva & Tavares-Dias 2024; Queiroz et al. 2025). In *C. macropomum*, therapeutic baths with 160 mg/L of *A. aculeatum* fixed oil increased plasma protein and glucose levels, indicating stress. However, control fish exposed to ethyl alcohol exhibited macrocytic-hypochromic anemia and decreased plasma protein levels, whereas treatments with 160 mg/L of *A. aculeatum* fixed oil increased MCV and decreased MCHC. Similarly, bath treatments with 500 mg/L of *C. guianensis* or iso-propyl alcohol (positive control) also increased plasma protein and glucose levels, as well as the total number of red blood cells. In contrast, these treatments decreased MCV in *C. macropomum* (Malheiros et al. 2023). Treatments with SLNs containing *A. aculeatum* fixed oil or myristic acid + Tween 80 (positive control) also decreased the total number of red blood cells in *C. macropomum* (Brito et al. 2026a). These results demonstrate that different solvents may also have harmful physiological effects on fish. On the other hand, bath treatments with essential oils of *Cymbopogon martini* or *Curcuma longa* did not alter the red blood cell parameters of *C. auratus* (Zhou et al. 2022). While examining the toxicity of *A. aculeatum* oil, which is composed of long-chain fatty acids, i.e., linoleic, stearic, palmitic, and oleic acids, we found that it was toxic to *C. macropomum*. To understand how absorption and other pharmacokinetic and pharmacodynamic processes occur, one must first understand their transport mechanisms across biological membranes, which are currently unknown to *A. aculeatum* oil. Jawaji et al. (2024) discussed how long-chain fatty acids appear to be less toxic than short-chain fatty acids because they are less soluble in water, which reduces their ability to penetrate cellular membranes. Nevertheless, the pharmacokinetic and pharmacodynamic processes of *A. aculeatum* fixed oil in fish require further investigation.

In conclusion, although bath treatments with 160 mg/L of *A. aculeatum* fixed oil were highly effective and did not affect the structure of *C. macropomum* gills, they were slightly stressful. Therefore, they should be used sparingly to control parasitic dactylogyrideans and avoid compromising fish physiology.

Author Contribution Bruna David Brito: analysis, design, material preparation, data collection, writing – original draft, writing – review & editing. Victor Hugo Souza Marinho: methodology, analysis, material preparation. Irlon Maciel Ferreira: methodology, analysis, material preparation analysis, design, Marcos Tavares-Dias: Supervision, financing, methodology, writing – review & editing.

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Data availability Clinical trial number: not applicable.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval This study was conducted in accordance with the principles established by the Brazilian College of Animal Experimentation (COBEA) and was approved by the Ethics Committee for Animal Use at Embrapa Amapá (# 026/2023-CEUA/CPAFAP).

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