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Zingiber officinale (Ginger), *Curcuma longa* (Turmeric) and *Cymbopogon nardus* (Lemon Grass) Essential Oils Have Anti-Dactylogyrideans Potential in *Colossoma macropomum* Cuvier 1816 (Tambaqui)

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ABSTRACT

Essential oils are composed of secondary metabolites derived from medicinal plants and have bioactive properties, such as antiparasitic activity. This study investigated the in vitro anti-dactylogyridean efficacy of the *Zingiber officinale*, *Curcuma longa* and *Cymbopogon nardus* essential oils, as well as the acute toxicity for *Colossoma macropomum* (tambaqui). The majority chemical components of the essential oils of *Z. officinale* (α-zingiberene, β-sesquiphellandrene, limonene and geranial), *C. longa* (ar-turmerone, α-turmerone, α-phellandrene, curlone and 1,8-cineole) and *C. nardus* (geraniol, geranial and neral) were analysed. All the essential oils exhibited dose-dependent efficacy against dactylogyrideans *Anacanthorus spathulatus*, *Notozothecium janauachensis* and *Mymarothecium boegeri*, and the mean effective concentration (EC_{50}) was 16.6 mg L^{-1} (3 h and 30 min) for *Z. officinale* essential oil, 30.9 mg L^{-1} (2 h and 15 min) for *C. longa* essential oil and 13.7 mg L^{-1} (30 min) for *C. nardus* essential oil. Effects of these oils on the ultrastructure of Anacanthorus dactylogyrideans exposed to the essential oils were also evaluated using scanning electron microscopy. This study shows for the first time the potential use of the *Z. officinale*, *C. longa* and *C. nardus* essential oils in controlling dactylogyridean parasites.

1 | Introduction

Aquaculture is the world's fastest growing food-producing sector, contributing most to food and nutrition security for the growing human population (FAO 2024). However, intensifying fish cultivation practices to increase production has also led to the emergence of parasitic fish diseases globally. These diseases are caused by monopisthocotylan and polyopisthocotylan parasites, which result in massive fish mortality and significant economic losses (Trasviña-Moreno et al. 2019; Alves et al. 2021; Mladineo et al. 2021; Zhou et al. 2021, 2022; Attia et al. 2022; Ávila-Castillo et al. 2024; Caña-Bozada et al. 2024). Therefore, in order to continue to develop and progress, the fish aquaculture industry must control such infections and prevent disease outbreaks. However, strategies for combating these parasites have focused on reducing financial losses, and practical solutions have mostly consisted of the use of short-term chemotherapeutant treatments targeting the direct elimination of these parasites or reducing their transmission.

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In tropical regions such as the Amazon, dactylogyrideans *Anacanthorus spathulatus* Kritsky, Thatcher & Kayton 1979; *Notozothecium janauachensis* Belmont-Jégui, Domingues & Martins 2004 and *Mymarothecium boegeri* Cohen & Kohn 2005 (Dactylogyridae) complete their direct life cycle rapidly, with direct transmission between host fish. This favours rapid dissemination in the intensive cultivation (Brazenor et al. 2020) of tambaqui *Colossoma macropomum* (Cuvier 1816) (Soares et al. 2017; Alves et al. 2021; Luz et al. 2021; Tavares-Dias et al. 2021). This Serrasalmidae fish species from the Amazon basin is of great economic importance to freshwater aquaculture in South American countries such as Brazil, Colombia, Peru and Venezuela. Recently, it has also been introduced to Central American countries, the United States and some Asian countries (China, the Philippines and Thailand) due to its excellent zootechnical attributes (FAO 2024; Santana et al. 2024; Cerqueira et al. 2025; PeixeBR 2025). However, infections caused by these ectoparasites cause severe alterations to *C. macropomum* gill filaments (Tavares-Dias et al. 2021). Controlling such gill ectoparasites in *C. macropomum* using chemotherapeutants (e.g., formalin, copper sulphate, potassium permanganate, praziquantel, albendazole, ivermectin, organophosphates and levamisole) is complex and difficult. Some are ineffective, negatively impact water quality, and are toxic to host fish. Furthermore, they have not been legalized (Hashimoto et al. 2016; Alves et al. 2021; Zhou et al. 2021, 2022), thus restricting their use. Therefore, using therapeutics that are more sustainable, appropriate and effective is crucial for *C. macropomum* aquaculture.

Recently, essential oils have attracted increased interest as an alternative to chemotherapeutants for controlling these ectoparasites in fish aquaculture due to their environmental biodegradability and friendliness (Soares et al. 2017; Alves et al. 2021; Luz et al. 2021; van et al. 2021; Zhou et al. 2022; Miri 2025). Ginger (*Zingiber officinale* Roscoe) and turmeric (*Curcuma longa* Linn.) are medicinal plants found in tropical and subtropical regions and cultivated in Asia (Avanço et al. 2017; Saccol et al. 2017; Moreira et al. 2024), as well as in Brazil. Essential oils and extracts of *Z. officinale* (Levy et al. 2015; Trasviña-Moreno et al. 2019; Costa et al. 2020; Fu et al. 2021; van et al. 2021; Saengsitthisak et al. 2025) and *C. longa* (Zhou et al. 2021, 2022) have been reported to exhibit parasiticidal and anthelmintic effectiveness in host fish. Lemon grass or citronella grass (*Cymbopogon nardus* Linn.) is a medicinal plant originally from Sri Lanka and Southern India that has spread throughout Asia, Africa and Latin America. It is used in traditional medicine due to its therapeutic properties (Barbas et al. 2017; Kaur et al. 2021). *C. nardus* essential oil exhibits bactericidal and antifungal activities in fish (Wei and Wee 2013; Kaur et al. 2021; Prado et al. 2024), and it is used as an antiparasitic agent in fish. Thus, this study investigated the in vitro efficacy of the *Z. officinale*, *C. longa* and *C. nardus* essential oils against parasitic dactylogyrideans in *C. macropomum*, as well as their acute toxicity for this fish.

2 | Materials and Methods

2.1 | Fish, Acclimation and Parasitic Dactylogyrideans

Three hundred fingerlings of *C. macropomum* were acquired from a commercial fish farm in Macapá, State of Amapá, Brazil, and were transported to the Aquaculture and Fisheries Laboratory of Embrapa, Macapá. 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^{-1}) for 20 days. They were fed twice a day with commercial feed containing 32% crude protein (Guabi, Brazil). Previously, 20 apparently healthy fish were examined for the presence of dactylogyrideans (mean intensity of 60 ± 21), and all were found with the infected gills (Figure 1). These naturally infected fish were used in the in vitro trials.

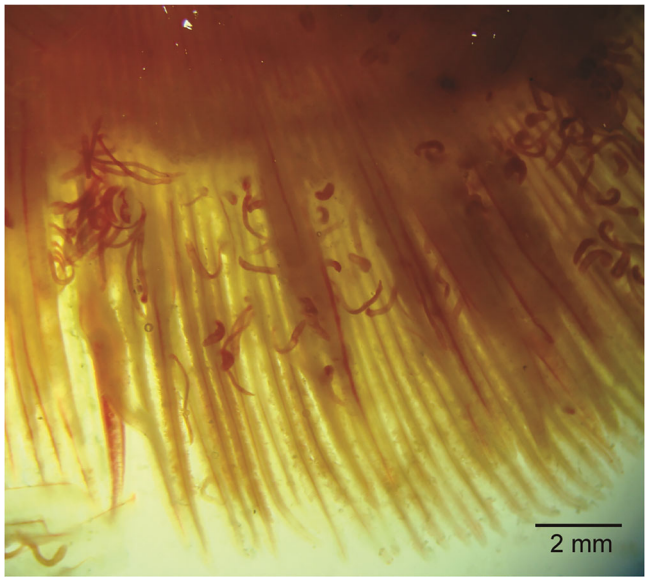

FIGURE 1 | Dactylogyridean parasites in the gills of *Colossoma macropomum*.

During the acclimation period of 20 days, the water parameters were monitored every 2 days to evaluate the temperature (29.9°C ± 0.2°C), dissolved oxygen (6.1 ± 0.2 mg L^{-1}), pH (6.2 ± 0.2), total ammonia (0.2 ± 0.1 mg L^{-1}), alkalinity (9.5 ± 0.002 mg L^{-1}) and hardness (10.0 ± 0.1 mg L^{-1}), with the aid of a multiparametric probe (Hanna, USA, model HI 9829). The tank was syphoned every week to remove accumulated organic material from the bottom.

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

2.2 | Obtaining Essential Oils of *Z. officinale, C. longa* and *C. nardus*, and Their Chemical Composition

The essential oils used in this study were obtained from the rhizomes of *Z. officinale* and *C. longa* specimens and the leaves of *C. nardus*, which were cultivated in the Medicinal Plants and Vegetables Sector of Embrapa Amazônia Ocidental in Manaus, state of Amazonas, Brazil. The oils were extracted by hydrodistillation using a Clevenger apparatus for 4 h. The chemical composition of the essential oils was determined using gas chromatography–mass spectrometry (GC–MS; Shimadzu QP5050A, Japan). Separation was performed using a silica SBP-

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5 capillary column composed of 5% phenylmethylpolysiloxane (30 m length × 0.25 mm i.d. and 0.25 μm phase thickness). The sample was dissolved in dichloromethane and analysed under the following conditions: split injection mode (ratio 1:40); injector temperature of 250°C; helium as the carrier gas; flow rate of 1.0 mL min^{-1} and oven temperature of 100°C for 5 min, then increased to 260°C at a rate of 4°C min^{-1}, finishing with an isothermal treatment of 20 min. Mass spectra were acquired in electron ionization mode at 70 eV, with a scan range of 40–350 m/z and a sampling rate of 1.0 scan s^{-1}. The ion source temperature was 200°C, the interface temperature was 250°C, and the solvent cut time was 2.5 min (Adams 2007).

2.3 | in vitro Assays of *Z. officinale, C. longa* and *C. nardus* Essential Oils Against Dactylogyrideans in *C. macropomum*

Six apparently healthy *C. macropomum* (184.2 ± 87.6 g and 21.7 ± 3.1 cm) and naturally infected by dactylogyrideans were euthanized by medullary section to collect all gill arches for i*n vitro* assays of each essential oil. Gills of six *C. macropomum* (184.2 ± 87.6 g and 21.7 ± 3.1 cm) that were naturally infected with dactylogyrideans were removed, and the gill arches were used for in vitro assays of each essential oil. All treatments were performed in triplicate, with one gill arch placed in a Petri dish. The essential oils from *Z. officinale*, *C. longa* and *C. nardus* were diluted in a solution of 70% ethyl alcohol and water from the fish cultivation tank (1:10 g). Then, each gill arch was carefully placed in a 5.5 cm Petri dish containing 500, 1000, 2000 or 3000 mg L^{-1} of *Z. officinale* essential oil; or 500, 1000, 2000 or 3000 mg L^{-1} of *C. longa* essential oil; or 250, 500, 1000 or 2000 mg L^{-1} of *C. nardus* essential oil. Two control groups were also used: one group with parasites exposed only to tank water and one group with parasites exposed to tank water plus 70% ethyl alcohol. The exposure time was between 15 min and 8 h, depending on the treatment (with or without oil exposure), and the mortality of the parasites was analysed.

All of the in vitro assays were performed at room temperature (26°C). Using stereomicroscopes with cold-light illumination, fields containing approximately ±20 parasites per view were examined. In each Petri dish containing a gill arch and an essential oil, and in both control groups, the parasites were observed every 15 min to quantify the number of dead and live individuals. Parasites were considered dead if they detached from the gill filaments or if they showed no mobility, even if they were still attached to the filaments (Hashimoto et al. 2016). The collected parasites were fixed in 5% formalin, conserved in 70% ethyl alcohol and prepared for species identification (Eiras et al. 2006), according to recommendations by Cohen et al. (2013). The in vitro efficacy for each essential oil was calculated using the methodology of Wang et al. (2008).

2.4 | Scanning Electron Microscopy of Dactylogyrideans Used for In Vitro Assays With Essential Oils

At the end of the in vitro assays, each gill arch used in treatments involving *Z. officinale* essential oil (500, 1000, 2000 and 3000 mg L^{-1}), *C. longa* essential oil (250, 500, 1000, 2000 and 3000 mg L^{-1}) and *C. nardus* essential oil (250, 500, 1000 and 2000 mg L^{-1}), as well as in both control groups (water from the cultivation tank and water from the cultivation tank plus 70% ethyl alcohol), was gently collected and fixed in 2.5% glutaraldehyde, which was prepared in a 0.1 M phosphate buffer solution (pH 7.2). The gill arches were then analysed using scanning electron microscopy to examine the effects of each essential oil on the dactylogyrideans. The gills were washed three times in 0.1 M phosphate buffer at 15 min intervals. Then, the samples were dehydrated in increasing concentrations of ethyl alcohol (at 70, 80, 90, 96 and twice at 100%) for 10 min at each concentration (Luz et al. 2021). The samples were analysed and photomicrographed using a scanning electron microscope (Hitachi, Tokyo, Japan, Mod. TM3030Plus) located in the Pharmaceutical Research Laboratory of the Federal University of Amapá (UNIFAP).

2.5 | Acute Toxicity in *C. macropomum* With Exposure to *Z. officinale, C. longa* and *C. nardus* Essential Oils

For acute toxicity trials with median lethal concentration (LC_{50}), healthy *C. macropomum* (26.1 ± 5.9 g and 11.0 ± 0.9 cm) were exposed to different concentrations of *Z. officinale*, *C. longa* or C. *nardus* essential oil (Table 3), using an aqueous static bioassay. All trials were carried out in 80 L tanks with 5 fish per tank (15 fish by treatment). The essential oils were prepared with dilution in a 1:10 (g) ratio of 70% ethyl alcohol. During exposure of fish to each essential oil, their survival, mortality and behavioural alterations were observed. After the exposure period ended to essential oils, the water flow for tanks was opened to completely remove the oil. The surviving fish were observed until they had completely recovered. Total recovery was considered when all the fish were actively swimming again.

2.6 | Statistical Analysis

The survival analysis of fish was conducted using the mean LC_{50} and its 95% confidence interval. The mean effective concentration (EC_{50}) and its 95% confidence interval were calculated for parasites. All analyses were calculated using the PROBIT procedure of GraphPad Prism software (Version 10.6).

3 | Results

3.1 | Composition of the Essential Oils From *Z. officinale*, *C. longa* and *C. nardus*

The essential oil from *Z. officinale* contained 50 chemical compounds that accounted for 99.0% of its composition. The majority components were α-zingiberene, β-sesquiphellandrene, limonene and geranial, accounting for 50.5% of the total composition. The essential oil from *C. longa* contained 46 chemical components, accounting for 99.0%, with ar-turmerone, α-turmerone, curlone, α-phellandrene and 1,8-cineole as the majority components, accounting for 74.8%. In the essential oil of *C. nardus*, 20 chemical compounds were identified (100%), and geraniol,

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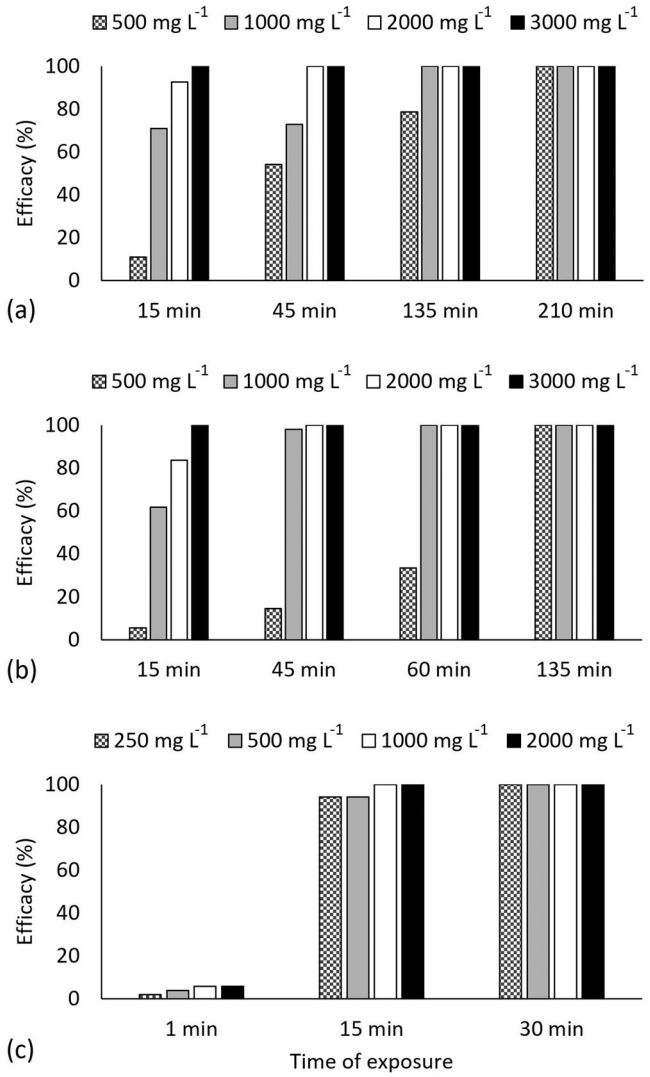

FIGURE 2 | In vitro anti-dactylogyridean efficacy of *Zingiber officinale* (a), *Curcuma longa* (b) and *Cymbopogon nardus* (c) essential oil at different concentrations and time exposures.

geranial and neral were the majority components, accounting for 84.7% (Table 1).

3.2 | in vitro Anti-Dactylogyridean Efficacy of *Z. officinale, C. longa* and *C. nardus* Essential Oils

The parasites of *C. macropomum* gills used for in vitro assays with each essential oil were identified as dactylogyrideans *A. spathulatus*, *N. janauachensis* and *M. boegeri*. All the concentrations of *Z. officinale*, *C. longa* and *C. nardus* essential oils caused the 100% immobilization of the parasites after exposure, but with varying times to achieve this complete effectiveness. In the highest concentrations, the 100% efficacy was achieved in a lower time of exposure (Figure 2) due to the variation of EC_{50} among essential oils. Essential oil of *C. longa* had the highest value of EC_{50}, whereas for *Z. officinale* and *C. nardus* essential oils, this was similar (Table 2). With the initial exposure to *Z. officinale*, *C. longa* or *C. nardus* essential oil, the dactylogyrideans were anesthetized for 3–5 min, which resulted in total immobilization and return of movement, and then death. In the control group exposed to cultivation tank water, total mortality (100%) of the parasites occurred after 8 h, whereas in the control group exposed to cultivation tank water plus ethyl alcohol, this occurred after 5 h.

3.3 | Scanning Electron Microscopy of Dactylogyrideans Exposed to the Essential Oils From *Z. officinale, C. longa* and *C. nardus* in in vitro Assays

Scanning electron microscopy analyses showed that the body surfaces of dactylogyrideans exposed only to cultivation tank water (Figure 3a) or cultivation tank water plus ethyl alcohol (Figure 3b) had minimal wrinkles compared to those exposed to essential oil treatments. Those exposed to 500 mg L^{-1} (Figure 3c) or 3000 mg L^{-1} (Figure 3d) of *Z. officinale* essential oil, or to 1000 mg L^{-1} (Figure 3e) or 3000 mg L^{-1} (Figure 3f) of *C. longa* essential oil, or 250 mg L^{-1} (Figure 3g) or 2000 mg L^{-1} (Figure 3h) of *C. nardus* essential oil exhibited tegument damages, such as deep wrinkles.

3.4 | Acute Toxicity to *C. macropomum* Exposed to *Z. officinale, C. longa* and *C. nardus* Essential Oils

The acute toxicity analysis showed that the LC_{50} values for *Z. officinale* and *C. longa* essential oils in *C. macropomum* were similar, while that for *C. nardus* essential oil was lower (Table 3). With exposure to 250 mg L^{-1} of *Z. officinale* or *C. longa*, as well as with exposure to 100 mg L^{-1} of *C. nardus* essential oil, none of the fish died or exhibited behavioural alterations, and they fully recovered 1 h after the essential oil was removed.

4 | Discussion

Essential oils are highly volatile substances composed of aromatic compounds extracted from various parts of plants, such as leaves, flowers and fruits. The chemical composition of essential oils can vary significantly according to the plant's geographical location, time of harvest, environmental conditions, extraction method (Tavares-Dias 2018; Costa et al. 2020; Kaur et al. 2021; Zhou et al. 2022; Prado et al. 2024) and species of medicinal plant.

In the essential oil of *Z. officinale*, we identified α-zingiberene, β-sesquiphellandrene, limonene and geranial as the majority components, similar to the results reported in previous studies (Chung et al. 2021; Costa et al. 2020). In *C. longa* essential oil, we found ar-turmerone, α-turmerone, curlone, α-phellandrene and 1,8-cineole to be the majority components, consistent with other studies (Saccol et al. 2017; Oliveira et al. 2021). However, Avanço et al. (2017) reported that the majority components of *C. longa* essential oil were α-turmerone, β-turmerone and ar-turmerone. Meanwhile, Cerqueira et al. (2025) found ar-turmerone and β-curcumin. In the *C. nardus* essential oil, we identified geraniol, geranial and neral as the majority components, similar to what Oliveira et al. (2021) found for this oil. Oliveira et al. (2021) reported that the concentration of geraniol is strongly influenced by the age of the leaves, with higher concentrations in older leaves than in younger ones. However, Wei and Wee (2013) identified predominance of 6-octenal, 3,7-dimethyl and citronellal as the

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TABLE 1 | Chemical composition of *Zingiber officinale*, *Curcuma longa* and *Cymbopogon nardus* essential oil.

Oil	*Zingiber officinale*			*Curcuma longa*			*Cymbopogon nardus*		
Peak	IRL exp.	Compounds	Content (%)	IRL exp.	Compounds	Content (%)	IRL exp.	Compounds	Content (%)
1	902	2-Heptanol	0.3	923	α-Thujene	—	1098	Linalool	1.2
2	920	Tricyclene	0.1	930	α-Pinene	0.5	1101	MO 152	0.2
3	930	α-Pinene	1.6	973	β-Pinene	0.1	1135	(*Z*,*Z*)-photocitral	0.1
4	944	Camphene	4.6	988	Myrcene	0.4	1145	*Trans*-chrysanthemal	0.2
5	969	Sabinene	0.1	999	Delta-2-carene	—	1149	Citronellal	1.9
6	973	β-Pinene	0.2	1003	α-Phellandrene	10.2	1162	Isoneral	0.3
7	987	Myrcene	1.5	1008	Delta-3-carene	0.3	1180	Isogeranial	0.5
8	1003	α-Phellandrene	0.4	1013	α-Terpinene	0.3	1226	Citronellol	4.7
9	1021	*p*-Cymene	0.1	1021	*p*-Cymene	1.7	1238	Neral	13.4
10	1025	Limonene	8.0	1024	Limonene	1.0	1256	Geraniol	53.3
11	1027	1.8-Cineole	2.8	1027	1.8-Cineole	7.0	1269	Geranial	18.0
12	1084	Terpinolene	0.2	1054	γ-Terpinene	0.4	1351	Citronellyl acetate	0.5
13	1094	Rosefuran	0.3	1084	Terpinolene	3.0	1381	Geranyl acetate	2.2
14	1098	Linalool	0.7	1173	Terpinen-4-ol	0.2	1385	β-Elemene	0.4
15	1160	Borneol	0.9	1187	α-Terpineol	0.3	1410	(*E*)-caryophyllene	1.5
16	1173	Terpinen-4-ol	0.1	1410	(*E*)-caryophyllene	0.4	1444	α-Humulene	0.2
17	1187	α-Terpineol	0.5	1437	ST 202	0.8	1472	Germacrene D	0.1
18	1226	Citronellol	0.5	1452	ST 204	0.2	1515	Delta-cadinene	0.2
19	1237	Neral	5.7	1473	γ-Curcumene	0.2	1572	Caryophyllene oxide	0.8
20	1267	Geranial	7.1	1476	Ar-curcumene	1.5	1645	α-Cadinol	0.4
21	1291	2-Undecanone	0.5	1489	α-Zingiberene	3.1		—	
22	1331	ST 204	0.1	1499	ST 200	0.1		—	
23	1356	ST 204	0.1	1502	β-Bisabolene	0.5		—	
24	1368	α-Copaene	0.4	1505	β-Curcumene	0.3		—	
25	1385	β-Elemene	0.6	1517	β-Sesquiphellandrene	3.0		—	
26	1400	Sesquithujene	0.2	1522	ST 204	0.3		—	

(Continues)

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TABLE 1 | (Continued)

Oil	*Zingiber officinale*			*Curcuma longa*			*Cymbopogon nardus*		
Peak	**IRL exp.**	**Compounds**	**Content (%)**	**IRL exp.**	**Compounds**	**Content (%)**	**IRL exp.**	**Compounds**	**Content (%)**
27	1426	*α-trans*-Bergamotene	0.1	1525	(*E*)-*γ*-bisabolene	0.2		—	
28	1429	ST 204	0.1	1529	n.i.	0.1		—	
29	1451	Allo-aromadendrene	0.8	1544	SO 220	0.2		—	
30	1465	ST 204	0.1	1553	SO 220	0.2		—	
31	1472	*γ*-Gurjunene	1.9	1559	SO 220	0.4		—	
32	1477	Germacrene D	4.1	1574	SO 218	1.0		—	
33	1483	Ar-curcumene	1.1	1585	Ar-turmerol	0.3		—	
34	1487	ST 204	1.9	1597	SO 220	1.0		—	
35	1491	*α*-Zingiberene	26.1	1602	SO 218	0.5		—	
36	1503	*β*-Bisabolene	5.8	1606	Ar-dihydro-turmerone	0.3		—	
37	1504	(*E*,*E*)-*α*-farnesene	7.2	1619	SO 218	0.2		—	
38	1507	ST 204	0.6	1624	SO 218	0.1		—	
39	1518	*β*-Sesquiphellandrene	9.3	1628	SO 218	0.7		—	
40	1525	(*E*)-*γ*-bisabolene	0.3	1631	SO 218	0.3		—	
41	1528	n.i.	0.1	1638	SO 218	0.2	—		
42	1542	Elemol	0.3	1652	SO 218	0.2	—		
43	1546	Germacrene B	0.7	1663	*α*-Turmerone	13.7	—		
44	1559	(*E*)-nerolidol	0.4	1666	Ar-turmerone	30.2	—		
45	1583	n.i.	0.3	1696	Curlone	13.7	—		
46	1606	n.i.	0.6	1748	SO 216	0.3	—		
47	1616	SO 222	0.3	—		—	—		
48	1624	SO 222	0.2	—		—	—		
49	1639	*β*-Eudesmol	0.2	—		—	—		
50	1679	SO 222	0.1	—		—	—		
Total compounds identified (%)			99.0		Total compounds identified (%)	99.9		Total compounds identified (%)	100

Abbreviation: n.i., not identified.

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TABLE 2 | Effective concentration (EC_{50}) of *Zingiber officinale*, *Curcuma longa* and *Cymbopogon nardus* essential oil against dactylogyrideans.

Essential oil	Time exposure	EC_{50} (95% CI, mg L^{-1})
Zingiber officinale	3 h and 30 min	16.6 (2.9–39.8)
Curcuma longa	2 h and 15 min	30.9 (4.4–75.7)
Cymbopogon nardus	30 min	13.7 (1.1–29.2)

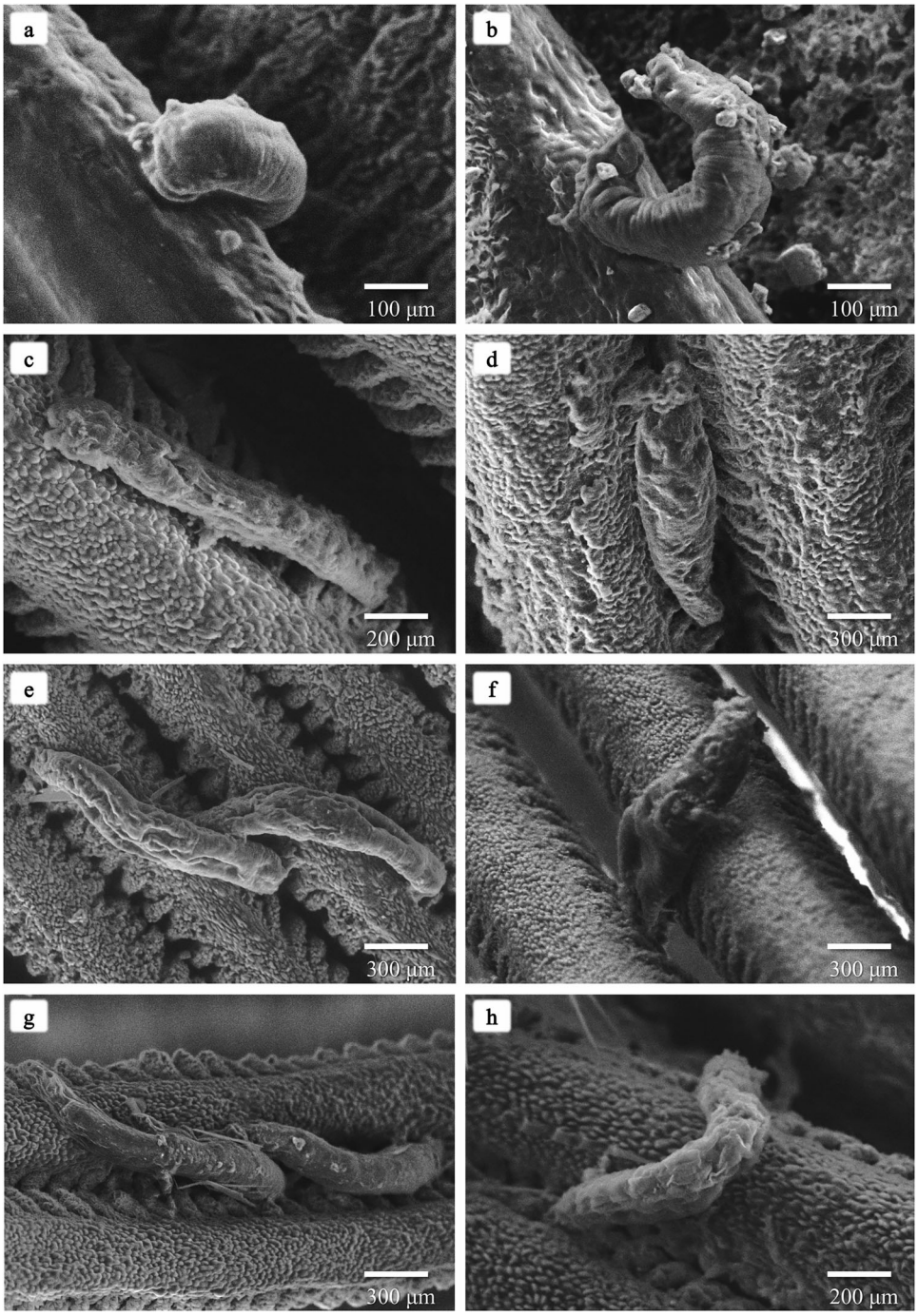

FIGURE 3 | Scanning electron microscopy of dactylogyrideans exposed to the essential oils. (a) Parasite after 8 h of exposure to water from the culture tank. (b) Parasite after 5 h of exposure to water from the culture tank + ethyl alcohol. (c) Parasite after 3 h and 30 min of exposure to 500 mg L^{-1} of *Zingiber officinale* essential oil. (d) Parasite after 15 min of exposure to 3000 mg L^{-1} of *Z. officinale* essential oil. (e) Parasite after 2 h of exposure to 1000 mg L^{-1} of *Curcuma longa* essential oil. (f) Parasite after 15 min of exposure to 3000 mg L^{-1} of *C. longa* essential oil. (g) Parasite after 30 min of exposure to 250 mg L^{-1} *Cymbopogon nardus* essential oil. (h) Parasite after 15 min of exposure to 2000 mg L^{-1} of *C. nardus* essential oil.

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TABLE 3 | Acute toxicity (LC_{50}—lethal concentration) and behavioural alterations for *Colossoma macropomum* after exposure of 30 min with *Zingiber officinale*, *Curcuma longa* and *Cymbopogon nardus* essential oils.

Essential oil	Concentration (mg L^{-1})	Exposure time (min)	LC_{50} (95% CI, mg L^{-1})	Alterations in fish behaviour
Zingiber officinale	250	10	498.2	Accelerated opercular beats, tumbling and excessive mucus secretion. Sedation after 3 min and recovery within 1 h after the end of exposure to 250 mg L^{-1}
	500	10		
	1000	30		
	2000	30		
	3000	30		
Curcuma longa	250	10	498.2	Agitation, accelerated opercular beats and jumping out of the tank. Sedation after 10 min and recovery within 1 h after the end of exposure to 250 mg L^{-1}
	500	10		
	1000	30		
	2000	30		
	3000	30		
Cymbopogon nardus	100	10	244.8	Irregular swimming, accelerated opercular beats. Sedation after 15 min and recovery 1 h and 30 min after cessation of exposure to 100 mg L^{-1}
	250	20		
	500	30		
	1000	30		
	2000	30		

majority components. Prado et al. (2024) identified citronellal, geraniol and citronellol as the majority components of the essential oil of *C. nardus* from different locations. Interestingly, it has been speculated that the majority components of these essential oils are primarily responsible for their antiparasitic activity (Zhou et al. 2022; Saengsitthisak et al. 2025). Nevertheless, further studies are needed to investigate this issue.

Parasitic infections by monopisthocotylans and polyopisthocotylans, as well as the development of parasitic resistance to chemotherapeutants, pose serious challenges to fish aquaculture. These challenges have stimulated the search for new alternatives to control these parasites, such as the use of essential oils (Hashimoto et al. 2016; Soares et al. 2017; Alves et al. 2021; Luz et al. 2021; Zhou et al. 2021, 2022). We observed in vitro efficacy, with a dose-dependent effect for *Z. officinale*, *C. nardus* and *C. longa* essential oils against *A. spathulatus, M. boegeri* and *N. janauachensis*. For these parasitic dactylogyrideans, the EC_{50} of *Z. officinale* essential oil was 16.6 mg L^{-1}, *C. longa* essential oil 30.9 mg L^{-1} and *C. nardus* oil 13.7 mg L^{-1}, demonstrating that the *C. longa* essential oil was the most toxicant. in vitro studies using 50–250 mg mL^{-1} of *Z. officinale* essential oil also demonstrated 100% efficacy against *Dactylogyrus* sp. (van et al. 2021). Zhou et al. (2022) reported EC_{100} of 12.0 mg L^{-1} after exposure for 24 h to *C. longa* essential oil against *Gyrodactylus kobayashii*. Extract of *C. longa* (100 mg L^{-1}) also had in vitro efficacy against *G. kobayashii* (Zhou et al. 2021). Therefore, applying these medicinal plant-derived products in fish aquaculture farms is of great interest. They have the advantages of having few toxic side effects on host fish and being environmentally friendly.

We examined the ultrastructural changes in *A. spathulatus*, *M. boegeri* and *N. janauachensis* after exposure to *Z. officinale*, *C. longa* and *C. nardus* essential oils. These oils caused damage and alterations to their tegument membranes due to their chemical constituents. Similar results were reported for the same dactylogyridean species exposed to essential oils from *Alpinia zerumbet*, *Piper callosum*, *Piper hispidum* and *Piper marginatum* (Alves et al. 2021; Luz et al. 2021), as well as for *G. kobayashii* exposed to dioscin, a compound isolated from the *Dioscorea collettii* plant (Zhou et al. 2021). However, the mechanisms involved in these ultrastructural damages are not well understood, although understanding them is crucial to explaining the direct effects of essential oils on these parasites. The tegument of monopisthocotylans and polyopisthocotylans is the primary surface that provides protection as well as helps maintain homeostasis, which is essential for survival. The tegument of these parasites is usually covered by fine wrinkles that are vital for their survival because they are the main site of energy for supplying adenosine triphosphate (ATP) (Zhang et al. 2020). Miri (2025) reported that the chemical components of essential oils can cause parasitic oxidation even when present in small amounts. This oxidation is primarily driven by a radical chain reaction, in which oxygen is incorporated into organic molecules, resulting in the formation of hydroperoxides, epoxides and other oxygen-containing derivatives. Therefore, these compounds appear to partially contribute to the mode of action of essential oils on these parasites (Steverding et al. 2005; Tavares-Dias 2018).

Acute toxicity evaluation of essential oils enables a preliminary selection of the most promising essential oils and safe concentrations for future use in baths for controlling dactylogyrideans. The evaluation showed that the essential oils were highly toxic to *C. macropomum*. The LC_{50} of both *Z. officinale* and *C. longa* essential oils was 498.2 mg L^{-1}, while that of *C. nardus* essential oil was 244.8 mg L^{-1}, and with 30 min. In contrast, for *Carassius auratus*,

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the $LC_{50-24\,h}$ of *C. longa* essential oil was 31.7 mg L^{-1} (Zhou et al. 2022). We observed that exposure to *Z. officinale*, *C. longa* and *C. nardus* essential oils had sedative effects on *C. macropomum*. The sedation and anaesthesia effects of *C. longa* (200–500 μL L^{-1}) and *C. nardus* (400–600 μL L^{-1}) essential oils have previously been demonstrated for *C. macropomum* (Barbas et al. 2017; Saccol et al. 2017). However, to our knowledge, no such studies have been performed on *Z. officinale* essential oil for *C. macropomum*, whereas sedative and anaesthetic effects of 200–400 mg L^{-1} on *Piaractus mesopotamicus* have been reported by Moreira et al. (2024). Consequently, the sedative effects of some essential oils may limit the use of high concentrations for therapeutic baths (Zhou et al. 2022), even though these concentrations are optimal for parasite control according to in vitro screening trials. For this reason, baths should be short and involve multiple applications. Moreover, these essential oils also had anaesthetic effects on *A. spathulatus*, *M. boegeri* and *N. janauachensis* in our in vitro studies. Zhou et al. (2022) reported that upon the initial exposure to *C. longa* essential oil, the anesthetized *G. kobayashii* detached from the host fish, thus increasing the anthelmintic efficacy. However, later they gradually recovered from anaesthesia and reinfected the host, thus leading to a decrease in anthelmintic efficacy.

In conclusion, we examined essential oils with different chemical compositions that exhibit similar bioactive activity against *A. spathulatus*, *M. boegeri* and *N. janauachensis*, though they differ in toxicity. Thus, given the therapeutic potential of *Z. officinale*, *C. nardus* and *C. longa* essential oils against these parasites, further research is needed to develop bath treatments that control parasitic infections while avoiding the toxicity to *C. macropomum*. Furthermore, these safer concentrations should be used for several days in therapeutic strategies to achieve good effectiveness.

Author Contributions

João G. R. Luz: execution of in vitro and in vivo trials and writing of the manuscript. **Dara A. P. Lacerda**: execution of in vitro and in vivo trials. **Eloísa F. L. Corrêa**: execution of in vitro and in vivo trials. **Gabriel F. Araújo**: execution of in vitro and in vivo trials. **Rafaela F. Araújo**: execution of in vitro and in vivo trials. **Francisco C. M. Chaves**: cultivation of medicinal plants and obtaining and identifying chemical components of essential oils. **Marcos Tavares-Dias**: coordination, financing and final writing of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data supporting the conclusions of this study are available from the corresponding author upon reasonable request.

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