



Anthelmintic efficacy of ethanolic extracts from two *Jatropha* species against monogenean gill parasites and hematological and histopathological effects on *Piaractus brachypomus*

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ABSTRACT

The present study evaluated the anthelmintic efficacy of ethanolic extracts from *Jatropha gossypifolia* and *Jatropha curcas* leaves for the control of gill monogeneans, as well as their hematological and histopathological effects on *Piaractus brachypomus*. The dried and ground leaves were macerated in 96 % ethyl alcohol, and the extract was obtained through filtration followed by solvent evaporation. The chemical composition of the extract was analyzed using liquid chromatography coupled with mass spectrometry. In the *in vitro* assays, gills of *P. brachypomus* were exposed to extracts of *J. gossypifolia* at concentrations of 500, 700, 1000, 1800, and 2000 mg L⁻¹, and to *J. curcas* at 500, 700, 1000, 1500, and 2000 mg L⁻¹. Two control groups were included: water and water with alcohol. For the treatment baths, five concentrations (50, 100, 500, 700 and 2000 mg L⁻¹) were tested. At the end of the exposure period, hematological and gill histological analyses were performed. The results indicated that the extracts contained phenolic compounds, peptides, and lipids. The *in vitro* assays demonstrated that all tested concentrations induced monogenean mortality, irrespective of dose or exposure time. Fish tolerated both 50 and 100 mg L⁻¹ concentrations of the extracts. *J. gossypifolia* reduced the mean abundance of parasites after six days of treatment, whereas *J. curcas* achieved a reduction after only three days, with efficacy exceeding 87 %. The extract of *J. curcas* induced milder alterations in hematological and histological parameters. These findings suggest that species of the genus *Jatropha* possess therapeutic potential for controlling parasitic infections in fish.

1. Introduction

The traditional use of medicinal plants to treat both emerging and common human diseases dates back centuries (Petrovska, 2012; Philipson, 2001). Pharmacology has since played a key role in isolating and enhancing the active compounds from these plants, leading to the development of important drugs in modern medicine (Balunas and Kinghorn, 2005). Nevertheless, traditional practices remain widely

employed for the treatment of dermatological, respiratory, neurological, parasitic, and gastrointestinal conditions, among others (Monigatti et al., 2013; Polesna et al., 2011; Ruiz et al., 2011). Bioactive compounds can be extracted from various sources, including essential oils, macerates, decoctions, and ethanolic, aqueous, or methanolic extracts. Among these, macerates, decoctions, and extracts are the most commonly used forms, owing to their low cost and ease of preparation (Sarquis et al., 2019).

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The Peruvian Amazon is home to numerous plant species with ethnobotanical applications (Ruíz et al., 2011). Among these, two species of the genus *Jatropha* stand out: *Jatropha gossypifolia* L. 1753 and *Jatropha curcas* L. 1753, both of which are traditionally used to treat fever, diarrhea, gastritis, dermatitis, oral infections, cancer-related conditions, and are also employed in spiritual practices (Rahu et al., 2021; Roumy et al., 2020; Sukohar et al., 2017; Quiroz et al., 2016; Tudela-Talavera, La Torre, 2016; Ruiz et al., 2011). Although originally native to tropical America, these species are now distributed across various countries due to their high adaptability (Moshobane et al., 2022; Wu et al., 2019; Divakara et al., 2010). They contain a variety of chemical compounds, including terpenoids, lignoids, steroids, flavonoids, alkaloids, glycosides, saponins, tannins, quinones, and phenolic compounds (Rahu et al., 2021; Wu et al., 2019). These bioactive constituents are associated with antifungal, antibacterial, antivenom, cytotoxic, and antioxidant activities (Rahu et al., 2021; Okoh et al., 2016; Félix-Silva et al., 2014; Huang et al., 2014). Several studies have demonstrated the potential of medicinal plants as alternative antiparasitic agents in cultured fish, due to their effectiveness, wide availability, accessibility and rich phytochemical composition (Ekanem et al., 2004; Soares and Tavares-Dias, 2013; Hashimoto et al., 2016; Meneses et al., 2018; Barriga et al., 2020). However, despite their natural origin, these compounds should be used with caution, as they may be toxic to fish and other aquatic organisms and can cause mortality if applied at inappropriate concentrations (Reverter et al., 2014). In aquaculture, the use of medicinal plants has been increasingly adopted for a range of applications, including as anesthetics, growth promoters, immunostimulants, and for the treatment of parasitic diseases through immersion baths (Malheiros et al., 2020; Gonzales et al., 2019; Corral et al., 2018; Valentim et al., 2018a, 2018b; Wu et al., 2011). The most commonly used forms are extracts, essential oils, latex, and oleoresins. However, recent advances in technology, such as the development of nanoemulsions, have enabled innovative approaches to the application of medicinal plant compounds. To date, at least 70 species of medicinal plants from the families *Myrtaceae*, *Lamiaceae*, *Verbenaceae*, *Fabaceae*, *Piperaceae*, and *Zingiberaceae* have been used to combat a variety of parasites affecting farmed fish. (Gonzales et al., 2022, 2020; Barriga et al., 2020; Zhang et al., 2020; Meneses et al., 2018; Valentim et al., 2018a; Soares et al., 2017a, 2017b; de Lima Boijink et al. 2016; Hashimoto et al., 2016; Sutili et al., 2014; Sharma et al., 2012; Steverding et al., 2005). Medicinal plants offer an alternative to the use of conventional chemical agents such as crystal violet, chloramphenicol, gentamicin, malachite green, and fluoroquinolone-enrofloxacin, among others (Conti et al., 2015), which are known to be highly toxic, environmentally contaminating, and pose significant risks to public health. Moreover, their use is prohibited in the European Union under Directive 96/23/EC (Soares et al., 2017a; Hoque et al., 2016; Conti et al., 2015; Quesada et al., 2013; Culp et al., 2002; Jung et al., 2001).

Piaractus brachipomus Cuvier, 1818 (Characidae) is one of the most widely cultivated fish species in countries of the Amazon region. In Peru, its cultivation is conducted on a semi-intensive scale and is also common in less developed communities, where it serves as a means of family subsistence. With a production volume of 3056 tons in 2023, it is the most extensively farmed native species, surpassed only by the introduced species *Oncorhynchus mykiss* Walbaum, 1792 and *Oreochromis niloticus* Linnaeus, 1758 (Produce, 2024). The success of *P. brachipomus* production, in addition to the desirable flavor of its meat, is attributed to its ease of adaptation to high-density culture systems, positive response to formulated feeds, and tolerance to different microclimates; factors that confer significant commercial value (de Souza e Silva et al. 2021). However, its cultivation can be compromised by outbreaks of monogenean parasites such as *Anacanthorus spathulatus*, *Mymarothecium viatorum*, and *Anacanthorus penilabiatus* (Negreiros, Tavares-Dias, 2019; Torres-Lozano et al., 2024), which spread rapidly among fish populations under inadequate farming and management conditions, due to their direct life cycle (Jerónimo et al. 2014). These parasites affect the

external surfaces of fish, leading to excessive mucus production, elevated mortality rates, reduced commercial value, and decreased production efficiency and economic returns. Consequently, monogeneans represent a significant threat to farmed fish and can cause substantial and potentially irreversible economic losses on a global scale. (Tavares-Dias and Martins, 2017). Therefore, the present study aims to evaluate the therapeutic potential of ethanolic leaf extracts of *J. gossypifolia* and *J. curcas* for the control of monogenean parasites in *P. brachipomus*, as well as to assess their hematological and histological effects.

2. Materials and methods

2.1. Preparation of plant material, extracts obtaining and chemical characterization

Leaves were collected in the morning from mature trees with well-formed foliage, while those in advanced stages of maturation were discarded (Fig. 1). *J. curcas* leaves were exclusively collected from the medicinal plant nursery of the National University of Madre de Dios. In contrast, *J. gossypifolia* leaves were collected from different points in the city of Puerto Maldonado, Madre de Dios region, Peru (Fig. 2). Samples of each species were deposited in the Alwyn Gentry-Hag Herbarium for taxonomic confirmation, under voucher numbers A. Gonzales – 001 and 002 HAG-UNAMAD.

The leaf material was transported to the Aquatic Biology Laboratory of the Peruvian Amazon Research Institute (IIAP-UTM: WGS 084, South; Zone 19 L East: 465119; North: 8601460) and dried in an air-conditioned environment for three days. To complete the drying process, the leaves were placed in an oven at 45 °C for one hour. Subsequently, they were weighed and ground using a cutting mill (Grindomix GM200, Retsch, Germany). The resulting powder was stored in airtight bags inside a desiccator, protected from light, until further use.

The ground leaves of each species were macerated in 96 % ethyl alcohol as a solvent at a 1:10 (w/v) ratio in an amber flask. The maceration process lasted seven days, with manual stirring of the solution three times a day to enhance the extraction of bioactive compounds. After maceration, the solution was filtered using a Büchner funnel with qualitative semi-slow flow filter paper (Ahlstrom, USA). The filtered solutions were transferred to glass trays, covered with aluminum foil, and protected with a 0.15 mm pore mesh until complete solvent evaporation. The resulting extract was collected using a spatula and stored at 4 °C until use. The extract yield percentage relative to the initial mass of plant material was calculated using the following formula:

$$\text{Extraction yield (\%)} = \frac{\text{Dry weight of extract recovered after extraction (g)}}{\text{Initial dry weight of ground plant material (g)}} \times 100$$

The chemical composition of the extracts was analyzed using ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS). A total of 10 mg of each extract was dissolved in 10 mL of methanol, and the mixture was subjected to an ultrasonic bath for five minutes. The solution was then filtered through 0.25 µm PVDF disc filters into an HPLC vial, and the analysis was performed using a Dionex Ultimate 3000 UHPLC system (Thermo Fisher Scientific, Bremen, Germany).

2.2. Fish origin and rearing conditions

P. brachipomus specimens were obtained through induced reproduction at the Amazonian Fish Reproduction Laboratory of the Peruvian Amazon Research Institute (IIAP), located in the Madre de Dios region, Peru. The fish were maintained in 1000 m³ earthen ponds until they reached the optimal size for the experiments (11.8 ± 1.2 cm and 34.6 ± 2.2 g).

A total of 500 fish were conditioned in two 1000 L (500 fish per tank)

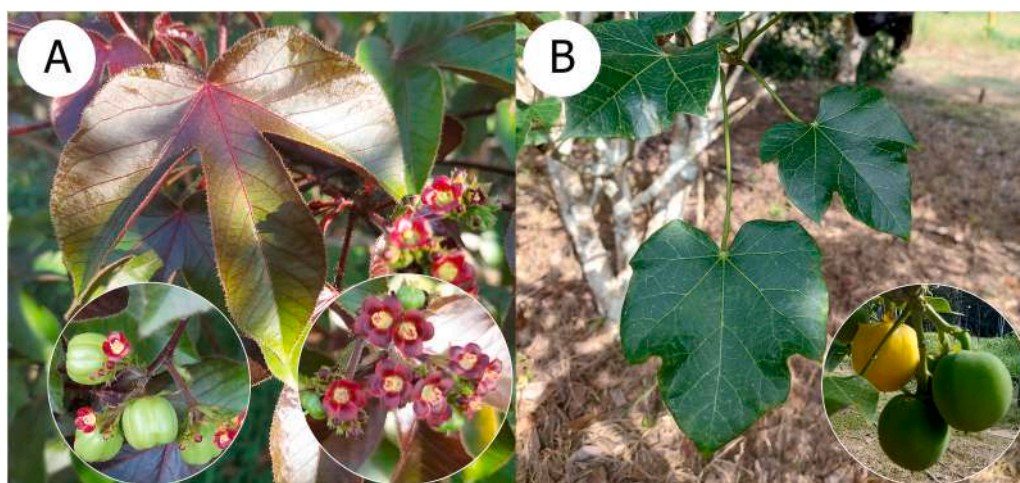


Fig. 1. Medicinal plant *Jatropa gossypifolia* (A), medicinal plant *Jatropa curcas* (B).

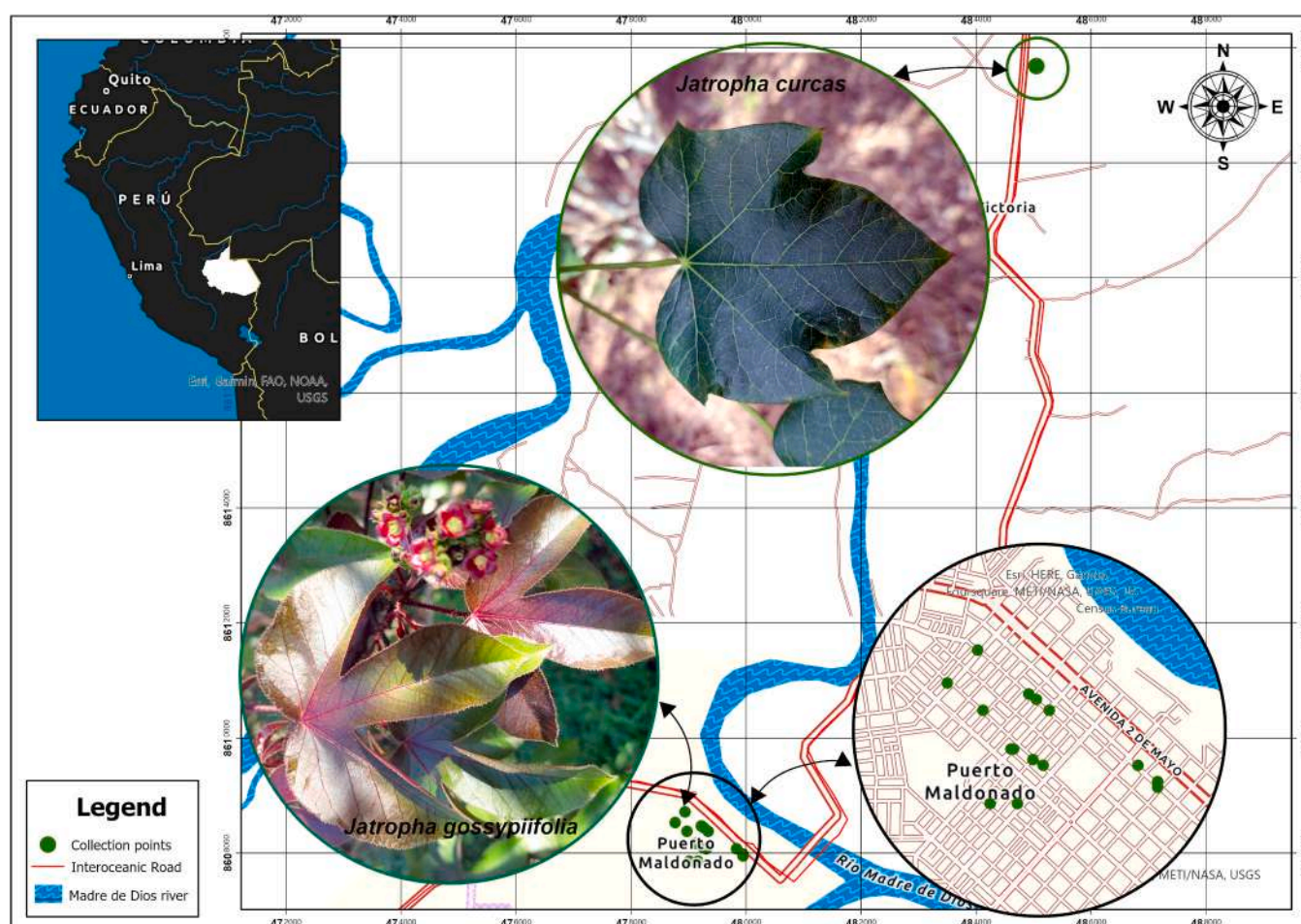


Fig. 2. Leaf collection site for *Jatropa curcas* and *Jatropa gossypifolia* in Puerto Maldonado, located in the southern Peruvian Amazon.

polypropylene tanks equipped with constant airflow and a closed water system. Tank cleaning was not performed, which increased fish susceptibility and created favorable conditions for the proliferation of monogeneans under controlled conditions. The fish were fed twice daily (at 08:00 and 16:00) with a commercial diet containing 36 % crude protein (CP). Water physicochemical parameters were monitored daily, including temperature ($27.5 \pm 1.3^\circ\text{C}$), dissolved oxygen (7.3

$\pm 2.1 \text{ mg L}^{-1}$), and pH (5.5 ± 0.7), using a portable multiparameter meter (HI 98196, Hanna Instruments, USA). Ammonia ($0.5 \pm 0.1 \text{ mg L}^{-1}$) and nitrite ($1.2 \pm 0.1 \text{ mg L}^{-1}$) concentrations were measured with a benchtop multiparameter photometer (HI 83399-01, Hanna Instruments, USA). After a seven-day acclimation period and prior to the experiments, ten fish naturally infected with monogeneans

were randomly sacrificed to confirm the infection and ensure that the parasitic load was adequate (> 20 monogeneans per gill arch). This period was sufficient to achieve 100 % infection of the fish, as required for antiparasitic efficacy assays (Gonzales et al., 2022).

All procedures in the present study were approved by the Animal Use Ethics Committee of the Universidad Nacional Mayor de San Marcos (N° 013–2021-CBE-FCB-UNMSM, N° 016–2022-CBE-FCB-UNMSM).

2.3. *In vitro* testing and Scanning Electron Microscopy (SEM)

A total of 38 specimens of *P. brachypomus* (17.0 ± 3.0 cm and 105.2 ± 56.4 g) were euthanized by cephalic concussion. Gills naturally parasitized by *A. spathulatus*, *A. penilabiatus*, and *M. viatorum* were isolated and exposed in triplicate to different concentrations of ethanolic extract of *J. gossypifolia* (500, 700, 1000, 1800, 2000 L⁻¹) and *J. curcas* (500, 700, 1000, 1500, 2000 L⁻¹). The concentrations were selected based on the dose–time relationship, following an approach similar to that used in previous studies (Soares et al., 2016; Gonzales et al., 2020). Two control groups were included: one consisting of culture tank water only, and another containing culture tank water with 96 % ethanol (20 mL L⁻¹). Extract concentrations were prepared separately for each plant at a 1:10 w/v ratio using 96 % ethanol. Before conducting the *in vitro* test, the gills were examined under a stereomicroscope (Stemi 305, Carl Zeiss, Germany) to identify a field of view containing at least 20 parasites. The gills were then subsequently immersed in the different extract concentrations and examined at five-minute intervals until 100 % parasite mortality was observed (Soares and Tavares-Dias, 2013). The efficacy of the treatments was calculated using the formula $AE = [(B - T) \times 100 \text{ \%}] / B$, where AE represents anthelmintic efficacy, B denotes the average number of live monogeneans in the control group, and T corresponds to the average number of live monogeneans in the extract-treated group (Wang et al., 2008).

Scanning Electron Microscopy (SEM) was employed to assess structural damage in monogenean parasites following exposure to ethanolic extracts. The gill tissues were fixed in 2.5 % glutaraldehyde buffered with 0.1 M sodium cacodylate and processed at the Federal University of São Paulo. After three washes with a 4 % osmium tetroxide solution, the samples were treated with 1 % tannic acid and gradually dehydrated through a graded ethanol series for 20 min at each concentration. The samples were then subjected to critical point drying using carbon dioxide and sputter-coated with platinum (Leica EM SCD 500, Germany). Observations were carried out using a scanning electron microscope (DSM 940, ZEISS, Germany) at an accelerating voltage of 15 kV (Gonzales et al., 2020).

2.4. Treatment bath and parasitological analysis

After determining the efficacy of the concentrations in the *in vitro* test, tolerance tests were conducted using ethanolic extracts of *J. gossypifolia* and *J. curcas* at concentrations of 50, 100, 500, 700, and 2000 mg L⁻¹. A total of 50 juvenile *P. brachypomus* were randomly distributed among ten 40 L glass aquaria, with five fish per aquarium. During the tests, the fish were maintained with constant aeration and without water renewal for 6 h in the *J. gossypifolia* treatment and 3 h in the *J. curcas* treatment. Behaviors such as erratic swimming, increased gill ventilation, lateral swimming, dilated eyes, loss of balance, reduced motility, jumping, and convulsions were observed (Gonzales et al., 2022, 2020). At the end of the trial, fish exposed to 50 and 100 mg L⁻¹ of both extracts maintained equilibrium and responded to external stimuli, unlike at higher concentrations, where loss of equilibrium and/or mortality were observed. Therefore, these concentrations were selected for the subsequent treatment baths.

An independent experiment was conducted for each ethanolic extract. A total of 288 specimens of *P. brachypomus* (12.5 ± 5.2 cm and 76.4 ± 40.0 g), naturally infected with monogeneans in the gills and otherwise showing no signs of disease, were randomly distributed into

24 tanks, each with a capacity of 250 L. Four treatments, each with three replicates consisting of 12 fish per replicate, were evaluated. The treatments included two control groups: T1 (culture tank water) and T2 (culture tank water with 20 mL L⁻¹ of 96 % ethanol), as well as two concentrations (50 mg L⁻¹ and 100 mg L⁻¹) for each of ethanolic extracts. Baths with *J. gossypifolia* extract were conducted for six hours over three consecutive days, while *J. curcas* baths were administered for 20 min over three consecutive days, with water replaced daily in all treatments. During the therapeutic baths, constant aeration was maintained and the water flow was kept closed. After the exposure period, fish were transferred to tanks containing extract-free water, where the water flow also remained closed and feeding was resumed four hours after the bath concluded.

After three days of treatment with the ethanolic extract of *J. gossypifolia*, six fish per replicate were euthanized for gill collection. In the *J. curcas* treatment, three fish per replicate were euthanized after three days of exposure, and their gills were collected. After six days of treatment, an additional four fish per replicate were euthanized for gill sampling. All gills were preserved in 5 % formalin for subsequent counting and identification of monogeneans, following the procedures described by Eiras et al. (2000). The prevalence and mean abundance of parasites were calculated according to the methodology described by Bush et al. (1997), and treatment efficacy was determined using the formula proposed by Wang et al. (2008).

2.5. Hematological analyses of *P. brachypomus* after treatment baths

Once the treatment baths had concluded, blood samples were collected from the caudal vein of the fish using pre-treated syringes with a 10 % EDTA anticoagulant solution (Gonzales-Flores et al., 2022). Erythrocyte counts were performed using a Neubauer hemocytometer. Hematocrit values were determined using the microhematocrit method, as described by Goldenfarb et al. (1971), and hemoglobin concentration was measured using the cyanomethemoglobin method. Based on these data, the hematimetric indices: mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the method described by Wintrobe (1934). For leukocyte differentiation and the determination of total leukocyte and thrombocyte counts (Tavares-Dias et al., 1999), blood smears were prepared and stained using a combination of May-Grünwald, Giemsa, and Wright stains, as described by Ranzani-Paiva et al. (2013). To assess biochemical parameters, 0.5 mL of blood was collected from each fish and centrifuged at 75 G for 10 min (HC-12A, Eurotech, China) to obtain plasma for the analysis of glucose and total protein levels. Glucose concentration was determined using the enzymatic-colorimetric glucose oxidase method at 500 nm, while total protein was measured using the Biuret method at 540 nm. Both analyses were performed using commercial kits (DiaSys Diagnostic Systems, Germany), and absorbance readings were obtained with a UV–visible spectrophotometer (VIS 721, Usamed, China).

2.6. Histological analyses of *P. brachypomus* gills after treatment baths

Gills from five fish per replicate were individually fixed in 10 % buffered formalin. After 24 h, the samples were transferred to 70 % ethanol for storage until processing. The gills were then placed in individual cassettes and passed through a graded ethanol series (75 %, 80 %, 95 %, and 100 %) followed by xylene substitutes, using an automated tissue processor (HistoCore Pearl, Leica Biosystems, USA). The samples were embedded in paraffin and sectioned at a thickness of 4 µm using a rotary microtome (HistoCore Autocut, Leica Biosystems, USA). The sections were transferred to a flotation bath and subsequently mounted onto glass slides. Staining was performed with hematoxylin and eosin (H&E). Histological images were captured using a binocular microscope (Primo Star iLED, Zeiss, Germany) and the Zeiss Zen Lite software, version 3.4.

2.7. Statistical analyses

Results were expressed as mean $\pm$ standard deviation. One-way analysis of variance (ANOVA) was performed, and when significant differences were detected among groups, Tukey's post hoc test ($p < 0.05$) was applied for multiple comparisons. All analyses were conducted after verifying the assumptions of normality (Shapiro–Wilk test) and homogeneity of variances (Levene's test), using SigmaPlot version 14 (Systat Software Inc.).

3. Results

3.1. Yield and chemical composition of ethanolic extracts of *Jatropha gossypifolia* and *Jatropha curcas*

The ethanolic extracts of both plant species were dark green in color, though they differed in texture: the extract of *J. gossypifolia* exhibited a solid, gum-like consistency, whereas the extract of *J. curcas* crystallized. The extraction yield of *J. gossypifolia* was lower (7.6 %) compared to that of *J. curcas* (10.3 %). *J. gossypifolia* extract contained phenolic compounds (59.4 %), lipids (21.9 %), peptides (6.2 %), carbohydrates (6.2 %), vitamin B5 (3.1 %), and nitrogenous compounds (3.1 %), whereas *J. curcas* extract contained phenolic compounds (66.6 %), lipids (29.2 %), and carbohydrates (4.2 %) (Tables 1 and 2).

3.2. In vitro antiparasitic activity

The ethanolic extract of *J. gossypifolia* at a concentration of 500 mg L⁻¹ resulted in 1.7 % mortality after just 10 min of exposure, reaching 100 % mortality after 1 h and 45 min. The 700 mg L⁻¹ concentration achieved total mortality within 1 h and 25 min, only 15 min longer than the time required at 1000 mg L⁻¹. Higher concentrations of 1800 and 2000 mg L⁻¹ resulted in complete mortality and 100 % efficacy after 50 and 45 min of exposure,

respectively (Table 3). During initial exposure to the ethanolic extracts, the monogeneans exhibited contraction and loss of motility; however, most resumed normal movement after approximately 10 s. Larger parasites were more susceptible, exhibiting rapid mortality, whereas smaller individuals demonstrated greater resistance and frequently sheltered among the secondary lamellae of the gills.

The ethanolic extract of *J. curcas*, at a concentration of 500 mg L⁻¹, began to induce monogenean mortality only after 20 min of exposure, which was 10 min later than the ethanolic extract of *J. gossypifolia*. Concentrations of 700 and 1000 mg L⁻¹ achieved 100 % mortality after 1 h and 25 min and 1 h and 10 min of exposure, respectively, durations that were comparable to those observed with *J. gossypifolia*. The 1500 and 2000 mg L⁻¹ concentrations induced parasite mortality within just 10 min of exposure, achieving 100 % efficacy in a shorter time frame compared to *J. gossypifolia* (Table 4). In the control treatments, parasite mortality was only observed after seven hours of exposure.

Scanning electron microscopy revealed that monogeneans exposed to water alone exhibited well-defined bodies with typical parasitic morphological features and no apparent alterations (Figs. 3A and 4A). In contrast, parasites exposed to ethanolic extract concentrations displayed superficial to deep tegumentary alterations, including lesions and burn-like damage (Figs. 3 and 4).

3.3. Tolerance test and in vivo antiparasitic efficacy of ethanolic extracts of *Jatropha gossypifolia* and *Jatropha curcas*

The tolerance test demonstrated that fish exhibited greater resistance to lower concentrations (50 mg L⁻¹ and 100 mg L⁻¹) of *J. gossypifolia* and *J. curcas* ethanolic extracts. Although signs of stress were observed, including rapid opercular movement, loss of equilibrium, and disruption of schooling behavior, no mortalities occurred. At concentrations of 500 and 700 mg L⁻¹, loss of equilibrium, dilated eyes, surface gasping, and lateral swimming were observed with both extracts. Specifically, at a concentration of 700 mg L⁻¹ of *J. gossypifolia* extract, 50 % mortality

Table 1
Compounds identified in the ethanolic extract of *Jatropha gossypifolia* using UHPLC-MS analysis.

N°	Retention Time (min)	Molecular Formula	Name of Compound	Compound Class
1	1.46	C12H22O11	Sucrose	Carbohydrates
2	1.55	C7H12O6	D- (-)-quinic acid	Phenolic
3	1.55	C5H11N3O2	4-Guanidinobutanoate	Nitrogenous compound
4	2.87	C9H17NO5	Pantothenic acid	Vitamin B5
5	6.60	C15H14O6	Catechin	Phenolic
6	8.77	C16H20O10	3-benzoyloxy-2-hydroxypropoxy]- 3,4,5-trihydroxyoxane-2-carboxylic acid	Phenolic
7	8.95	C27H30O15	Isovitexin-7-O-glucoside	Phenolic
8	9.0	C26H28O14	Apigenin 6-c-pentosyl-8-c-hexosyl	Phenolic
9	9.19	C21H20O11	Luteolin - 4 - C -glucoside	Phenolic
10	9.31	C21H20O11	Luteolin - 6 - C -glucoside	Phenolic
11	9.38	C27H30O15	Vitexin - 2'' - O -glucoside	Phenolic
12	9.63	C27H30O14	Vitexin - 2'' - O -rhamnoside	Phenolic
13	9.86	C21H20O10	Isovitexin	Phenolic
14	10.00	C21H20O12	Hyperoside	Carbohydrates
15	10.05	C21H20O11	Luteolin-7-O-glucoside	Phenolic
16	10.46	C27H30O14	Rhoifolin	Phenolic
17	10.67	C21H20O11	Homoorientin	Phenolic
18	12.15	C15H12O6	Eriodictyol	Phenolic
19	12.54	C15H10O6	Luteloil	Phenolic
20	13.12	C18H32O5	9,12,13-Trihydroxy-10,15- octadecadienoic acid (isomer 1)	Lipid
21	13.25	C15H12O5	Naringenin	Phenolic
22	13.25	C17H16O7	3,5,7-Trihydroxy-4',8- dimethoxyflavanone	Phenolic
23	13.43	C16H14O6	Homoeriodictiol	Phenolic
24	13.59	C19H34O5	9,12,13 -TriHOME (isomer 1)	Lipid
25	13.90	C18H32O5	9,12,13 -Trihydroxy -10,15 - octadecadienoic acid (isomer 2)	Lipid
26	14.11	C17H14O7	3,7 -Dimethylquercetin	Phenolic
27	14.3 2	C19H34O5	9,12,13 -TriHOME (isomer 2)	Lipid
28	14.5 8	C17H36N2O	N -[3 - (dimethylamino)propyl] dodecanamide	Peptide
29	14.8 3	C19H39N2O3	Lauramidopropyl betaine	Lipid
30	15.42	C37H56N8O8	Cyclogossin A	Peptide
31	16.30	C33H56O14	DGMG 18:3	Lipid
32	17.84	C18H30O3	(9Z,11E,15Z)-13-hydroxyoctadeca9,11,15-trienoic acid	Lipid

Table 2Compounds identified in the ethanolic extract of *Jatropha curcas* using UHPLC-MS analysis.

N°	Retention Time (min)	Molecular Formula	Name of Compound	Compound Class
1	1.81	C12H22O11	Sucrose	Carbohydrates
2	2.33	C10H15NO	Hordenine	Phenolic
3	5.10	C30H26O12	Procyanidin B2	Phenolic
4	6.60	C15H14O6	Catechin	Phenolic
5	7.78	C30H26O12	Procyanidin B1	Phenolic
6	8.28	C15H14O6	Epicatechin	Phenolic
7	8.33	C19H32O8	Icariside B5	Phenolic
8	8.57	C26H28O15	luteloin 6-c-pentosyl-8-c-hexosyl	Phenolic
9	8.93	C27H30O15	Isovitexin-7-O-glucoside	Phenolic
10	9.0	C26H28O14	apigenin 6-c-pentosyl-8-c-hexosyl	Phenolic
11	9.16	C21H20O11	Homoorientin	Phenolic
12	9.83	C21H20O10	Isovitexin	Phenolic
13	9.92	C27H30O15	Kaempferol-7-O-neohesperidoside	Phenolic
14	10.05	C21H20O11	Luteolin-glucoside (isomer 1)	Phenolic
15	10.46	C27H30O14	Rhoifolin	Phenolic
16	10.67	C21H20O11	Luteolin-glucoside (isomer 2)	Phenolic
17	10.90	C21H20O10	Vitexin	Phenolic
18	13.01	C18H32O5	9,12,13-Trihydroxy-10,15-octadecadienoic acid (isomer 1)	Lipid
19	13.59	C19H34O5	9,12,13-TriHOME (isomer 1)	Lipid
20	13.90	C18H32O5	9,12,13-Trihydroxy-10,15-octadecadienoic acid (isomer 2)	Lipid
21	14.36	C19H34O5	9,12,13-TriHOME (isomer 2)	Lipid
22	14.54	C17H36N2O	N-[3-(dimethylamino)propyl] dodecanamide	Lipid
23	14.86	C19H39N2O3	Lauramidopropyl betaine	Lipid
24	17.85	C18H30O3	(9Z,11E,15Z)-13-hydroxyoctadeca9,11,15-trienoic acid	Lipid

was observed. Finally, at 2000 mg L⁻¹ of both extracts, fish exhibited loss of equilibrium, erratic swimming, jumping, convulsions, and 100 % mortality.

The parasites found in the gills of *P. brachypomus* belonged to the family Dactylogyridae, including the species *Anacanthorus spathulatus*, *Anacanthorus penilabiatus*, and *Mimarotectum viatorum*, with *A. spathulatus* being the most abundant. The ethanolic extract of *J. gossypifolia* demonstrated high efficacy in controlling the parasites, showing a 99.97 % reduction at a concentration of 50 mg L⁻¹ and 100 % efficacy at 100 mg L⁻¹ after three days of treatment. The mean abundance of *A. spathulatus*, *M. viatorum*, and *A. penilabiatus* was significantly reduced ($p < 0.05$) in the treatments with 50 and 100 mg L⁻¹ compared to the control groups. Prevalence reached 0 % in fish treated with 100 mg L⁻¹ for all three species (Table 5). The efficacy of *J. curcas* reached 87.15 % at 50 mg L⁻¹ and 90.35 % at 100 mg L⁻¹ after three days of treatment. After six days, the efficacy increased to 94 % at 100 mg L⁻¹ and 96.77 % at 50 mg L⁻¹. *J. curcas* extract significantly reduced ($p < 0.05$) the mean abundance of *A. spathulatus* and *A. penilabiatus* at both three and six days of treatment compared to control groups. For *M. viatorum*, abundance was significantly lower ($p < 0.05$) compared to the water alcohol control group at both three and six post-treatment (Table 6). Prevalence decreased in fish treated with 50 and 100 mg L⁻¹ at 3 h and was further reduced at 6 h.

3.4. Hematological effects on *Piaractus brachypomus* after therapeutic baths with ethanolic extracts of *Jatropha gossypifolia* and *Jatropha curcas*

Treatment baths with ethanolic extract of *J. gossypifolia* at concentrations of 50 and 100 mg L⁻¹ significantly ($p < 0.05$) reduced hemoglobin levels, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) in the fish. At 100 mg L⁻¹, there was a significant ($p < 0.05$) increase in total erythrocyte and leukocyte counts. Hematocrit values were significantly ($p < 0.05$) lower in fish treated with 50 mg L⁻¹. Additionally, fish exposed to 100 mg L⁻¹ showed a significant rise in glucose levels compared to those treated with 50 mg L⁻¹ and the water control group. Total protein levels were reduced in fish exposed to 50 mg L⁻¹ relative to the controls (Fig. 5).

Treatment baths with the ethanolic extract of *J. curcas* at both concentrations did not affect hematocrit or erythrocyte production in the fish. Hemoglobin levels were reduced only when compared to the

alcohol control group. The 50 mg L⁻¹ concentration significantly ($p < 0.05$) lowered the mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) relative to the alcohol control. Total leukocyte counts showed variation only among the control groups. Alcohol exposure significantly ($p < 0.05$) increased plasma glucose levels compared to the water control group, while both extract concentrations reduced total protein levels in the fish (Fig. 6).

Five types of leukocytes were identified in the blood of *P. brachypomus*, with monocytes, lymphocytes, and neutrophils being the most prevalent. A significant ($p < 0.05$) reduction in lymphocyte and monocyte counts, accompanied by an increase in neutrophils, was observed in fish treated with 50 and 100 mg L⁻¹ of the ethanolic extract of *J. gossypifolia* (Table 7). Treatment baths with 50 mg L⁻¹ of the *J. curcas* extract led to a significant ($p < 0.05$) decrease in monocyte counts compared to the water control, a significant ($p < 0.05$) increase in neutrophils compared to the alcohol control, and no changes in lymphocyte levels (Table 8). Both extract concentrations also reduced the number of thrombocytes and decreased LG-PAS-positive leukocytes when compared to the water control group.

3.5. Gill histopathological changes in *Piaractus brachypomus* after therapeutic baths with ethanolic extracts of *Jatropha gossypifolia* and *Jatropha curcas*

Histological analysis of the gill sections from fish subjected to extract-free treatments revealed only minor alterations in some samples, including primary lamella congestion, epithelial hyperplasia, and displacement of the secondary lamellae, along with the presence of monogenean parasites (Figs. 7A and 7B). In the gill tissues of fish treated with extract concentrations, a higher frequency of secondary lamellae displacement, epithelial hyperplasia, and lamellar fusion was observed. Hemorrhages were detected in some sections from fish exposed to treatment baths with 50 mg L⁻¹ of the *J. curcas* extract, while aneurysms were identified in fish treated with 100 mg L⁻¹ of the ethanolic extract of *J. gossypifolia* (Figs. 7C and 7F).

4. Discussion

Medicinal plants represent a sustainable alternative for addressing these challenges, owing to their content of secondary metabolites synthesized through pathways such as the shikimic acid and acetyl-CoA

Table 3
In vitro anthelmintic efficacy of varying concentrations of ethanolic extract of *Jatropha gossypifolia* against monogenean parasites of *Piaractus brachypomus*.

Exposure Time	Treatment	Number of Live Parasites	Mortality (%)
0 h	Water Treatment	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		20.0 ± 0.0	0.0
30 min		20.0 ± 0.0	0.0
45 min		20.0 ± 0.0	0.0
50 min		19.7 ± 2.4	1.7
1.10 min		19.7 ± 2.4	1.7
1.25 min		19.7 ± 2.4	1.7
1.45 min		19.7 ± 2.4	1.7
2 h		18.3 ± 2.4	8.3
5 h		13.7 ± 9.4	31.7
7 h		0.0 ± 0.0	100
0 h	Alcohol Treatment	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
30 min		19.3 ± 4.7	3.3
40 min		19.3 ± 4.7	3.3
45 min		19.3 ± 4.7	3.3
50 min		19.3 ± 4.7	3.3
1.10 min		16.0 ± 8.2	18.3
1.25 min		16.3 ± 6.2	18.3
1.45 min		16.3 ± 6.2	18.3
2 h		14 ± 8.2	30.0
5 h		5.0 ± 14.7	75.0
7 h		0.0 ± 0.0	100
0 h	500 mg L ⁻¹	20.0 ± 0.0	0
10 min		19.7 ± 2.4	1.7
20 min		19.7 ± 2.4	1.7
30 min		18.7 ± 2.4	6.7
45 min		16.0 ± 10.8	20.0
50 min		14.0 ± 8.2	30.0
1.10 min		10.7 ± 2.4	46.7
1.25 min		6.3 ± 8.5	68.3
1.45 min		0.0 ± 0.0	100.0
0 h	700 mg L ⁻¹	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		20.0 ± 0.0	0.0
30 min		19.7 ± 2.3	1.7
45 min		14.0 ± 24.8	30.0
50 min		3.7 ± 20.5	43.3
1.10 min		11.3 ± 25.9	81.7
1.25 min		0.0 ± 0.0	100
0 h	1000 mg L ⁻¹	20.0 ± 0.0	0
10 min		19.3 ± 2.4	3.3
20 min		18.0 ± 4.1	10.0
30 min		17.0 ± 4.1	15.0
45 min		13.3 ± 15.5	33.3
50 min		12 ± 10.8	40.0
1.10 min		0.0 ± 0.0	100
0 h	1800 mg/L ⁻¹	20.0 ± 0.0	0
10 min		18.7 ± 4.2	6.6
20 min		12.7 ± 4.2	36.6
30 min		8.0 ± 4.7	60.0
45 min		1.7 ± 0.8	91.6
50 min		0.0 ± 0.0	100
0 h	20000 mg/L ⁻¹	20.0 ± 0.0	0.0
10 min		17.7 ± 8.5	11.7
20 min		7.0 ± 10.8	65.0
30 min		0.3 ± 2.4	98.3
45 min		0.0 ± 0.0	100

pathways (Goyal et al., 2012; Ramawat et al., 2009). Flavonoids such as apigenin, isovitexin, vitexin, luteolin, kaempferol, and catechin had previously been reported in leaf extracts of *J. gossypifolia* and *J. curcas* (Cavalcante et al., 2020; Silveira et al., 2020; Wu et al., 2019; Huang et al., 2014; Shahi and Singh, 2011). However, this study identified several compounds that had not been previously reported in these two plant species. In the *Jatropha* genus, lignans, neolignans, sesqueneolignans, coumarins, and coumarinolignoids are commonly found in seeds, roots, and stems; flavonoids are typically present in the leaves, stems, and roots; and alkaloids have been detected in the leaves, stems, flowers, fruits, rhizomes, and exudates (Cavalcante et al., 2020). Our findings

Table 4
In vitro anthelmintic efficacy of varying concentrations of ethanolic extract of *Jatropha curcas* against monogenean parasites of *Piaractus brachypomus*.

Exposure Time	Treatment	Number of live parasites	Mortality (%)
0 h	Water Treatment	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		20.0 ± 0.0	0.0
30 min		20.0 ± 0.0	0.0
45 min		20.0 ± 0.0	0.0
50 min		19.7 ± 2.4	1.7
1.10 min		19.7 ± 2.4	1.7
1.25 min		19.7 ± 2.4	1.7
1.45 min		19.7 ± 2.4	1.7
2 h		18.3 ± 2.4	8.3
5 h		13.7 ± 9.4	31.7
7 h		0.0 ± 0.0	100
0 h	Alcohol Treatment	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		19.3 ± 4.7	3.3
30 min		19.3 ± 4.7	3.3
45 min		19.3 ± 4.7	3.3
50 min		19.3 ± 4.7	3.3
1.10 min		16.3 ± 6.2	18.3
1.25 min		16.3 ± 6.2	18.3
1.45 min		16.3 ± 6.2	18.3
2 h		14 ± 8.1	30.0
5 h		5.0 ± 14.7	75.0
7 h		0.0 ± 0.0	100
0 h	500 mg L ⁻¹	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		19.6 ± 2.8	1.7
30 min		17.6 ± 7.6	11.7
45 min		16.0 ± 10.0	20.0
50 min		14.6 ± 212.5	26.7
1.10 min		8.6 ± 7.6	56.7
1.25 min		3.0 ± 0.0	85.0
1.45 min		0.0 ± 0.0	100
0 h	700 mg L ⁻¹	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		20.0 ± 0.0	0.0
30 min		17.6 ± 2.3	11.7
45 min		14.0 ± 8.1	30.0
50 min		13.0 ± 4.0	35.0
1.10 min		1.6 ± 6.2	91.7
1.25 min		0.0 ± 0.0	100
0 h	1000 mg L ⁻¹	20.0 ± 0.0	0.0
10 min		20.0 ± 0.0	0.0
20 min		20.0 ± 0.0	0.0
30 min		13.0 ± 0.0	35.0
45 min		5.0 ± 16.3	75.0
50 min		4.0 ± 12.2	80.0
1.10 min		0.0 ± 0.0	100
0 h	1500 mg L ⁻¹	20.0 ± 0.0	0.0
10 min		19.6 ± 2.3	1.7
20 min		11.6 ± 26.5	41.7
30 min		0.0 ± 0.0	100
0 h	2000 L ⁻¹	20.0 ± 0.0	0.0
10 min		19.6 ± 1.6	1.7
20 min		0.0 ± 2.3	100

support the hypothesis that the presence of phytoconstituents vary according to several factors, including the type of solvent used, the extraction and processing methodologies, the specific plant part analyzed, the season of collection, plant maturity, genetic differences, and even the geographical origin of the plant (Silveira et al., 2020; Igbinosa et al., 2011).

The use of *J. curcas* and *J. gossypifolia* in traditional medicine is widespread and well documented in the Peruvian Amazon. However, their application as anthelmintic agents in aquaculture has not been previously reported. In the present *in vitro* assays, all tested concentrations of the ethanolic extracts of *J. gossypifolia* and *J. curcas* achieved 100 % mortality and efficacy against monogenean parasites, with the effects being both concentration- and exposure time-dependent. These findings are consistent with previous studies that have demonstrated the

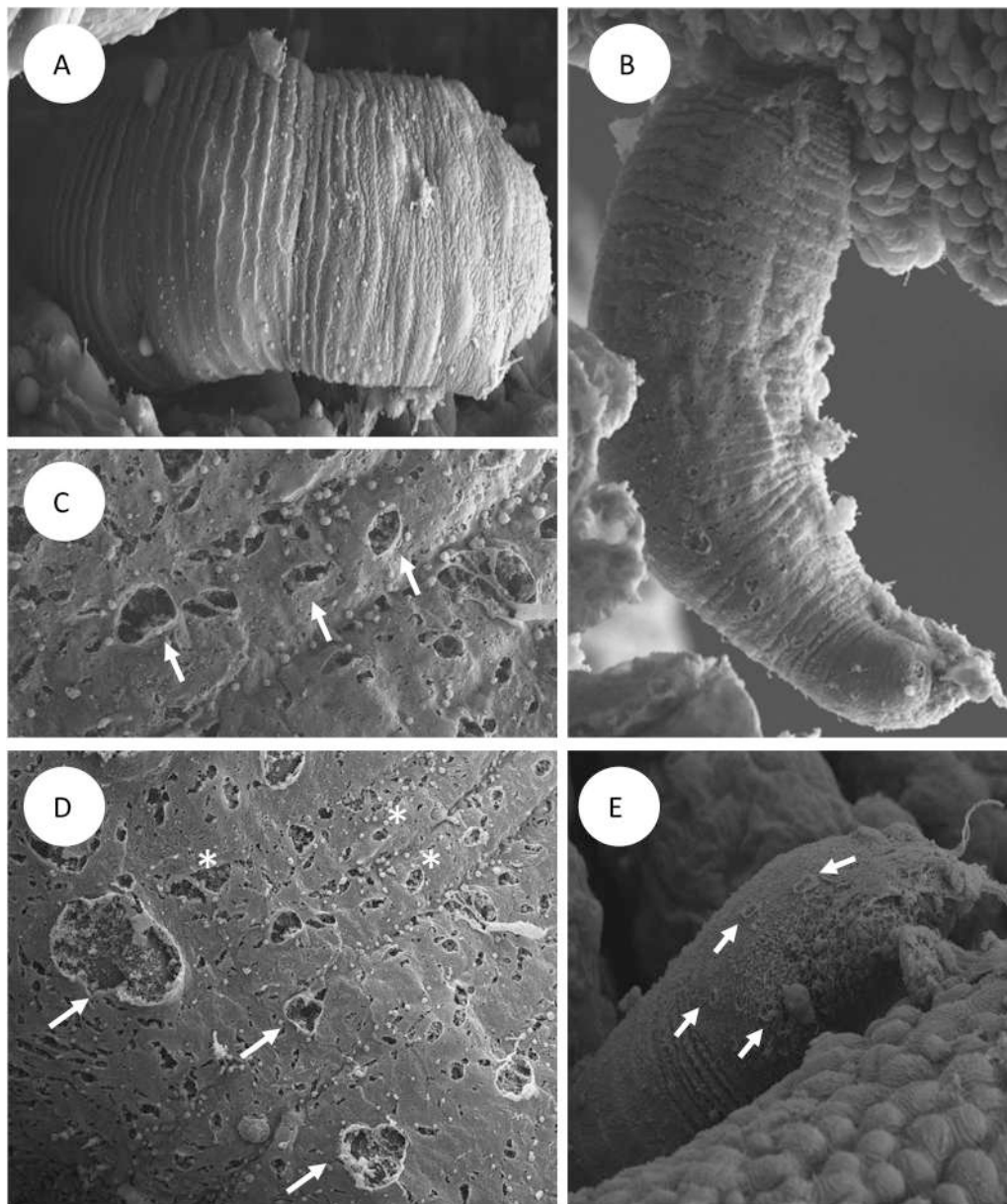


Fig. 3. Scanning electron microscopy (SEM) images of monogeneans exposed to the ethanolic extract of *Jatropha gossypifolia*. Parasite exposed to water, showing characteristic surface ornamentation (A). Monogeneans attached to the parasite following exposure to 600 mg L⁻¹ (B). Presence of deep tissue alterations (arrow) and surface alterations (asterisk) in monogeneans exposed to 600 mg L⁻¹ (C–D). Perforation of the integument (arrows) observed in monogeneans exposed to 2000 mg L⁻¹ (E).

antiparasitic efficacy of essential oils (Gonzales et al., 2022, 2020; Barriga et al., 2020; Huang et al., 2013; Ekanem et al., 2004).

Before administering treatment baths, it is crucial to assess the tolerance of fish to the concentrations identified as ideal during *in vitro* testing. Key criteria for evaluation include fish species, size, extract concentration, and exposure duration, all of which are essential to ensuring animal welfare (Gonzales et al., 2020). During the tolerance assessment, the tested concentrations were observed to induce initial behavioral changes in the fish, likely associated with an acute stress response and the activation of homeostatic regulatory mechanisms under adverse conditions. As the concentrations increased, the intensity of these behaviors also escalated, likely due to the higher availability of bioactive compounds in the medium, which can lead to elevated mortality rates (Shahi and Singh, 2011). The extracts evaluated in this study did not exhibit anesthetic effects in fish, in contrast to findings commonly reported in studies involving essential oils (Gonzales et al.,

2022, 2020; Barriga et al., 2020). Such anesthetic properties are more frequently associated with aromatic plants that contain compounds like menthol, eugenol, geraniol, neral, linalool, citronellal, myrcene, and thymol (Aydin, Barbas, 2020).

In therapeutic baths, ethanolic extracts demonstrated over 90 % efficacy against gill monogeneans at both tested concentrations, surpassing the 70 % threshold considered suitable for field application (Tavares-Dias, 2018). The antiparasitic effect of both plant species may be primarily associated with the activity of vitexin and catechin, compounds that have shown efficacy against the monogenean *Dactylogyrus intermedius* in fish and the protozoan *Trypanosoma cruzi* in *in vitro* studies (Ferri et al., 2023; Ji et al., 2025). Efficacy varies among different plant species and is influenced by factors such as the type of bioactive compounds present, concentration, solvent used, exposure time, and the specific parasite targeted. These parameters are critical in determining the effectiveness of phytotherapeutic agents (de Queiroz et al., 2022;

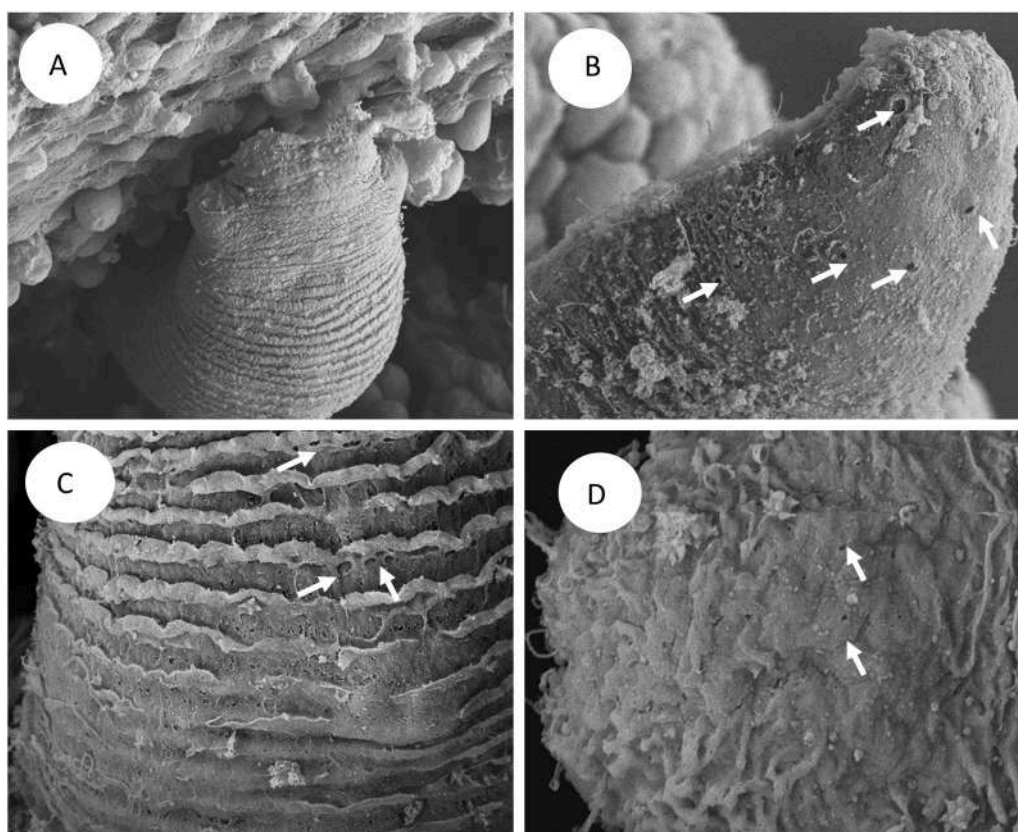


Fig. 4. Scanning electron microscopy (SEM) images of monogeneans exposed to the ethanolic extract of *Jatropha curcas*. Parasite exposed to water treatment, showing normal morphology (A). Deep perforations (arrow) on the ventral side of the body of monogeneans exposed to 1000 mg L⁻¹ (B). Superficial surface alterations (arrows) on monogeneans exposed to 500 mg L⁻¹ (C). Deep tissue perforations in monogeneans exposed to 500 mg L⁻¹ (D).

Table 5

Parasite prevalence (P%) and mean abundance (MA) of monogenean gill parasites in *Piaractus brachypomus* subjected to three-day therapeutic baths with ethanolic extract of *Jatropha gossypifolia*.

Treatments	Water		Water + Alcohol		50 mg L ⁻¹		100 mg L ⁻¹	
Parasite species	P (%)	MA	P (%)	MA	P (%)	MA	P (%)	MA
<i>A. spathulatus</i>	100	376.4 ± 60.9 ^a	100	256.2 ± 62.1 ^b	6.7	0.1 ± 0.1 ^c	0	0.0 ± 0.0 ^c
<i>M. viatorum</i>	100	3.5 ± 1.1 ^a	86.7	5.8 ± 2.9 ^a	0.0	0.0 ± 0.0 ^b	0	10. ± 0.0 ^b
<i>A. penilabiatus</i>	93	6.2 ± 3.7 ^a	73.3	6.1 ± 6.1 ^a	6.7	0.1 ± 0.1 ^b	0	0.0 ± 0.0 ^b

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < 0.05$).

Table 6

Parasite prevalence (P%) and mean abundance (MA) of monogenean gill parasites in *Piaractus brachypomus* subjected to three- and six-day therapeutic baths with ethanolic extract of *Jatropha curcas*.

Treatments	Water		Water + Alcohol		50 mg L ⁻¹		100 mg L ⁻¹	
Parasite species	P (%)	MA	P (%)	MA	P (%)	MA	P (%)	MA
Three-day								
<i>A. spathulatus</i>	100	90.8 ± 10.6 ^a	100	90.1 ± 16.5 ^a	100	10.7 ± 9.91 ^b	88.9	8.22 ± 5.9 ^b
<i>M. viatorum</i>	66.7	1.00 ± 0.67 ^{ab}	66.7	2.89 ± 0.8 ^b	22.2	0.22 ± 0.19 ^a	11.1	0.11 ± 0.1 ^a
<i>A. penilabiatus</i>	100	8.33 ± 2.8 ^a	88.9	8.33 ± 6.7 ^a	66.7	1.89 ± 0.3 ^b	33.3	1.33 ± 1.7 ^b
Six-day								
<i>A. spathulatus</i>	100	85.0 ± 6.4 ^a	100	79.7 ± 13.8 ^a	83.3	2.33 ± 0.9 ^b	83.3	5.67 ± 2.2 ^b
<i>M. viatorum</i>	66.7	2.25 ± 1.6 ^{ab}	66.7	5.17 ± 2.1 ^a	25.0	0.25 ± 0.2 ^{bc}	8.3	0.08 ± 0.1 ^c
<i>A. penilabiatus</i>	100	9.58 ± 2.6 ^a	83.3	10.0 ± 7.8 ^a	16.7	0.58 ± 0.6 ^b	25.0	0.50 ± 0.5 ^b

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < 0.05$).

Zhang et al., 2014; Huang et al., 2013; Ekanem et al., 2004). Understanding the mechanisms of action of secondary metabolites against monogeneans remains a promising area of research, with some studies employing scanning and transmission electron microscopy to elucidate structural and ultrastructural changes. In the present study,

monogeneans exhibited cuticular perforations and burn-like lesions, ranging from mild to severe, following exposure to ethanolic extracts of *J. curcas* and *J. gossypifolia*. Similar alterations, including perforations and cuticular wrinkling, have been documented in monogeneans treated with *Copaifera reticulata* oleoresin and the essential oils of *Cymbopogon*

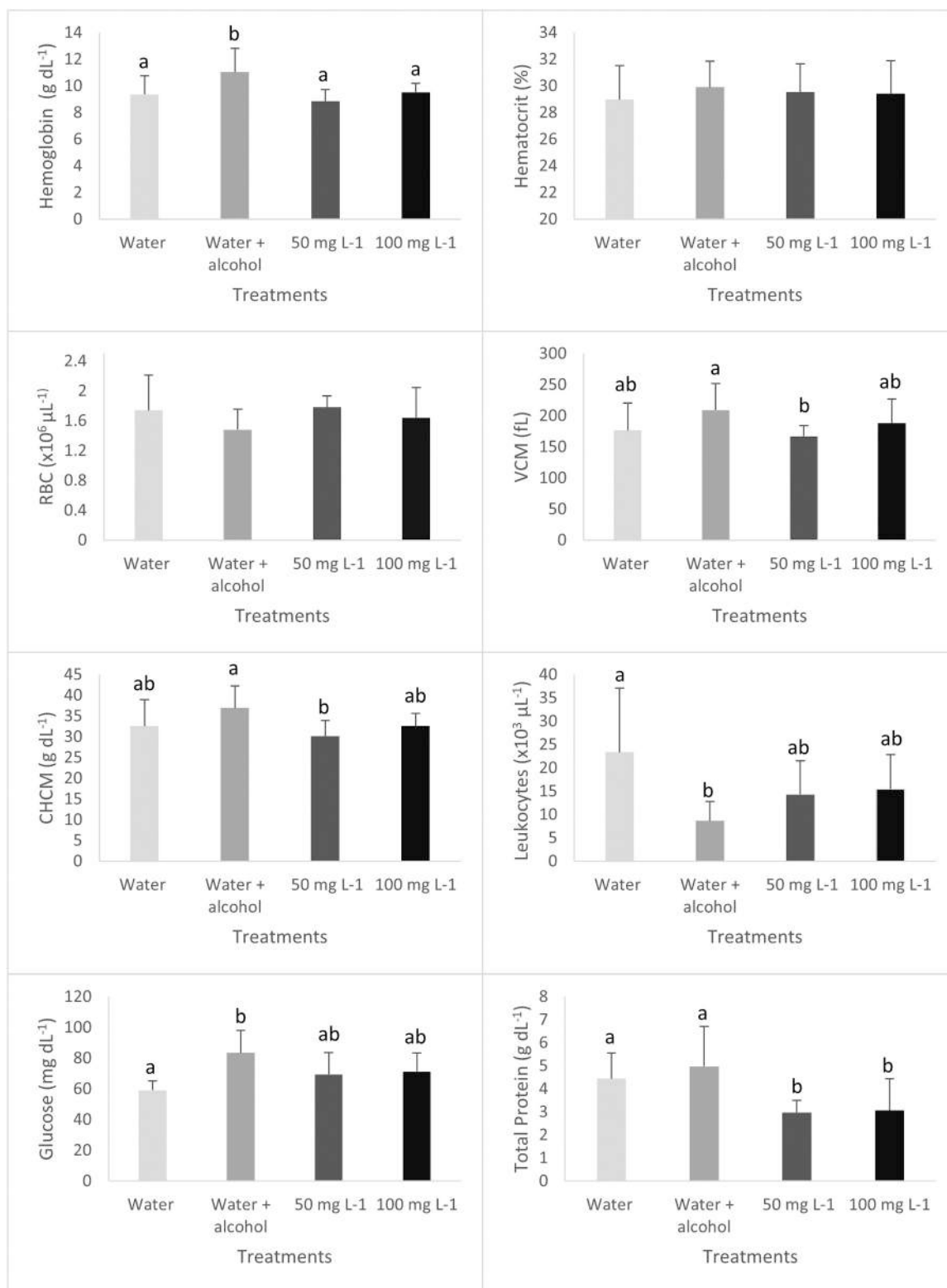


Fig. 5. Hematological and biochemical parameters of *Piaraetus brachypomus* after six consecutive days of therapeutic baths with ethanolic extract of *Jatropha curcas*.

citratus, *Piper callosum*, *P. hispidum*, and *P. marginatum* (Alves et al., 2021; Malheiros et al., 2020; Gonzales et al., 2020). In adult Digenea, *Schistosoma mansoni* exposed to ethanolic extracts of *J. gossypifolia* and *P. arboreum* demonstrated esophageal dilation, abnormal body contractions, internal tissue damage, and destruction of sensory papillae (da Silva Alves et al. 2020). The flavonoids present in extracts of the *Jatropha* genus play a significant role in the mechanism of action against

monogeneans by targeting the parasite's tegument. These compounds interfere with the biosynthesis of fatty acids and functional biomolecules essential for parasite survival, inducing oxidative stress, cellular damage, and tissue alterations that can ultimately lead to parasite death (Rudrapal and Chetia, 2017). Flavonoids such as vitexin, rhoifolin, catechin, luteolin, apigenin, and especially orientin, which are found in the *Jatropha* genus, have been shown to possess free radical scavenging

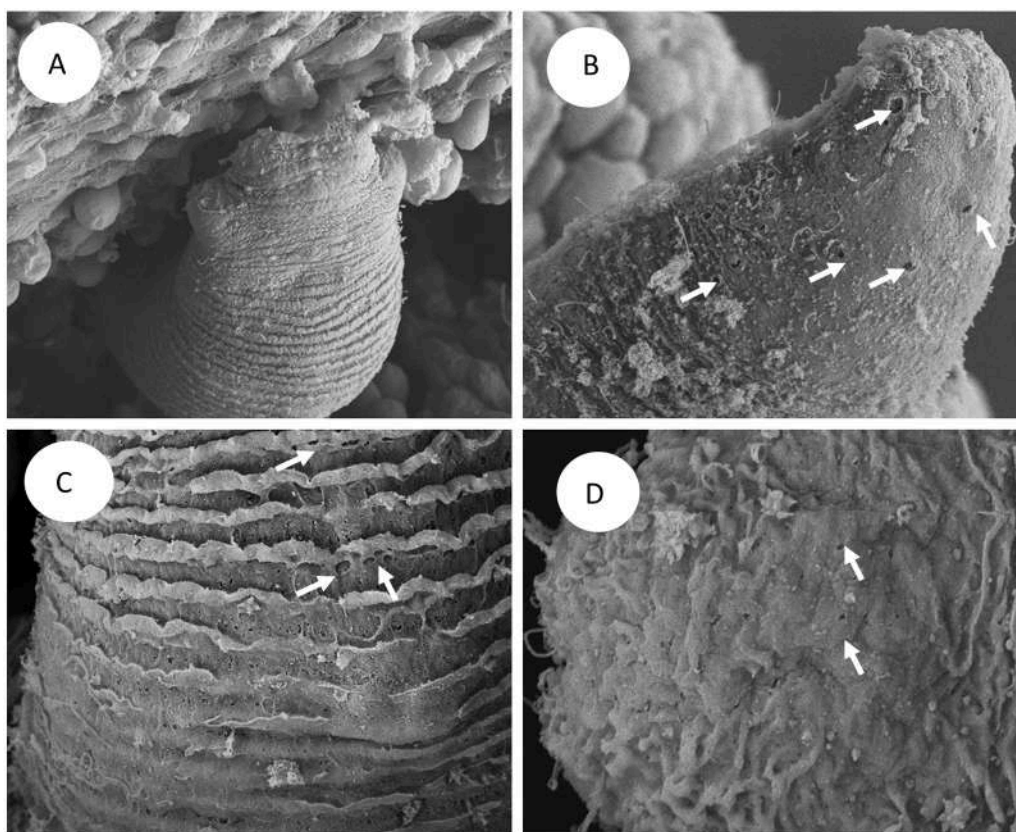


Fig. 6. Hematological and biochemical parameters of *Piaractus brachypomus* after three consecutive days of therapeutic baths with ethanolic extract of *Jatropha gossypifolia*.

Table 7

Thrombocytes and leukocyte differentials in *Piaractus brachypomus* after six days of treatment with *Jatropha gossypifolia* ethanolic extract.

Parameter	Water	Water + Alcohol	50 mg L ⁻¹	100 mg L ⁻¹
Thrombocytes (μL)	62517 ± 21812 ^a	54332 ± 12399 ^a	53711 ± 30641 ^a	73155 ± 42427 ^a
Lymphocytes (μL ⁻³)	11842 ± 9749 ^a	4984 ± 3957 ^{ab}	3354 ± 4300 ^b	2609 ± 1791 ^b
Monocytes (μL ⁻³)	2989 ± 1655 ^a	1146 ± 679 ^b	1549 ± 1325 ^b	1551 ± 1912 ^b
Neutrophils (μL ⁻³)	4864 ± 2743 ^b	3577 ± 3183 ^b	27713 ± 9370 ^a	36839 ± 20589 ^a
PAS-GL (μL ⁻³)	1313 ± 1101 ^a	694 ± 732 ^a	963 ± 1326 ^a	2324 ± 2476 ^a
Eosinophils (μL ⁻³)	24.69 ± 71.93 ^a	71.84 ± 197.8 ^a	18.74 ± 49.75 ^a	39.57 ± 94.96 ^a

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < 0.05$).

Table 8

Thrombocytes and leukocyte differentials in *Piaractus brachypomus* after six days of treatment with *Jatropha curcas* ethanolic extract.

Parameter	Water	Water + Alcohol	50 mg L ⁻¹	100 mg L ⁻¹
Thrombocytes (μL)	60841 ± 25373 ^a	58544 ± 15946 ^a	29195 ± 10634 ^b	36795 ± 14354 ^b
Lymphocytes (μL)	12773 ± 10444 ^a	4095 ± 2831 ^b	6285 ± 3790 ^{ab}	7753 ± 3600 ^{ab}
Monocytes (μL)	3021 ± 1458 ^a	1224 ± 1035 ^b	1354 ± 597 ^b	2395 ± 1175 ^{ab}
Neutrophils (μL)	5188 ± 2657 ^a	2767 ± 2194 ^b	6422 ± 3874 ^a	4995 ± 3816 ^a
PAS-GL (μL)	1105 ± 635 ^a	502 ± 237 ^{ab}	208 ± 259 ^b	342 ± 440 ^b
Eosinophils (μL)	29.6 ± 78.2 ^a	30.7 ± 106 ^a	33.9 ± 68.1 ^a	0.0 ± 0.0 ^a

Values are presented as mean ± SE. Values in the same row with different superscripts are significantly different ($P < 0.05$).

activity. In addition, these compounds exhibit cytotoxic, antivenom, antimalarial, antifungal, antiplasmodial, antidiabetic, antibacterial, and insecticidal properties (Tian et al., 2021; Cavalcante et al., 2020; Almeida et al., 2016; Félix-Silva et al., 2014; Huang et al., 2014). Consequently, their ability to combat monogeneans in fish is aligned with these broader biological activities.

Hematology is widely employed to assess animal welfare, as various factors can influence blood and plasma parameters in fish (Witeska et al., 2022; Soares et al., 2016). Among the two plants evaluated, *J. gossypifolia* induced more pronounced alterations in specific

hematological parameters, indicating a higher level of toxicity in fish. The agitation and immobilization observed during treatments represent initial stress responses, which lead to increased energy demand for oxygen transport at the cellular level (Witeska et al., 2022). The reduction in hemoglobin and mean corpuscular hemoglobin concentration (MCHC) observed in fish exposed to *J. gossypifolia* is associated with gill hemorrhaging and anemia induced by the treatment baths, as previously documented (Witeska et al., 2022; Soares et al., 2016). Glucose levels were elevated in fish treated with 100 mg L⁻¹ of ethanolic extract of *J. gossypifolia* compared to the water control group, whereas total

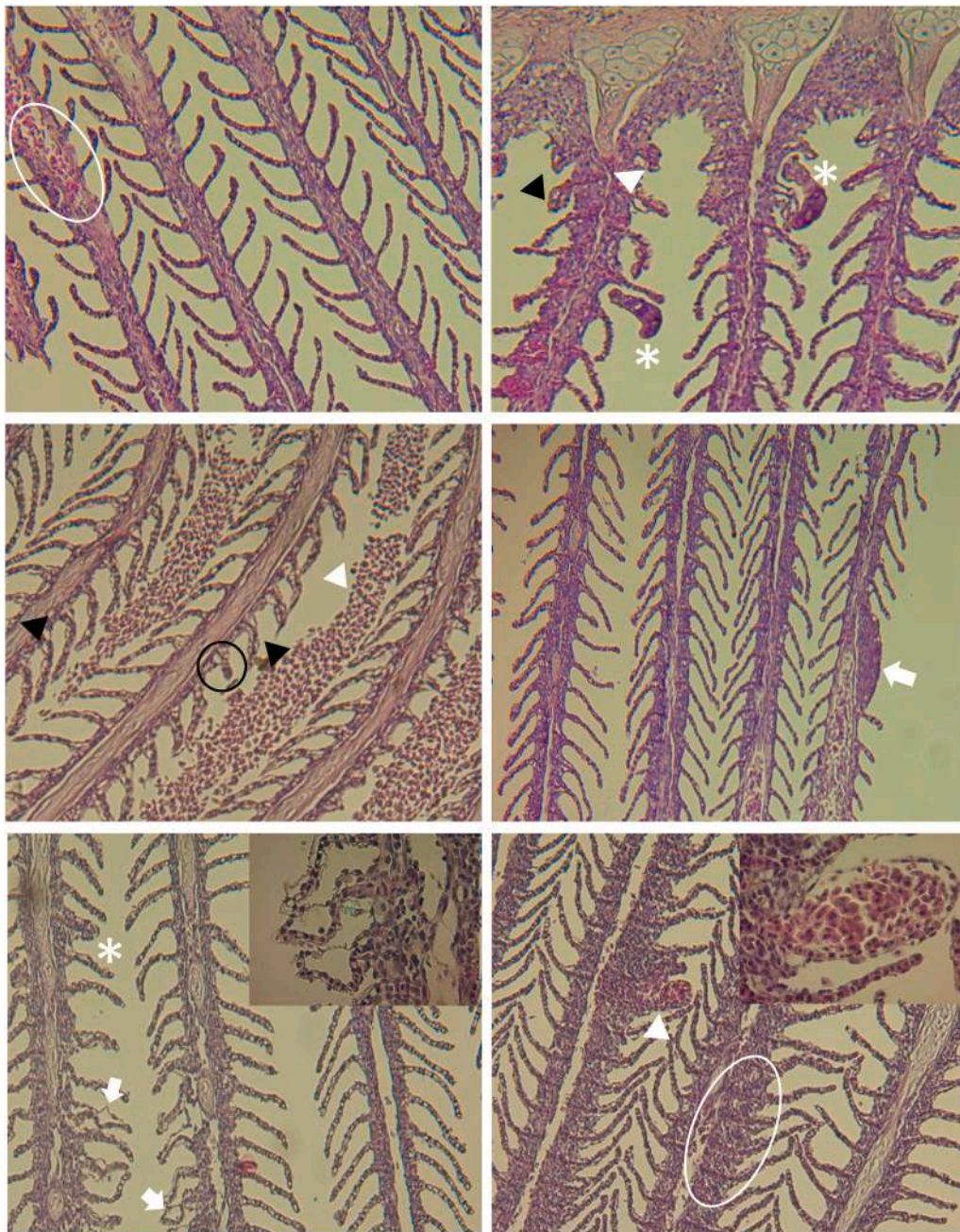


Fig. 7. Gills of fish exposed to water (control), showing congestion of the primary lamellae (circle) (A). Hyperplasia of the secondary lamellae (black triangle), lamellar displacement (white triangle), and presence of monogenean parasites (*) in the gills of fish exposed to the water + ethanol control (B). Hemorrhage due to epithelial rupture (white triangle), displacement of secondary lamellae (black triangle), and epithelial hyperplasia (circle) in fish exposed to 50 mg L⁻¹ of ethanolic extract of *Jatropha curcas* (C). Hyperplasia with fusion of secondary lamellae (arrow) in fish exposed to 100 mg L⁻¹ of ethanolic extract of *J. curcas* (D). Epithelial hyperplasia (*), displacement of secondary lamellae (arrows) in fish exposed to 50 mg L⁻¹ of ethanolic extract of *Jatropha gossypifolia* (E). Fusion of secondary lamellae (circle) and aneurysm (arrow) in fish exposed to 100 mg L⁻¹ of ethanolic extract of *J. gossypifolia* (F).

protein levels decreased at a concentration of 50 mg L⁻¹ and in fish exposed to both concentrations of *J. curcas* extract. Alterations in plasma parameters reflect a rapid physiological response to stress, often leading to increased metabolic energy demand and potential hepatic damage, as reported in studies examining the effects of medicinal plants, toxic agents, and heavy metals (Hashimoto et al., 2016; Sánchez et al., 2017; de Queiroz et al., 2022). Alcohol has been widely employed as a solvent in phytotherapeutic studies. In the present investigation, alcohol significantly increased hemoglobin and glucose levels in fish, consistent with findings from previous studies involving therapeutic baths (Gonzales et al., 2020; Soares et al., 2016). These results suggest that

alcohol may induce anoxic conditions and, when combined with bioactive compounds from certain plant species, could potentially enhance overall toxicity (Soares et al., 2017b; Soivio and Oikari, 1976).

The gills are among the most vital organs in fish, and gill lesions can serve as indicators of environmental pollution, thereby acting as reliable biomarkers for assessing the toxicity of treatments administered to fish (Poleksic and Mitrovic-Tutundzic, 1994). In the present study, fish exposed to ethanolic extracts exhibited a higher frequency of gill histopathological alterations, including epithelial hyperplasia, displacement and fusion of secondary lamellae and even aneurysms, which were exclusively observed in fish treated with the highest concentration of

J. gossypifolia extract. Although medicinal plants are of natural origin, treatment baths can induce gill disorders in fish that range from mild to severe, regardless of the concentration applied (Barriga et al., 2020; Gonzales et al., 2020; Soares et al., 2017a, 2017b, 2016). Most alterations reported in previous studies are classified as stage I or II and are considered reversible with the improvement of water quality parameters (Poleksic and Mitrovic-Tutundzic, 1994). In this study, the use of alcohol as a solvent resulted in gill lesions similar to those observed following exposure to plant extracts. Several laboratory studies have indicated that alcohol can cause gill damage, suggesting that its use should be carefully moderated or replaced with less aggressive solvents (Soares et al., 2017a, 2017b, 2016).

5. Conclusion

The ethanolic extracts of *J. gossypifolia* and *J. curcas* demonstrated over 90 % efficacy against gill monogeneans in *P. brachypomus* following treatment baths. However, both extracts caused alterations in fish behavior, gill tissues and blood parameters as a rapid stress response, indicating a potential toxic effect associated with the concentrations used. Therefore, the therapeutic use of both ethanolic extracts should be applied with caution. Further research is recommended to determine the median lethal concentration (LC₅₀) of the extracts to ensure their safety in fish and other aquatic organisms.

Data sharing statement

The authors emphasize the availability of data and materials.

CRediT authorship contribution statement

Alexandra Cornejo-Rigaud: Methodology, Investigation, Data curation. **Fernandez-Mendez Christian:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Flores Gonzales Anai:** Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Omar Mertins:** Writing – review & editing, Methodology, Conceptualization. **Marcos Tavares-Dias:** Writing – review & editing, Validation, Conceptualization. **Marian Paredes:** Methodology, Investigation, Data curation. **Patrick Mathews Delgado:** Writing – review & editing, Methodology, Conceptualization. **Noelia Estela:** Methodology, Investigation, Data curation.

Consent for publication

The authors approved this version of the manuscript for publication.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Anai Flores Gonzales reports financial support was provided by National Council of Science Technology and Technology Innovation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

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