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Original Article

# Anti-dactylogyridean efficacy of solid lipid nanoparticles of *Pentaclethra macroloba* oleoresin in *Colossoma macropomum*, with hematology and gill histopathology assessment

## Eficácia anti-dactologirídeos de nanopartículas lipídicas sólidas de oleorresina de *Pentaclethra macroloba* em *Colossoma macropomum*, com avaliação hematológica e histopatológica de brânquias

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**Abstract**

This study investigated, for the first time, the efficacy against *Anacanthorus spathulatus*, *Notozothecium janauachensis,* and *Mymarothecium boegeri* of solid lipid nanoparticles (SLNs) containing *Pentaclethra macroloba* oleoresin, and the effects of therapeutic baths on the hematology (glucose, total proteins, and erythrocyte parameters) and gill histopathology of tambaqui *Colossoma macropomum*. The oleoresin used had a predominant composition of monounsaturated fatty acids in their composition, with oleic acid present at a relative abundance of 56.4% and behenic acid present at 15.8%. Tolerance trials with different concentrations of SLNs (500, 750, 1,000, and 2,000 mg/L) showed that the fish could tolerate 500 mg/L. This concentration was used for the five 1-h therapeutic baths per day. Two control groups were also used: one with water from the culture tank and the other with Tween 80 + myristic acid. The therapeutic baths' efficacy against dactylogyridean parasites was similar between the group with SLNs containing 500 mg/L of *P. macroloba* oleoresin and the control group with Tween 80 + myristic acid. The therapeutic baths only increased plasma total protein levels. Histopathological analyses of tambaqui gills showed moderate changes that did not affect respiratory function in fish subjected to baths with SLNs containing *P. macroloba* oleoresin. Although the fish could tolerate 500 mg/L of SLNs with *P. macroloba* oleoresin without experiencing adverse physiological effects, this treatment was therapeutically ineffective. The factors responsible for this ineffectiveness were discussed.

**Keywords:** fish, monogeneans, oil, parasites, phytotherapy, pracaxi, treatment.

**Resumo**

Este estudo investigou, pela primeira vez, a eficácia anti-*Anacanthorus spathulatus*, *Notozothecium janauachensis e Mymarothecium boegeri* de nanopartículas lipídicas sólidas (NLSs) com oleorresina de *Pentaclethra macroloba*, e os efeitos dos banhos terapêuticos na hematologia (glicose, proteínas totais e parâmetros eritrocitários) e histopatologia branquial de tambaqui *Colossoma macropomum*. Oleorresina utilizada apresentou predominância de ácidos graxos monoinsaturados em sua composição, com ácido oleico em maior abundância relativa (56,4%) e ácido behênico com abundância de 15,8%. Ensaios de tolerância com diferentes concentrações de NLSs com oleorresina de *P. macroloba* (500, 750, 1.000 e 2.000 mg/L) mostraram que os peixes toleraram 500 mg/L, e essa concentração tolerada foi utilizada nos cinco banhos terapêuticos de 1 hora por dia. Dois grupos controles também foram utilizados: um com água do tanque de cultivo e outro com Tween 80 + ácido mirístico. A eficácia dos banhos terapêuticos com NLSs contendo 500 mg/L de oleorresina de *P. macroloba* contra parasitas dactilogirídeos foi semelhante ao controle com Tween 80 + ácido mirístico. Banhos terapêuticos resultaram apenas em aumento nos níveis plasmáticos de proteína total, e análises histopatológicas das brânquias de tambaqui mostraram alterações moderadas que não afetaram a função respiratória submetida a banhos com NLSs de oleorresina de *P. macroloba*. Embora os peixes tenham tolerado 500 mg/L de NLSs com oleorresina de *P. macroloba* sem efeitos fisiológicos adversos, esse tratamento não foi terapeuticamente eficaz, e os fatores responsáveis por essa ineficácia foram discutidos.

**Palavras-chave:** peixe, monogenéticos, óleo, parasitas, fitoterapia, pracaxi, tratamento.

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## 1. Introduction

Derivatives of medicinal plants (e.g., oils, extracts, and teas) have long been used in traditional medicine worldwide to treat various diseases. Many drugs have also been developed from the major chemical constituents of these plants (Guimarães et al., 2016; Javid et al., 2020; Sinda et al., 2021). Since the late 1970s, the World Health Organization has emphasized the importance of medicinal plants as valuable therapeutic resources. Currently, phytotherapy is also widely recommended for controlling and treating parasitic diseases affecting fish aquaculture as an alternative to chemotherapeutics that are usually used (Malheiros et al., 2020, 2022, 2023; Tavares-Dias et al., 2021a; Alves et al., 2024). This perspective has motivated the search for species of oils derived from medicinal plants for use in veterinary medicine, as has already occurred for the treatment of several human diseases since ancient times with herbal therapy (Javid et al., 2020; Sinda et al., 2021). The Amazon region is known for its incredible diversity of medicinal plant species (Guimarães et al., 2016), which have therapeutic potential for use in various fish aquaculture sectors due to their pharmacological properties and diverse biological activities (Ribeiro et al., 2025).

*Pentaclethra macroloba* (Willd.) Kuntze, a species of the family Fabaceae, is a medicinal plant from the Amazon region that is popularly known as the "Gavilán tree," "pracaxi," or "pracachy." This Fabaceae species is distributed throughout Brazil, Colombia, Guyana, Peru, Suriname, and Venezuela. It is a predominant Neotropical tree in the Amazon basin, particularly in the floodplain forests of the Amazon River estuary. The production of toxic secondary compounds by *P. macroloba* seeds has been suggested as a factor explaining their dominance in Central American and Amazonian forests. This species is also found in Costa Rica, Cuba, Honduras, Jamaica, Nicaragua, Panama, and Trinidad and Tobago (Costa et al., 2014; Guimarães et al., 2016; Teixeira et al., 2020; Eberhart et al., 2023; Ribeiro et al., 2025). The seeds of the *P. macroloba* tree produce an oleoresin that is used by Amazon riverside populations for different purposes and is of importance for the economy of the region, because oleoresins generally exhibit bioactivity (Teixeira et al., 2020; Ibiapina et al., 2021; Eberhart et al., 2023) and toxicant effects. This oleoresin is used in various industries to produce biofuels, cooking oils, margarines, lubricants, soaps, cosmetics, and medicines due to the presence of different acids in its chemical composition (Oliveira et al., 2013; Costa et al., 2014; Teixeira et al., 2020; Dantas et al., 2024; Ribeiro et al., 2025). It has also been used in clinical medicine to treat dermatological issues, moisturize the skin, and promote cell renewal. Additionally, it is used to treat ulcers, asthma, bronchitis, and inflammation due to its antihemorrhagic and antiproteolytic properties (Teixeira et al., 2020; Huh et al., 2024; Dantas et al., 2024; Ribeiro et al., 2025). These properties are attributed to their bioactive compounds, particularly pectic compounds and long-chain fatty acids.

The seeds of *P. macroloba* are edible and contain 45–48% oil, which is rich in fatty acids (Eberhart et al., 2023). The fruit contains significant amounts of nutrients, including lipids, carbohydrates, and proteins (Ibiapina et al., 2021). The oleoresin extracted from these seeds is generally composed of fatty acids such as oleic, behenic, and linoleic acids, with predominance of oleic acid (Ribeiro et al., 2025). *Pentaclethra macroloba* extracts screened for *in vitro* antibacterial activity have demonstrated efficacy against different species due to their major chemical constituents (Leal et al., 2011; Oliveira et al., 2013; Gioster-Ramos et al., 2023; Ribeiro et al., 2025). However, the oil has failed to inhibit *Staphylococcus aureus* Rosenbach, 1884 growth (Guimarães et al., 2016). However, the oleoresin is insoluble in water, which makes it difficult to use in aquaculture as a parasiticide against fish ectoparasites

Nanotechnology has shown significant potential as an innovative tool for aquaculture (Partridge et al., 2019; Ahmed et al., 2023; Abdelkarim et al., 2025), including combating parasite infections that cause diseases in different animal species (Partridge et al., 2019; Mogahed et al., 2023; Nemati et al., 2024; Abdelkarim et al., 2025). Currently, nanotechnology is being used to improve the efficacy of different drugs by increasing their permeability through the absorptive membranes, which reduces the required dose, because it exerts better therapeutic efficacy with improved selectivity and reduced toxicity (Mogahed et al., 2023; Nemati et al., 2024). For instance, solid lipid nanoparticles (SLNs) are commonly used in human medical and health research (Partridge et al., 2019; Ahmed et al., 2023; Abdelkarim et al., 2025). SLNs are colloidal particles (50-1,000 nm) made from lipids, which are solid at room temperature, and containing surfactants as stabilizers or emulsifiers (Wissing et al., 2004; Partridge et al., 2019; Nemati et al., 2024; Abdelkarim et al., 2025). However, the exact mechanisms by which drugs delivered by in SLNs improve bioavailability remain largely unknown (Partridge et al., 2019), and SLNs have rarely been used to control and treat parasitic infections in fish species (Abdelkarim et al., 2025).

Recently, Brabec et al. (2023) proposed new classes for the Monopisthocotyla and Polyopisthocotyla parasites, thus extinguishing the previously accepted Monogenea class for this taxon of Platyhelminthes worms. Monopisthocotylan parasites cause severe health damage to farmed marine and freshwater fish, consequently harming fish production and negatively affecting the aquaculture industry, resulting in economic losses due to mortality (Tavares-Dias et al., 2021a; Malheiros et al., 2023; Ávila-Castillo et al., 2024; Caña-Bozada et al., 2024; Alves et al., 2024), requiring adequate control. For this reason, studies have indicated the use of oleoresin from *Copaifera reticulata* Ducke (Malheiros et al., 2020, 2022), fixed oil from *Carapa guianensis* (Aublet) Steudel (Malheiros et al., 2023), and essential oil from *Piper callosum* Ruiz & Pav for the control and treatment of infections caused by monopisthocotylan species in tambaqui *Colossoma macropomum* (Cuvier, 1816) (Characiformes: Serrasalmidae). *Anacanthorus spathulatus* Kritsky, Thatcher & Kayton 1979; *Notozothecium janauachensis* Belmont-Jégui, Domingues & Martins 2004, and *Mymarothecium boegeri* Kritsky, Thatcher & Kayton 1979 (Monopisthocotyla: Dactylogyridae) are parasites that frequently infect the gills of tambaqui, a native fish to the Amazon River basin. However, *Linguadactyloides brinkmanni*

Thatcher & Krytsky, 1983, has also occasionally been reported infecting *C. macropomum* (Tavares-Dias et al., 2021a, b; Malheiros et al., 2023; Alves et al., 2024). Many species of these ectoparasites feed on gill mucus and/or blood, which causes damage to the delicate gill lamellae, possibly due to suction or the application of digestive secretions (Tavares-Dias et al., 2021b; Malheiros et al., 2023; Ávila-Castillo et al., 2024).

Since *P. macroloba* oleoresin is insoluble in water, making its use in antiparasitic baths in fish aquaculture difficult, it is necessary to increase its solubility. Thus, this study aimed to investigate the efficacy of SLNs with *P. macroloba* oleoresin against dactylogyridean parasites of *C. macroppomum* gills, as well as evaluate its effects on blood and histopathological parameters.

## 2. Materials and Methods

### *2.1. Obtaining P. macroloba oleoresin*

The *P. macroloba* oleoresin was supplied by the Kamukaia Project from Embrapa Amapá, state of Amapá, Brazil. The project aimed to improve the quality and production of this oleoresin among riverside communities in the state of Amapá.

### *2.2. Composition analysis of fatty acids in Pentaclethra macroloba oleoresin*

An aliquot of *P. macroloba* oleoresin was analyzed for its fatty acid composition and converted into fatty acid methyl esters (FAMEs) using the methodology described by Hartman and Lago (1973). After extraction, the materials were analyzed using a gas chromatograph equipped with a flame ionization detector (GC2010 Plus, Shimadzu, Kyoto, Japan) and a stationary phase biscianopropyl-polydimethylsiloxane capillary column (SP2560, 100 m × 0.25 mm, df 0.20, Supelco®, Bellefonte, PA, USA). The oven temperature of the column was as follows: the initial temperature was maintained at 80°C, then increased to 180 °C at 11 °C/min, and finally to 220 °C at 5 °C/min, and then held for 23 min. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min. The split ratio was 1:30, and the injector and detector temperatures were 220 °C. The FAMEs were identified by comparing their retention times with those of a previously injected fatty acid standard mix (code CRM47885, Supelco®, Bellefonte, PA, USA) using the same methodology. The contribution of each compound to the mixture was determined by the relative area (%) of its respective peak in the chromatogram (FAME, Supelco®, Bellefonte, PA, USA).

### *2.3. Preparation of solid lipid nanoparticles with Pentaclethra macroloba oleoresin*

Solid lipid nanoparticles (SLNs) with *P. macroloba* oleoresin were prepared according to the methodology described by Oliveira et al. (2022), with a few modifications. The aqueous phase consisted of the surfactant Tween 80 (5% by weight), which was dissolved in distilled water (89% by weight). The lipid phase consisted of the co-surfactant myristic acid (1% by weight) dissolved in *P. macroloba* oleoresin (5% by weight) at 65 ºC for 45 min. Subsequently, the lipid and aqueous phases were mixed and stirred in a vortex for 10 min, and then final concentrations of *P. macroloba* oleoresin were prepared according to the desired concentrations.

Polysorbate 80 (Tween 80®) and myristic acid were purchased from Sigma-Aldrich (São Paulo, Brazil).

### *2.4. Analyses of particle size, PDI and zeta potential of the solid lipid nanoparticles*

The droplet size, polydispersity index and zeta potential of the SLNs with *P. macroloba* oleoresin were determined using a ZS zetasizer (Malvern, United Kingdom). Each sample was diluted with distilled water (1:10 g ratio) to avoid multiple scattering effects, in accordance with Oliveira et al. (2022). All analyses were performed at 25 ºC. Measurements were taken in triplicate, and the mean droplet size was expressed as the mean diameter.

### *2.5. Fish acclimatization, and parasites*

Fingerlings of *C. macropomum* weighing approximately 10 g were obtained from a commercial fish farm in Macapá, state of Amapá, Brazil. They were kept in a 500-L tank with constant aeration and continuous water flow at the Aquaculture and Fisheries Laboratory of Embrapa Amapá in Macapá (Brazil). Fingerlings were fed ad libitum four times a day with a commercial ration containing 32% crude protein (Guabi®, São Paulo, Brazil). The fish were naturally parasitized by dactylogyridean monopisthocotylans and were used in all trials of this study.

The tank was siphoned weekly to remove the accumulated organic matter from the bottom. The following water quality parameters were monitored every two days using a multiparameter probe (YSI, USA): temperature (29.9 ± 0.2 °C), dissolved oxygen (5.6 ± 0.2 mg/L), pH (5.7 ± 0.1), ammonia (0.4 ± 0.2 mg/L), alkalinity (10.0 ± 0.001 mg/L), and hardness (10.0 ± 0 mg/L).

This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and was authorized by the Embrapa Amapá Ethics Committee for the Use of Animals (Protocol No. 013- CEUA-Embrapa Amapá).

### *2.6. Tolerance of C. macropomum to different concentrations of SLNs with P. macroloba oleoresin*

Tolerance tests were performed using different concentrations of SLNs with *P. macroloba* oleoresin (500; 750; 1,000, and 2,000 mg/L) for *C. macropomum* baths (18.2 ± 2.6 cm and 97.1 ± 29.9 g). Each concentration of SLNs with *P. macroloba* oleoresin was prepared as previously described. To evaluate fish tolerance to each concentration of SLNs with *P. macroloba* oleoresin, five fish per replicate were used (three replicates), for a total of 15 fish per treatment, distributed in 100-L tanks. During the evaluation, we analyzed the fish's behavior and mortality during 3 h exposure to the tested concentrations.

### *2.7. Therapeutic baths of C. macropomum using SLNs with P. macroloba oleoresin*

A total of 117 *C. macropomum* specimens (17.8 ± 1.2 cm and 70.5 ± 15.5 g) were randomly distributed into 100-L

tanks for therapeutic baths with SLNs of *P. macroloba* oleoresin aimed to evaluate the anti-dactylogyridean efficacy on the gills of this host fish. The experimental design of the baths consisted of three treatments, each with three replicates of 10 fish (30 fish per treatment). During all therapeutic baths, the tanks were aerated constantly, and the continuous water flow was shut off. The treatments were as follows: one with 500 mg/L of SLNs containing *P. macroloba* oleoresin, and two control groups (one with water from the culture tank, and one with Tween 80 + myristic acid). The solution of 500 mg/L of SLNs with *P. macroloba* oleoresin was prepared as previously described. The fish underwent therapeutic baths for 1 h daily for five consecutive days. After the baths, the water in each tank was completely replaced and kept under continuous flow until the next bath. All baths were carried out in the morning, and the fish were fed *ad libitum* in the afternoon.

After five days of therapeutic bathing, the fish in each treatment group were euthanized by medullary section to collect their gills. The gills were then fixed in 5% formalin to quantify and identify the parasites (Eiras et al., 2006). After quantifying the parasites, we calculated the prevalence and mean abundance (Bush et al., 1997) and the effectiveness of the therapeutic baths (Wang et al., 2008).

### *2.8. Blood parameters of C. macropomum after therapeutic baths using SLNs with P. macroloba oleoresin*

After five therapeutic baths with SLNs containing 500 mg/L of *P. macroloba*, five fish from each replicate (15 fish per treatment) were collected for evaluation of blood parameters. One blood sample was collected from each fish by puncturing the caudal vein with a 3 mL syringe containing 10% EDTA. Blood was used to determine the hematocrit (Hct) using the microhematocrit method, the total erythrocyte number (RBC) using a Neubauer chamber, and the hemoglobin concentration ([Hb]) using the cyanmethemoglobin method. Hematimetric indices, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were calculated using Hct, RBC, and [Hb] values.

The remaining blood was centrifuged at 75 G (Centrifuge MCD-2000, Brazil) to obtain the blood plasma, which was used to determine glucose levels using the enzymatic-colorimetric glucose oxidase method and total protein levels using the biuret method with commercial kits (Doles, GO, Brazil) and a UV/visible spectrophotometer (Model K37-VS, Kasvi, Brazil) for these analyses.

### *2.9. Histopathology of C. macropomum gills after therapeutic baths using SLNs with P. macroloba oleoresin*

After the five therapeutic baths with SLNs of *P. macroloba* oleoresin, three fish from each replicate (9 fish per treatment) were euthanized by medullary section. A gill arch was then removed from each side (right and left) for histopathological analysis. All 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 100% xylene, impregnated, and embedded in paraffin to obtain blocks. The paraffin blocks were cut using a microtome (Leica DM 1000) to a thickness of 5 µm. After preparing duplicate slides, the gill arches were stained with hematoxylin and eosin (Behmer et al., 1976). Images were captured with a digital camera (Moticam 2300 3.0 M Pixel) attached to a common optical microscope connected to a computer.

Alterations were evaluated semiquantitatively by ranking the severity of gill lesions. Ranking was evaluated as follows: grade 1 = no pathological alterations, grade 2 = focal mild to moderate changes, and grade 3 = extensive severe pathological alterations. This ranking was used to determine an overall assessment value of histopathological gill lesions in each fish. Based on these data, the mean assessment value (MAV) of gill lesions was calculated for each treatment group (Schwaiger et al., 1997). The histopathological alteration index (HAI) was calculated as follows. The gills of each fish submitted to histopathological analyses were examined using a calculation based on the degree of change observed in each gill. The scale of values is used to associate the effects of the damages as follows: values of 0–10 indicate normal function of fish gills; values of 11–20 indicate slightly to moderately damaged gills; 11–50 indicate moderate to severe damage. When the values are greater than 100, the gill damage is irreparable (Poleksić and Mitrović-Tutundžić, 1994).

### *2.10. Statistical analysis*

The abundance of parasites, blood, 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, the Kruskal-Walli's test was used. The Dunn test was applied to compare the differences between the medians (Zar, 2010), with IBM SPSS Statistics 24.0 software, and a statistical difference was accepted at $p< 0.05$.

## 3. Results

### *3.1. Chemical composition of the P. macroloba oleoresin*

The gas chromatographic analysis revealed that *P. macroloba* oleoresin is predominantly composed of monounsaturated fatty acids, with oleic acid (C18:1. ʊ-9) accounting for 56.4% relative abundance, and polyunsaturated fatty acids, such as linoleic acid (C18:2. ʊ-6), accounting for 11.3%. Saturated fatty acids were also identified, including behenic acid (C22:0), which had a relative abundance of 15.8% and lignoceric acid (C24:0) with a relative abundance of 9.4%. Lower abundances of other saturated fatty acids were present in the oleoresin, such as palmitic acid (C16:0) and stearic acid (C18:0) (Table 1).

### *3.2. Physicochemical characteristics of the SLNs with P. macroloba oleoresin*

The SLNs of *P. macroloba* oleoresin were light in color, monodisperse, and had a particle size of 390.9 ± 1.7 nm. They also had a PDI value of 0.311 and a zeta potential of -42.8 ± 0.7 mV.

### 3.3. Tolerance of *C. macropomum* to different concentrations of SLNs with *P. macroloba* oleoresin

During tolerance trials with *C. macropomum*, behavioral changes were observed when exposed to 750; 1,000, or 2,000 mg/L of SLNs of *P. macroloba* oleoresin. However, these changes were not observed in fish exposed to 500 mg/L of SLNs of *P. macroloba* oleoresin. While these high concentrations of SLNs with *P. macroloba* oleoresin provoked behavioral changes in the fish, they did not cause death in any of the treatments during the entire exposure period (Table 2). After exposure, the fish were actively swimming.

**Table 1.** Fatty acid composition of the *Pentaclethra macroloba* oleoresin.

| Fatty acids | Relative concentration (%) |
|---|---|
| Palmitic (C16:0) | 1.5 ± 0.2 |
| Stearic (C18:0) | 2.6 ± 0.7 |
| Oleic (C18:1 n9) | 56.4 ± 0.5 |
| Linoleic (C18:2) | 11.3 ± 0.2 |
| Arachidic (C20:0) | 1.1 ± 0.1 |
| Linolenic (18:3) | 1.2 ± 0.1 |
| Behenic (22:0) | 15.8 ± 0.4 |
| Lignoceric (C24:0) | 9.4 ± 0.6 |
| Total identified compounds | 99.3 |
| Total saturated | 30.4 |
| Total monounsaturated | 56.4 |
| Total polyunsaturated | 12.5 |

### 3.4. Therapeutic baths against parasites in *C. macropomum* gills with SLNs of *P. macroloba* oleoresin

No fish died during the 5 days of therapeutic baths with SLNs of *P. macroloba* oleoresin. The ectoparasites on the gills of *C. macropomum* were identified as dactylogyrideans *A. spathulatus*, *N. janauachensis*, and *M. boegeri*. The gills of the control group fish exposed to water from the culture tank showed a 100% prevalence of these ectoparasites. In contrast, the prevalence was low among fish exposed to Tween 80 + myristic acid. These parasites were not found on the gills of fish subjected to five baths with 500 mg/L of SLNs containing *P. macroloba* oleoresin. The mean abundance of dactylogyridean parasites was higher in the control group fish exposed to the culture tank water than in fish exposed to Tween 80 + myristic acid or SLNs of *P. macroloba* oleoresin. Fish exposed to Tween 80 + myristic acid or SLNs of *P. macroloba* oleoresin exhibited high, similar therapeutic efficacy (Table 3), indicating the potent anthelmintic action of these surfactants.

### 3.5. Blood parameters of *C. macropomum* after therapeutic baths with SLNs of *P. macroloba* oleoresin

In fish subjected to five therapeutic baths with 500 mg/L of SLNs with *P. macroloba* oleoresin, plasma total protein levels increased ($p<0.05$) compared to the control groups (culture tank water and Tween 80 + myristic acid). However, there were no significant differences ($p>0.05$) in plasma levels of glucose, hemoglobin, hematocrit, total number of erythrocytes, MVC, and MCHC between these treatments (Table 4).

**Table 2.** Tolerance of *Colossoma macropomum* after 3 h of exposure to different concentrations of solid lipid nanoparticles containing *Pentaclethra macroloba* oleoresin.

| Concentration | N | Mortality (%) | Behavioral alterations |
|---|---|---|---|
| 500 mg/L | 15 | 0 | No alteration in behavior |
| 750 mg/L | 15 | 0 | Agitation, swimming on the surface of the tank searching for air, increased opercular beats, and erratic swimming |
| 1,000 mg/L | 15 | 0 | Agitation, jumping out of the water, increased opercular beats, swimming to the tank surface for air, erratic swimming, and sinking to the bottom of the tank |
| 2,000 mg/L | 15 | 0 | Agitation, jumping out of the water, increased opercular beats, swimming to the tank surface for air, erratic swimming, and sinking to the bottom of the tank |

N: Sample number

**Table 3.** Parasitological indices of dactylogyridean parasites in *Colossoma macropomum* gills after therapeutic baths with solid lipid nanoparticles containing *Pentaclethra macroloba* oleoresin, and therapeutic efficacy.

| Treatments | N | Prevalence (%) | Mean abundance | Efficacy (%) |
|---|---|---|---|---|
| Culture tank water | 30 | 100 | $19.9 \pm 13.8^{a}$ | - |
| Tween 80 + myristic acid | 30 | 13.3 | $0.3 \pm 0.9^{b}$ | 98.3 |
| 500 mg/L | 30 | 0 | $0^{b}$ | 100 |

Data are expressed as the mean ± standard deviation. Different letters, in the same column, indicate differences by the Dunn test ($p<0.05$). N: Sample number.

**Table 4.** Blood parameters of *Colossoma macropomum* after five therapeutic baths with solid lipid nanoparticles containing *Pentaclethra macroloba* oleoresin.

| Parameters | Water (N = 15) | Tween 80 + myristic acid (N = 15) | 500 mg/L (N = 15) |
|---|---|---|---|
| Glucose (mg/dL) | 72.3 ± 13.2[a] | 87.2 ± 26.41[a] | 84.7 ± 11.15[a] |
| Total protein (g/dL) | 2.8 ± 0.54[a,c] | 2.7 ± 0.36[c] | 6.0 ± 1.1[b] |
| Hemoglobin (g/dL) | 6.2 ± 0.56[a] | 6.2 ± 1.42[a] | 5.7 ± 0.93[a] |
| Hematocrit (%) | 25.8 ± 2.1[a] | 26.1 ± 3.2[a] | 25.0 ± 2.6[a] |
| Erythrocytes (x$10^6$/µL) | 2.8 ± 0.34[a] | 2.9 ± 0.43[a] | 2.9 ± 0.35[a] |
| MCV (fL) | 93.2 ± 13.7[a] | 93.8 ± 18.1[a] | 90.6 ± 12.1[a] |
| MCHC (g/dL) | 23.9 ± 1.90[a] | 23.2 ± 4.18[a] | 22.1 ± 3.8[a] |

Data are expressed as the mean ± standard deviation. Different letters, in the same row, indicate differences by the Dunn test ($p<0.05$). N: Sample number. MCV: Mean Corpuscular Volume. MCHC: Mean Corpuscular Hemoglobin Concentration.

**Table 5.** Histopathological alteration index (HAI) and mean assessment values (MAV) for gills of *Colossoma macropomum* after therapeutic baths with solid lipid nanoparticles containing *Pentaclethra macroloba* oleoresin.

| Treatments | N | MAV | HAI | Severity of lesions according to HAI |
|---|---|---|---|---|
| Culture tank water | 9 | 11.0 ± 2.3[a] | 11.4 ± 2.3[a] | Mild to moderate |
| Tween 80® + myristic acid | 9 | 12.2 ± 1.6[a] | 19.4 ± 5.4[a] | Mild to moderate |
| 500 mg/L | 9 | 10.4 ± 3.2[a] | 17.0 ± 5.5[a] | Mild to moderate |

Data are expressed as the mean ± standard deviation. Different letters, in the same column, indicate differences by the Dunn test ($p<0.05$). N: Sample number.

### *3.6. Histopathology of C. macropomum gills after therapeutic baths with SLNs of P. macroloba oleoresin*

Analyses of gill histopathology revealed mid damage in fish subjected to therapeutic baths with 500 mg/L of SLNs containing *P. macroloba* oleoresin, as well as in fish from both control groups. However, these damages did not affect gill function, as indicated by the histopathological alteration index (HAI) values of the three experimental groups. Furthermore, there were no significant differences ($p>0.05$) in mean assessment values (MAV) between fish subjected to baths with *P. macroloba* oleoresin and the two control groups (Table 5). The main histopathological alterations in the gills of fish exposed to 500 mg/L of SLNs containing *P. macroloba* oleoresin were hyperplasia, aneurysms, hypertrophy, epithelial detachment, and total or partial fusion of secondary lamellae. In the gills of control fish exposed to water from the culture tank, we observed hyperplasia with fusion of secondary lamellae, aneurysms, epithelial detachment, and hypertrophy. In the gills of control fish exposed to Tween 80 + myristic acid, alterations included hyperplasia with fusion of secondary lamellae, epithelial detachment, and partial fusion of secondary lamellae (Figure 1).

## 4. Discussion

Amazonian vegetable oils are a source of essential fatty acids and liposoluble bioactive compounds, with increasing demand for these oils as an industrial alternative to other phytotherapy resources. Fatty acids are usually categorized as saturated, monounsaturated, or polyunsaturated (Huh et al., 2024), and it is well known that oleoresins are rich in monounsaturated fatty acids, as well as diverse fatty acids (Teixeira et al., 2020; Eberhart et al., 2023; Ribeiro et al., 2025), accounting for over 90% of their composition (Teixeira et al., 2020; Eberhart et al., 2023). In the analyzed *P. macroloba* oleoresin, we identified a fatty acid composition of 99.3%, with a predominance of saturated (30.4%) and monounsaturated (56.4%) fatty acids, which are constituted by oleic (56.4%), behenic (15.8%), linoleic (11.3%), and lignoceric acids (9.4%). Palmitic acid (1.5%), stearic acid (2.6%), and arachidic acid (1.1%) were present in lower amounts. Similarly, Costa et al. (2014) and Teixeira et al. (2020) reported that the major constituents of *P. macroloba* oleoresin were oleic (53.5%), behenic (16.1%), linolenic (13.0%), and lignoceric (10.4%) acids. (2020). However, Costa et al. (2014) also identified lower percentages of arachidic acid (1%), lauric acid (0.03%), myristic acid (0.06%), linolenic acid (0.12%), and palmitic acid (1.8%). Nevertheless, lauric and myristic acids were not identified in the *P. macroloba* oleoresin in the present study. Conversely, Ribeiro et al. (2025) reported higher levels of oleic acid (65.2%) and behenic acid (17.2%), and lower levels of linoleic acid (8.45%) than in the present study. Differences in the fatty acid composition of *P. macroloba* oleoresin usually may be influenced by factors such as the extraction method and the complex interactions between genetic and environmental factors, leading to different responses among Amazonian plant populations to environmental conditions (Teixeira et al., 2020; Eberhart et al., 2023; Huh et al., 2024; Ribeiro et al., 2025).

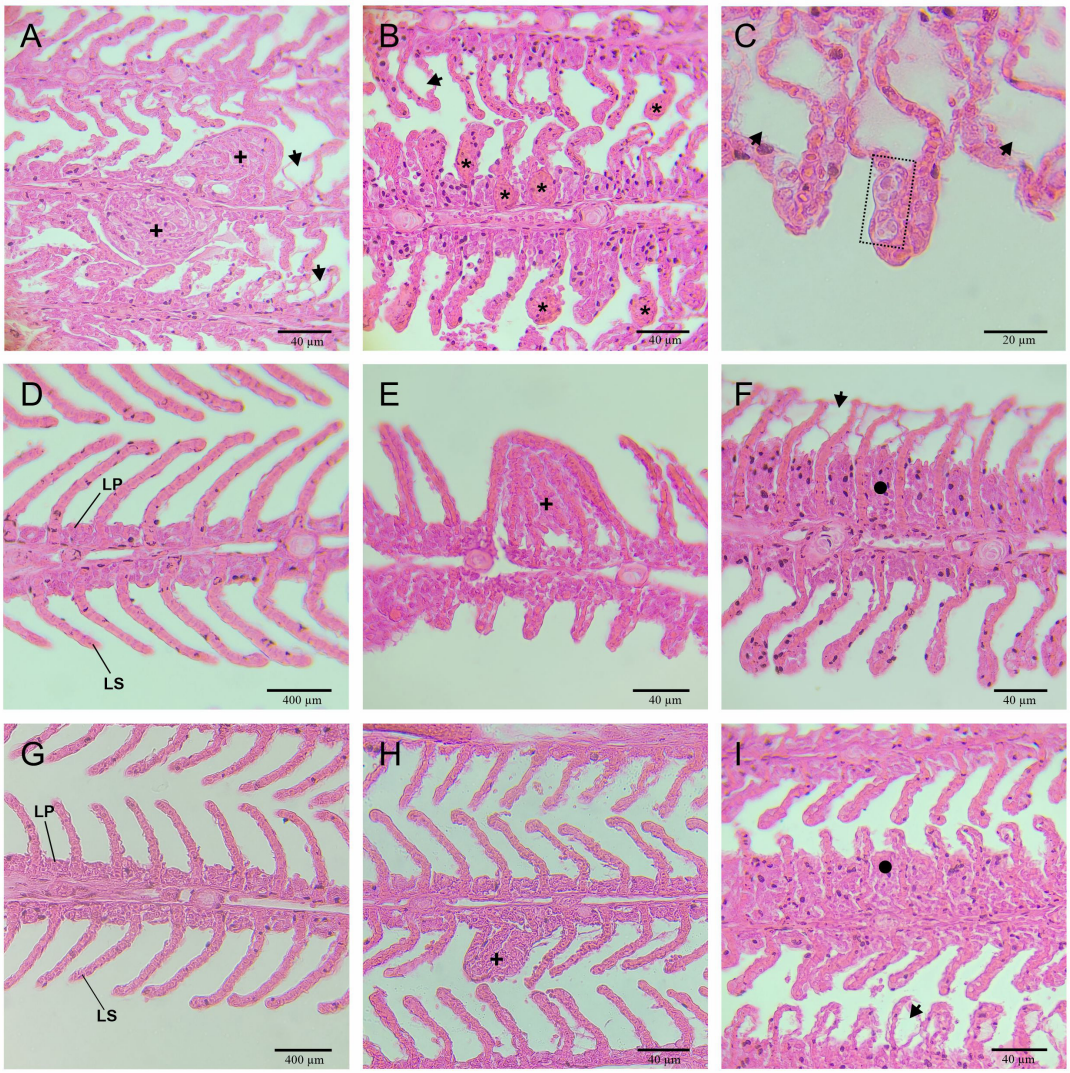


**Figure 1.** Histopathology of the *Colossoma macropomum* gills. A-C: Control fish exposed to water from the culture tank. D-F: Control fish exposed to Tween 80 + myristic acid. G-I: Fish submitted to therapeutic baths with 500 mg/L of solid lipid nanoparticles containing *Pentaclethra macroloba* oleoresin. **LP**: Primary lamellar epithelium. **LS**: Secondary lamellar epithelium. **✚**: Hyperplasia with fusion of the secondary lamellar epithelium. **●**: Hyperplasia with partial fusion of the secondary lamellar epithelium. Arrow: Aneurysm. Dotted rectangle: Hypertrophy. *: Detachment of the lamellar epithelium. Coloration: Hematoxylin and Eosin (HE).

The SLNs with a solid lipid core and surfactants, and/or co-surfactants as stabilizers are widely used nanoformulations. They have many advantages, such as biocompatibility and biodegradability. They also improve therapeutic efficacy due to their ability to easily disperse into the cell membranes of targeted organisms (Wissing et al., 2004; Nemati et al., 2024; Abdelkarim et al., 2025). For nanoformulations, the zeta potential is a significantly important parameter because it relates to the surface potential of the droplets, and high values are associated with kinetically stable systems. In addition, the polydispersity index is a parameter associated with the homogeneity of particle populations, with desirable values within the range of 0 to 0.500 (monodispersed and relatively broad distributions, respectively), while acceptable polydispersity index values are below 0.700 (Wissing et al., 2004; Oliveira et al., 2022). Therefore, the SLNs of *P. macroloba oleoresin* oil had a zeta potential, polydispersity index, and particle size indicating a relatively narrow particle size distribution, kinetic stability, and satisfactory size for this nanoformulation (Oliveira et al., 2022).

Since phytotherapeutics may be toxic to fish, their toxicity can be evaluated using tolerance tests, observation of clinical signs, and assessment of adverse physiological and histological effects of exposure (Tavares-Dias, 2018; Malheiros et al., 2023; Alves et al., 2024). These procedures are important for predicting the toxic effects of phytotherapeutics on fish (Tavares-Dias, 2018;

Eberhart et al., 2023; Malheiros et al., 2023; Alves et al., 2024). In this first study on the exposure of *C. macropomum* to 500, 750, 1,000, or 2,000 mg/L of SLNs containing *P. macroloba* oleoresin, no mortality occurred during or after treatment. However, *C. macropomum* tolerated 500 mg/L of SLNs of *P. macroloba* oleoresin better than higher concentrations because the latter provoked behavioral alterations that were not observed in fish exposed to the former. Exposure to the highest concentrations of SLNs containing *P. macroloba* oleoresin caused agitation in these fish, including jumping out of the water, increased opercular beats, swimming to the surface of the tanks for air, erratic swimming, and falling to the bottom of the tanks. Similar behavioral changes have been reported in *C. macropomum* exposed to 50–250 mg/L of *C. reticulata* oleoresin (Malheiros et al., 2020), 800–1,000 mg/L of *C. guianensis* fixed oil (Malheiros et al., 2023), and 100 mg/L of essential oil of *Piper hispidum* Swartz (Alves et al., 2024). However, Wistar rats orally exposed to *P. macroloba* oleoresin showed no changes in the clinical signs investigated (Eberhart et al., 2023).

Five daily therapeutic baths with SLNs containing 500 mg/L of *P. macroloba* oleoresin did not demonstrate efficacy against *A. spathulatus*, *M. boegeri*, or *N. janauachensis* on *C. macropomum* gills, because the results were similar to the control with Tween 80 + myristic acid. In contrast, antibacterial activity of *P. macroloba* extracts has been reported due to their major chemical constituents (Leal et al., 2011; Oliveira et al., 2013; Gioster-Ramos et al., 2023; Ribeiro et al., 2025). Since oils, especially oleoresins like the *P. macroloba* used here, are insoluble in water, different solvents or surfactants have been used to improve their solubility, but these may also exhibit varied anthelmintic activity (Tavares-Dias, 2018). Isopropanol, when used as a solvent for *C. guianensis* fixed oil, showed anti-dactylogyridean effects, though the efficacy was low at 33.2% (Malheiros et al., 2023). Likewise, 70% ethanol, when used as a solvent for *P. hispidum* essential oil, achieved an efficacy of 12.7% (Alves et al., 2024). Tween 80, when used as a solvent for *Melaleuca alternifolia* (Maiden & Betche) Cheel oil, exhibited parasiticidal activity when used alone against *Gyrodactylus* spp. in *Gasterosteus aculeatus* Linnaeus, 1758, thus increasing the oil's effectiveness (Steverding et al., 2005). Therefore, these contrasting results are likely due to the solvents and their concentrations used. Notably, the anti-dactylogyridean efficacy of SLNs containing 500 mg/L of *P. macroloba* in this study was increased due to myristic acid, which also possesses pharmacological properties, including antibacterial, antifungal, antiviral, and antiparasitic activity (Rehmana et al., 2017; Javid et al., 2020). Therefore, the anthelmintic activity of myristic acid requires further investigation.

In fish aquaculture, evaluating the health of relies heavily on the evaluation of physiological parameters, which provide valuable information about the oxygen-carrying capacity, metabolic processes, nutritional well-being, and overall condition after treatments with phytotherapics (Malheiros et al., 2022, 2023; Madhulika et al., 2024; Alves et al., 2024). Additionally, total plasma protein levels are one of the most common and useful blood parameters for determining the health status of farmed fish (Malheiros et al., 2022, 2023; Alves et al., 2024). Proteins perform various functions such as maintaining osmotic pressure and pH, transporting metabolites, and interacting with the immune system. They play a key role in fish humoral immunity and the innate immune response. Therefore, total plasma levels of protein levels depend on intracellular mechanisms and specific proteins, they may be significantly affected by various types of stressors that affect fish (Malheiros et al., 2022; Madhulika et al., 2024; Alves et al., 2024), including exposure to phytotherapeutics. In *C. macropomum*, five therapeutic baths with 500 mg/L of SLNs containing *P. macroloba* oleoresin increased the total plasma protein levels. Similarly, increases in plasma levels of total protein were reported in *C. macropomum* subjected to therapeutic baths with 100 mg/L of *C. reticulata* oleoresin and 250 mg/L of nanoemulsion with this oleoresin (Malheiros et al, 2022) as well as with 500 mg/L of *C. guianensis* fixed oil (Malheiros et al, 2022). However, therapeutic baths with 100 mg/L of *P. hispidum* essential oil did not affect the plasma levels of total proteins in *C. macropomum* exposed (Alves et al., 2024).

Piscine gills play a fundamental role in the respiratory process and other systems of these animals. They protect the filaments of this organ and absorb oxygen dissolved in water, thus allowing a constant flow of this gas through the respiratory epithelium. Gills also enable the elimination of carbon dioxide from the blood, maintaining acid-base equilibrium in fish species (Schwaiger et al., 1997; Tavares-Dias et al., 2021b; Malheiros et al., 2023; Alves et al., 2024), and respond to dactylogyridean parasites (Tavares-Dias et al., 2021b; Ávila-Castillo et al., 2024). Therapeutic baths with SLNs containing 500 mg/L of *P. macroloba* oleoresin caused moderate damage to the gills of *C. macropomum*, similar to the control group exposed to water from the culture tank or Tween 80 + myristic acid. This indicates that these treatments were not the cause of the observed gill alterations. Hyperplasia, aneurysm, hypertrophy, epithelial detachment, and total and partial lamellar fusion of the secondary filaments were observed in the gills of fish from three treatments. These gill damages in *C. macropomum* were therefore caused by dactylogyridean parasites, because the damages were similar to those reported by Tavares-Dias et al. (2021b) for the same fish species naturally infected by *A. spathulatus*, *M. boegeri,* and *N. janauachensis*. Ávila-Castillo et al. (2024) also demonstrated similar damage caused by dactylogyridean parasites (*Cichlidogyrus bifurcatus* Paperna, 1960; *Cichlidogyrus dossoui* Douëllou, 1993; *Cichlidogyrus halli* (Price & Kirk, 1967) Paperna, 1979; *Cichlidogyrus haplochromii* Paperna & Thurston, 1969; *Cichlidogyrus longicornis* Paperna & Thurston, 1969; *Cichlidogyrus sclerosus* Paperna & Thurston, 1969; *Cichlidogyrus thurstonae* Ergens, 1981; *Cichlidogyrus tilapiae* Paperna, 1960 and *Cichlidogyrus* sp.) in the gills of *Oreochromis niloticus* (Linnaeus, 1758). Histological studies of the livers and kidneys of Wistar rats exposed to *P. macroloba* oleoresin administered orally reported mild alterations due to toxicity as the dose of oleoresin increased (Eberhart et al., 2023). Conversely, therapeutic baths with 100 mg/L of *P. hispidum* essential oil caused moderate tissue alterations to the gills of *C. macropomum* that did not compromise

their function (Alves et al., 2024). Our results are valuable because there is currently no information on the toxicity or parasiticide potential of *P. macroloba* oleoresin in fish or nanoformulation containing this oleoresin.

In conclusion, five baths with 500 mg/L of SLNs containing *P. macroloba* oleoresin were well tolerated by *C. macropomum* without damaging their gills or affecting their physiology. However, these baths were ineffective against dactylogyridean parasites. *Pentaclethra macroloba* oleoresin likely has anthelmintic properties, although we were unable to demonstrate this due to the difficulty of diluting this oleoresin in water. Therefore, further studies are required to evaluate the anti-dactylogyridean potential of *P. macroloba* oleoresin, which could be achieved by using different nanoformulations, such as nanoemulsions.

## Data Availability Statement

All data from this study are available upon request.

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