Turk. J. Fish.& Aquat. Sci. 26(4), TRJFAS28287
https://doi.org/10.4194/TRJFAS28287

RESEARCH PAPER

Turkish Journal of FISHERIES and AQUATIC SCIENCES

Dual Antioxidant and Antiparasitic Activities of *Melia azedarach* Ethanol Extract in Enhancing Health and Growth Performance of Freshwater Catfish

Thi Phuong Nhung Tran[1,*], Thi Hoan Vu[1]

[1]Industrial University of Ho Chi Minh City, Institute of Biotechnology and Food Technology, Ho Chi Minh city, Vietnam (700000).

How to Cite

Tran, T.P.N., Vu, T.H. (2026). Dual Antioxidant and Antiparasitic Activities of *Melia azedarach* Ethanol Extract in Enhancing Health and Growth Performance of Freshwater Catfish. *Turkish Journal of Fisheries and Aquatic Sciences*, *26(4), TRJFAS28287.* https://doi.org/10.4194/TRJFAS28287

Article History
Received 28 April 2025
Accepted 08 October 2025
First Online 15 October 2025

Corresponding Author
E-mail: tranthiphuongnhung@iuh.edu.vn

Keywords
Aquaculture,
Clarias fuscus,
Melia azedarach,
Oxidative stress,
Trichodina nigra.

Abstract

To evaluate the dual antioxidant and antiparasitic effects of *Melia azedarach* leaf ethanol extract (EMAE) in whitespotted catfish (*Clarias fuscus*). In vitro, six groups assessed DPPH/FRAP and *Trichodina nigra* growth inhibition; in vivo, infected fish received formalin (100 mg/L, 30 min/day ×3) or EMAE baths (800-1000 ppm, 60 min/day ×3). EMAE showed dose-dependent antioxidant activity (EMAE1000: DPPH 49.43%; FRAP 0.56 mmol Fe^{2+}/g). In vitro, EMAE1000 inhibited parasite growth (20.98% at 72 h). In vivo, EMAE reduced *T. nigra* intensity (skin 18.67; gills 27.67 at 1000 ppm) and improved growth (BWG 37.86%; SGR 1.24% day^{-1}). Oxidative stress markers (NO, O_2^-) decreased, while antioxidant enzymes (PON, TrxR, POD) increased toward control levels. EMAE provides measurable antiparasitic and redox-modulating benefits in *C. fuscus*, supporting its potential as a sustainable adjunct to conventional treatments.

Introduction

Oxidative stress and parasitic infections are major constraints in aquaculture, undermining fish immunity, growth, and survival (Buchmann, 2022; Nisa et al., 2024). Oxidative stress reflects an imbalance between the generation of reactive oxygen species (ROS) and the capacity of antioxidant defenses to neutralize them (Bernd et al., 2022). Although ROS arise as by-products of cellular metabolism and are detoxified by superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Marta et al., 2024), excessive ROS induced by environmental stressors (pollution, temperature shifts, suboptimal water conditions) overwhelm these systems and damage lipids, proteins, and DNA (Samar et al., 2023), thereby compromising fish health and increasing susceptibility to infection with significant economic losses (Changyou et al., 2023; Mahmoud et al., 2020). Against this backdrop, we targeted the whitespotted clarias (*Clarias fuscus*), an ecologically and economically important species widely farmed in southern Viet Nam, where trichodinid ciliates (e.g., *Trichodina* spp.) are recognized drivers of morbidity and growth depression in intensive culture; testing a plant-derived therapy against *T. nigra* in this host is therefore directly relevant to production needs (Tran and Le, 2025).

Synthetic chemicals are widely employed to control oxidative stress and parasitic diseases. However, their continued use is linked to ecological harm, drug resistance, and bioaccumulation in aquatic ecosystems (Nhung and Buu, 2023; Jayaseela et al., 2022; Gholami et al., 2021). These concerns underscore the urgent need for eco-friendly and effective alternatives that promote fish health while ensuring environmental safety (Velusamy et al., 2021; Melba et al., 2023).

Melia azedarach Linn. (Meliaceae), native to Asia and now widely distributed across tropical and

subtropical regions worldwide (Ramírez et al., 2024), is common in Viet Nam (Burgess et al., 2020) and has garnered attention for its antioxidant and antiparasitic properties; in this study, leaves were collected in Song Trau Commune, Trang Bom District, Đong Nai Province, Vietnam. *M. azedarach* leaves, seeds, and bark contain flavonoids, phenolics, saponins, and alkaloids, compounds recognized for redox-modulating and antimicrobial activities (Marzie et al., 2024). Ethanol leaf extracts show strong radical-scavenging capacity and inhibit lipid peroxidation (Bahaa et al., 2023), while modulating antioxidant enzymes, an effect attributed to bioactives such as oleamide and fatty acids (Maria et al., 2022). This assertion is supported by chemical antioxidant assays, DPPH (radical-scavenging capacity; lower IC_{50} indicates stronger activity) and FRAP (ferric-reducing power; higher Fe^{2+} equivalents indicate stronger activity), which consistently demonstrate concentration-dependent responses in *M. azedarach* leaf extracts, consistent with their phenolic/flavonoid content (Bahaa et al., 2023; Marzie et al., 2024). In parallel, reports of selective cytotoxicity, low or negligible toxicity to non-tumor/host cell lines, and acceptable safety in acute/subacute exposure while retaining activity against target pathogens support a favorable safety margin (Martha et al., 2020; Maria et al., 2020).

In addition to its antioxidant effects, *Melia azedarach* has demonstrated antiparasitic efficacy against *Trichodina nigra* (a trichodinid ciliate) in *Clarias fuscus* (whitespotted clarias) (Nhung and Buu, 2023). Similar effects have been reported in other hosts, including common carp (*Cyprinus carpio*) infected with monogenean ectoparasites treated effectively with *M. azedarach* fruit extracts, and goldfish (*Carassius auratus*) infected with *Gyrodactylus kobayashii* treated with cortex *Meliae* (*Melia* bark) extracts, etc; pharmacopoeial sources attribute this crude drug to *M. toosendan* or *M. azedarach* (Faranak et al., 2023; Adak and Kumar, 2022; Meihong et al., 2022; Hoda et al., 2019). Suggested mechanisms include inhibition of parasite enzymes, disruption of cellular membranes, and host immune modulation (Luise et al., 2022; Liuhong et al., 2024). Integrating phytotherapeutics like *M. azedara*ch into disease control strategies aligns with the principles of sustainable aquaculture by reducing chemical inputs, minimizing ecological footprint, and promoting fish welfare through natural means (Claudia et al., 2023; Melba et al., 2023).

Accordingly, this study aims to investigate the antioxidant and antiparasitic efficacy of ethanol extract from *Melia azedarach* leaves using *in vitro* and *in vivo* approaches in whitespotted clarias (*Clarias fuscus*), contributing to environmentally responsible and sustainable aquaculture practices. In aquaculture, plant-derived candidates typically progress through a two-stage pipeline: (i) *in vitro* assays (e.g., DPPH/FRAP for antioxidant capacity; parasite motility/paralysis-/mortality tests to estimate LC_{50}/EC_{50}; and cytotoxicity screens in fish cells or early-life stages) and (ii) *in vivo* infection trials in the target species to confirm efficacy and safety under realistic conditions (reductions in gill/skin parasite load, improved clinical signs and survival, and normalization of oxidative-stress and hematological biomarkers). This workflow is consistent with current fish-parasitology practice and recent studies that pair *in vitro* results with *in vivo* validation for sustainable disease control (Khetsha et al., 2024).

Materials and Methods

Collection of Plant Material and Preparation of the Extract

Fresh and healthy leaves of *Melia azedarach* were collected in September 2024 from Song Trau Commune, Trang Bom District, Dong Nai Province, Vietnam. A voucher specimen (MA110924VST) was deposited at the Department of Biotechnology, Institute of Biotechnology and Food Technology, Ho Chi Minh City University of Industry. The collected leaves were thoroughly washed with distilled water, air-dried at ambient temperature (25±1°C), and ground into fine powder using a mechanical grinder (TMND-A23, Tan Minh Co., Ltd., Vietnam), then sieved to 60-mesh (~250 µm).

A total of 200 g of powdered material was macerated in 2000 mL of 70% ethanol for 72 hours at room temperature (25±1°C) under continuous stirring. The mixture was filtered through Whatman No. 4 filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator (RV 10 Digital V-C, IKA, Germany). The obtained ethanol extract (designated EMAE) was stored in airtight amber glass containers at 4°C until further use.

The extract was not standardized to a single marker compound; batch quality was controlled by total polyphenols and total flavonoids (TPC/TFC), and all experiments were run with the same extract lot.

Qualitative Phytochemical Screening

A preliminary phytochemical analysis was performed to detect the presence of major bioactive classes, including terpenoids, steroids, alkaloids, tannins, polyphenols, flavonoids, saponins, and cardiac glycosides, using standard colorimetric methods as described by Nhung et al. (2023). The detection relied on visual color changes and precipitate formation in the presence of specific chemical reagents. A concise summary of the detection methods is presented in Table 1. All reagents and media were sourced as follows: Folin-Ciocalteu reagent (Merck KGaA, Darmstadt, Germany); sodium carbonate (Merck KGaA, Darmstadt, Germany); 2,2-diphenyl-1-picrylhydrazyl, DPPH (Sigma-Aldrich, St. Louis, MO, USA); 2,4,6-tripyridyl-s-triazine, TPTZ (Sigma-Aldrich, St. Louis, MO, USA); ferric chloride hexahydrate, $FeCl_3 \cdot 6H_2O$ (Merck KGaA, Darmstadt,

Germany); RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, MA, USA); and tricaine methanesulfonate, MS-222 (Sigma-Aldrich, St. Louis, MO, USA).

Quantification of Major Phytochemical Constituents

Total Polyphenol Content (TPC)

Polyphenol content was determined using the Folin-Ciocalteu method. Briefly, 100 µL of extract (100 µg/mL) was mixed with 500 µL of 10% Folin-Ciocalteu reagent and 4 mL of 7.5% sodium carbonate solution. The mixture was incubated for 60 minutes at room temperature (25±1°C), and absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV-1900i, Shimadzu, Japan). Results were expressed as mg gallic acid equivalents (GAE) per gram of extract (Khetsha et al., 2024).

Total Flavonoid Content (TFC)

The total flavonoid content was measured based on the aluminum chloride colorimetric assay. One milliliter of extract was mixed with 1 mL of 2% aluminum chloride in methanol, followed by the addition of 2 mL of 1 M sodium hydroxide after 10 minutes of incubation. The mixture was allowed to react for 30 minutes before absorbance was recorded at 430 nm. Results were expressed as mg quercetin equivalents (QE) per gram of extract (Khetsha et al., 2024).

Saponin Content

Saponin concentration was determined using the vanillin–sulfuric acid method. One milliliter of extract was reacted with 1 mL of vanillin-sulfuric acid reagent and incubated at 60°C for 30 minutes. After cooling, absorbance was measured at 544 nm. The results were calculated as mg saponin equivalents (SE) per gram of extract (Nhung, 2025).

Tannin Content

Tannin content was assessed using the vanillin–hydrochloric acid assay. One milliliter of extract (10 mg/mL) was mixed with 1 mL of 4% vanillin and 1 mL of concentrated HCl. The mixture was incubated at 40°C for 30 minutes, and absorbance was measured at 550 nm. Tannin content was reported as mg tannin equivalents (TE) per gram of extract (Nhung et al., 2023).

Research Design and Treatment Groups (in Vitro & in Vivo)

Overview. The study comprised parallel *in vitro* antiparasitic/antioxidant assays and an *in vivo* bath-therapy trial in infected fish. Designs were pre-specified, randomized across positions/tanks, and aligned in time points to enable cross-level comparisons (baseline, 24, 48, 72 h). Detailed procedures for parasite culture, assays, diet/feeding, and biomarker measurements are provided in Sections 2.5 and 2.6.

In Vitro Design (anti-Trichodina Assay and Antioxidant Tests)

Groups (n= 6*)*: (1) Blank (RPMI-1640), (2) Distilled water, (3) Formalin 100 ppm (positive control), and (4-6) EMAE at 800, 900, and 1000 ppm.

Replication: Three independent wells per group (n= 3). Approximately 10^4 *T. nigra* were inoculated per test vessel.

Conditions: 25±1°C, pH 7.0-7.4, dissolved oxygen (DO) 5-6 mg L^{-1}; identical aeration and partial water renewal as needed. *Time points*: 24, 48, and 72 h.

Primary endpoints: Percentage inhibition of *T. nigra* growth and parasite counts (automated counter

Table 1. Phytochemical screening and quantification of the ethanol extract of *Melia azedarach* leaves (EMAE)

Phytoconstituents	Test description	Observation	Detection	Quantification (mg/g extract)
Tannins	2 mL EMAE + 2 mL H_2O + 2–3 drops $FeCl_3$ (5%)	Green precipitate	Present	8.03±0.34 (mg TE/g)
Flavonoids	1 mL EMAE + 1 mL $Pb(OAc)_4$ (10%)	Yellow coloration	Present	36.66±1.03 (mg QE/g)
Terpenoids	2 mL EMAE + 2 mL $(CH_3CO)_2O$ + 2–3 drops conc. H_2SO_4	Deep red coloration	Present	–
Polyphenols	2 mL EMAE + 2 mL $FeCl_3$	Bluish-green appearance	Present	65.42±1.34 (mg GAE/g)
Saponins	5 mL EMAE + 5 mL H_2O, heated	Froth formation	Present	14.59±0.37 (mg SE/g)
Steroids	2 mL EMAE + 2 mL $CHCl_3$ + 2 mL H_2SO_4 (conc.)	Reddish-brown ring at the junction	Present	–
Cardiac glycosides	2 mL EMAE + 2 mL $CHCl_3$ + 2 mL CH_3COOH	Violet to blue-green coloration	Absent	–
Alkaloids	2 mL EMAE + a few drops of Hager's reagent	Yellow precipitate	Present	–

Quantification values are expressed as mean±standard deviation (SD) (n= 3). Abbreviations: TE= tannin equivalents; QE= quercetin equivalents; GAE= gallic acid equivalents; SE= saponin equivalents. Values are mean±SD; in vitro n= 3 wells per group per time point; in vivo n= 10 fish per group. Units mg equivalents g^{-1} extract.

with microscopic verification). Growth inhibition (%) was computed as

Growth inhibition (%) = [(Control – Treated) / Control] x 100

Secondary endpoints: Antioxidant activity by DPPH (A_{517}) and FRAP (A_{593}) with standard controls/standards and IC_{50} or equivalent summary metrics.

Bias control: Randomization of replicate placement to minimize positional effects.

Further methods: Parasite culture and assay procedures are detailed in 2.5.1-2.5.4.

In Vivo Design (Bath Therapy in Infected Fish)

Experimental animals: White-spotted catfish (*Clarias fuscus*) clinically healthy at enrollment; screening, husbandry, and ethics approvals as in 2.6.1.

*Groups (*n= 6*;* 10 fish/group*)*: (1) Normal control (uninfected, vehicle), (2) Disease control (infected, vehicle), (3) Formalin 100 mg L^{-1} for 30 min day^{-1} × 3 (positive control), and (4-6) EMAE at 800, 900, and 1000 ppm for 60 min day^{-1} × 3. Groups (2-6) were challenged with *T. nigra* before treatment per 2.6.1.

Conditions and feeding: Uniform water quality across tanks; diet/feeding rate, storage, logs, and performance indices per 2.6.2 (and calculations in 2.6.4).

Schedule and sampling: Assessments at baseline (0 h), 24, 48, and 72 h after the first treatment; at each time point, n= 3 fish per group were sampled.

Primary endpoint: T. nigra load on gills and skin.

Secondary endpoints: Survival and clinical signs; oxidative-stress and hematological biomarkers (e.g., NO, O_2^-, PON, TrxR, POD, GSSG), and growth indices (BWG, DWG, SGR).

Bias control: Random allocation to tanks; identical husbandry to minimize positional/environmental bias.

Further methods: Infection procedure, sampling, biomarker analyses, and index calculations are detailed in 2.6.1-2.6.4.

In Vitro Evaluation of Antioxidant and Antiparasitic Properties

Design is summarized in Section 2.4; detailed procedures for parasite culture, assays, and readouts are provided below (Sections 2.5.1-2.5.4).

In Vitro Culture of Trichodina nigra

T. nigra specimens were obtained from the Department of Biotechnology, Faculty of Natural Sciences, Vietnam National University, Hanoi. Parasites were cultured in RPMI 1640 medium at 26 ± 1°C, pH 7.4, with a dissolved oxygen level of 6 mg/L. When the culture reached a density of 10^4 parasites/mL, cells were harvested and stored at 4°C for subsequent use (Nhung and Buu, 2023).

Antioxidant Activity Assays

DPPH radical scavenging assay: A 0.1 mM DPPH methanolic solution was mixed with EMAE (10-100 µg/mL) at a 1:1 (v/v) ratio, incubated for 30 min in the dark at 25±1°C, and the absorbance was read at 517 nm (UV-1900i, Shimadzu, Japan). DPPH is a stable free radical (purple) that is reduced to the non-radical DPPH-H (yellow) when an antioxidant donates a hydrogen atom/electron; thus, the decrease in A517 is proportional to radical-scavenging capacity. A methanol blank and a DPPH control (no extract) were included, Trolox served as a reference standard, and reactions were run in triplicate with IC_{50} values derived from concentration-response curves.

DPPH scavenging activity (%) = $[(A_{control} - A_{sample})/A_{control}]$ x 100 (Natividad et al., 2020)

Ferric reducing antioxidant power (FRAP): The FRAP working reagent (300 mM sodium acetate buffer, pH 3.6; 10 mM TPTZ dissolved in 40 mM HCl; and 20 mM $FeCl_3 \cdot 6H_2O$ mixed at 25:2.5:2.5, v/v/v) was freshly prepared and pre-warmed to 37°C. Extracts (10-100 µg/mL) were mixed with the reagent (1:2, v/v) and incubated for 30 min at 37°C. Absorbance was read at 593 nm, and results were expressed as mmol Fe^{2+}/g extract. TPTZ (2,4,6-tripyridyl-s-triazine) is the chromogenic ligand in FRAP; antioxidants reduce the Fe(III)-TPTZ complex to Fe(II)-TPTZ, forming an intense blue complex with λmax= 593 nm. The increase in A_{593} is therefore proportional to the sample's ferric-reducing/antioxidant power (Natividad et al., 2020).

Rotary evaporator (RV 10 Digital V-C; IKA, Staufen, Germany); UV-Vis spectrophotometer (UV-1900i; Shimadzu, Kyoto, Japan); automated cell counter (E7500-E; Accuris, Edison, NJ, USA); compound microscope (CX23; Olympus, Tokyo, Japan); analytical balance (Entris; Sartorius, Göttingen, Germany); multiparameter water-quality meter (ProDSS; YSI Inc., Yellow Springs, OH, USA).

Growth Inhibition Assay for T. nigra

Approximately 10^4 *T. nigra* cells were inoculated into each treatment well. All treatments and the Normal control were incubated under identical water conditions (25±1°C; pH 7.0-7.4; DO 5-6 mg/L) for 24-72 h. Parameters were checked at 0/24/48/72 h and maintained via standardized aeration and partial water renewal. Parasite counts were performed using an E7500-E automated cell counter (Accuris, USA), and growth inhibition was calculated as:

Growth inhibition (%) = $[(N_{Control} - N_{Treated}) / N_{Control}]$ x 100 (Nhung and Buu, 2023)

Microscopic Examination

Aliquots from each treatment were mounted on glass slides, covered with coverslips, and examined at 400× magnification using an Olympus CX23 microscope. Parasite morphology and counts were documented over 24, 48, and 72 hours.

In Vivo Assessment of Extract Efficacy

The consolidated in vivo research design is presented in Section 2.4; this section details husbandry, infection, treatments, sampling schedule, biomarkers, and index calculations (Sections 2.6.1-2.6.6).

Experimental Animals and Conditions

White-spotted catfish (*Clarias fuscus*, Lacepède, 1803) weighing 45±2 g were obtained from Tư Hải fish farm (Củ Chi, Ho Chi Minh City, Vietnam). After 3 days of acclimatization, 60 clinically healthy fish, defined by normal swimming/feeding, intact skin/fins/gills without lesions, normal opercular rate, and negative skin/gill wet-mounts for ectoparasites, with abnormal or moribund individuals excluded, were randomly assigned to six groups (10 fish per group). Fish were fed a commercial diet supplemented with frozen bloodworms (1% body weight/day). Water quality was maintained at 29.0±0.5°C, pH 7.55±0.11, DO 4.35±0.21 mg L^{-1}, and alkalinity 75.52±0.16 mg L^{-1}; parameters were measured with a calibrated multiparameter meter (ProDSS; YSI Inc., Yellow Springs, OH, USA) following the manufacturer's QA/calibration procedures. Experimental procedures complied with CPCSEA animal care guidelines (Government of India, 2021). All experimental protocols involving animals were reviewed and approved by the Institutional Animal Ethics Committee (Approval No. 205HD/DHNN), in compliance with national and international guidelines for animal experimentation. Before inoculation, each fish was screened and confirmed parasite-free: after light anesthesia with MS-222 (100 mg/L), wet-mount skin and gill scrapes were prepared and examined under a light microscope (100× and 400×; e.g., CX23, Olympus, Japan); any individual with detectable ectoparasites was excluded from the trial.

Diet and Feeding Management

Fish were fed a commercial floating, extruded catfish pellet (grower formulation for Clarias spp.; 2.5-mm diameter; produced by a certified feed mill in Viet Nam; lot number as stated on the accompanying COA). According to the manufacturer's certificate of analysis (COA), the as-fed proximate composition was approximately crude protein 35–38%, crude lipid 6–8%, crude fiber ≤5%, ash 10–12%, and moisture ≤10%. The daily ration was set at 1.0% body weight (BW) per day, divided into two equal meals (09:00 and 17:00 h). Rations were recalculated every 3 days from bulk biomass and temperature-adjusted (reduced by ~50% when water temperature <24°C and withheld when <20°C) to reflect the feeding behavior and metabolic rate of catfish.

Pellets were stored in their original, airtight bags at 4°C and used within ≤4 weeks after opening; for longer storage, unopened bags were kept at −20°C. Feed was brought to room temperature before use. To stimulate initial feeding, frozen bloodworms were provided at ~10–15% of the daily ration for the first 2 days only; the combined pellet + bloodworm amount did not exceed the preset %BW. Uneaten feed was siphoned 30 minutes after each meal and recorded. Feeding logs (offered feed, refusals, biomass updates, and mortality corrections) were used to calculate FCR and PER as described in Section 2.6.4.

Infection and Treatment Protocol

T. nigra (50 parasites/fish) were inoculated onto fish skin. Sampling intervals were 0, 24, 48, and 72 h post-first treatment. Six groups were established: uninfected control, untreated infected, 100 ppm formalin, and EMAE at 800, 900, and 1000 ppm. Treatments were administered once daily for 3 consecutive days; assessments were performed at 0 (baseline), 24, 48, and 72 h after the first treatment (total duration: 72 h). Sampling was performed at defined intervals in accordance with established guidance to minimize stress: fish were lightly anesthetized in buffered MS-222 (≈80-120 mg/L; pH-adjusted to tank water) before mucus/gill scrapes and biomarker sampling; handling time was limited to ≤2 min per fish, fish were kept in temperature-matched, aerated water, handled with soft nets/wet gloves, and returned to recovery tanks immediately. This refinement (brief, buffered tricaine anesthesia and gentle handling) reduces struggling and distress while improving data quality; reporting follows ARRIVE 2.0 recommendations (Aurora, 2022; Nils et al., 2024; Nathalie et al., 2020).

Evaluation of Trichodina nigra Elimination on Fish Skin and Gills

Assessment of *T. nigra* infection intensity in fish skin and gills: Skin samples were collected using sterile swabs or scalpels, placed into test tubes with phosphate buffer, and diluted (1:30) for microscopic examination. Gills were similarly sampled by scraping or excision. A drop of each diluted sample was placed on a slide, covered with a coverslip, and observed under a compound microscope at 400× magnification (Olympus CX23, Japan). Parasite counts were conducted after 24, 48, and 72 hours of treatment to assess the antiparasitic efficacy of the extract.

Each time point involved 3 randomly selected fish per treatment group (n= 3). Counts were expressed as

the number of *T. nigra* per field of view. Parasites were identified on fresh wet-mounts (100×/400×) by size, shape, and motility, when needed, on air-dried smears after silver-nitrate impregnation to visualize the adhesive disc and denticle ring; morphometrics included body and disc diameters, border-membrane width, denticle number/shape, and radial pins, following contemporary trichodinid taxonomy. Infection indicators were evaluated, including:

Prevalence (%) = (Number of infected fish / Total number examined) x 100

Mean intensity = Total number of parasites / Number of infected fish

Eradication rate (%) = [(Parasite count in control – Parasite count in treatment) / Parasite count in control] x 100

All microscopic analyses were performed under consistent environmental conditions (room temperature 25±1°C, pH 7.4, dissolved oxygen 5-6 mg/L).

Growth Performance Evaluation

Fish were lightly anesthetized with MS-222 (100 mg L^{-1}; Sigma-Aldrich, St. Louis, MO, USA), blotted dry, weighed to the nearest 0.01 g on an analytical balance (Entris; Sartorius, Göttingen, Germany), and measured for total length to the nearest 0.1 cm using a digital caliper (500-196-30 Absolute Digimatic; Mitutoyo, Kawasaki, Japan). Body weight was recorded biweekly using an Entris digital scale (Germany). Growth indices were calculated as follows:

BWG (%) = [(Final weight – Initial weight) / Initial weight] x 100

DWG (g/day) = [(Final weight – Initial weight) / Days] x 100

SGR (%/day) = [(Ln final weight – Ln initial weight)/Days] x 100

FCR = Total feed intake (g) / Total fish biomass gained (g)

PER = Body weight gain (g) / Total protein intake (g)

Where, BWG= body weight gain (%); DWG= daily weight gain (g day^{-1}); SGR= specific growth rate (% day^{-1}); FCR= feed conversion ratio; PER= protein efficiency ratio; GAE= gallic acid equivalents; QE= quercetin equivalents; SE= saponin equivalents; TE= tannin equivalents.

Antioxidant Enzyme Assays

Fish tissues (gills, skin, liver) were stored at -80°C, homogenized in PBS, and analyzed for the following markers using spectrophotometric methods:

Nitric oxide (NO), Griess assay (540 nm); superoxide anion (O_2^-), NBT reduction (560 nm); glutathione disulfide (GSSG), DTNB method; paraoxonase (PON), paraoxon hydrolysis (405 nm); thioredoxin reductase (TrxR), Ellman's method (340 nm); peroxidase (POD), chromogenic assay (470 nm). All values were normalized to protein by the Lowry method.

All values were normalized to protein content measured by the Lowry method (Hasin et al., 2024).

Statistical Analysis

All data were expressed as mean±standard deviation (SD). Statistical analyses were performed using Statgraphics Centurion XIX. One-way analysis of variance (ANOVA) was applied to compare differences among experimental groups, followed by appropriate post-hoc tests when applicable. For non-normally distributed variables, the Mann–Whitney U test was employed based on the guidelines of Zar (1999). A p-value less than 0.05 was considered statistically significant.

Results

Phytochemical Composition of *Melia azedarach* Ethanol Extract

Table 1 summarizes the qualitative and quantitative phytochemical profile of the ethanol extract of *Melia azedarach* leaves (EMAE). The extract tested positive for flavonoids, polyphenols, saponins, tannins, terpenoids, steroids, and alkaloids, while cardiac glycosides were not detected. Quantitative results indicated high levels of polyphenols (65.42±1.34 mg GAE/g) and flavonoids (36.66±1.03 mg QE/g), suggesting potent antioxidant potential. Saponins (14.59±0.37 mg SE/g) and tannins (8.03±0.34 mg TE/g) were also present in significant concentrations. These profiles indicate a polyphenol/flavonoid-rich extract, providing a mechanistic basis for the antioxidant readouts and potentially contributing to antiparasitic activity via redox modulation.

In Vitro Antioxidant and Antiparasitic Activity

The antioxidant efficacy of EMAE was assessed using DPPH and FRAP assays (Figures 1 and 2). DPPH radical scavenging activity demonstrated a clear concentration-dependent trend, with EMAE1000 achieving the highest activity at 49.43% ($P<0.05$), followed by EMAE900 (32.66%) and EMAE800 (16.61%). The positive control exhibited 56.78% activity, while the untreated control showed a negligible effect. FRAP analysis further confirmed these findings, with EMAE1000 exhibiting the strongest reducing capacity (0.56 mmol Fe^{2+}/g, $P<0.05$). Together, the concordant DPPH and FRAP responses show a robust concentration-response consistent with the extract's phenolic content, justifying progression to in vivo validation.

In Vitro Antiparasitic Activity Against *Trichodina nigra*

Table 2 presents the growth inhibition percentages and parasite counts at 24, 48, and 72 hours post-treatment. Blank and distilled-water controls showed negligible inhibition, whereas the positive control (formalin) produced the strongest antiparasitic effect. Formalin exhibited the strongest antiparasitic efficacy (22.67% inhibition at 72 h, parasite count reduced to 9.67±2.08). Among the EMAE-treated groups, a dose-dependent trend was observed: EMAE800 showed moderate inhibition (12.18%), EMAE900 performed better (12.68%), and EMAE1000 showed the greatest effect (20.98% inhibition, parasite count reduced to 13.33±1.53). Although formalin remained superior, the monotonic improvement from 800→1000 ppm indicates true pharmacological activity of EMAE against *T. nigra*, rather than a vehicle or handling effect.

In Vivo Antiparasitic and Growth-promoting Effects

Table 3 and Figure 3 show that EMAE reduced *Trichodina nigra* infection intensity on the skin and gills of *Clarias fuscus* in a concentration-dependent manner. Fish treated with EMAE1000 had significantly lower parasite counts (skin: 18.67 *T. nigra* individuals; gills: 27.67 *T. nigra* individuals) and better growth performance (BWG: 37.86% day^{-1}; SGR: 1.24% day^{-1}; $P<0.05$) than EMAE800 and EMAE900. The positive control still performed best across all outcomes. Parasite burdens were quantified separately on skin and gills for each group; additionally, parasite morphometrics (body and adhesive-disc diameters) from representative wet mounts showed no significant differences among groups or time points ($P>0.05$), indicating that EMAE primarily reduces *T. nigra* abundance rather than parasite size. The concomitant

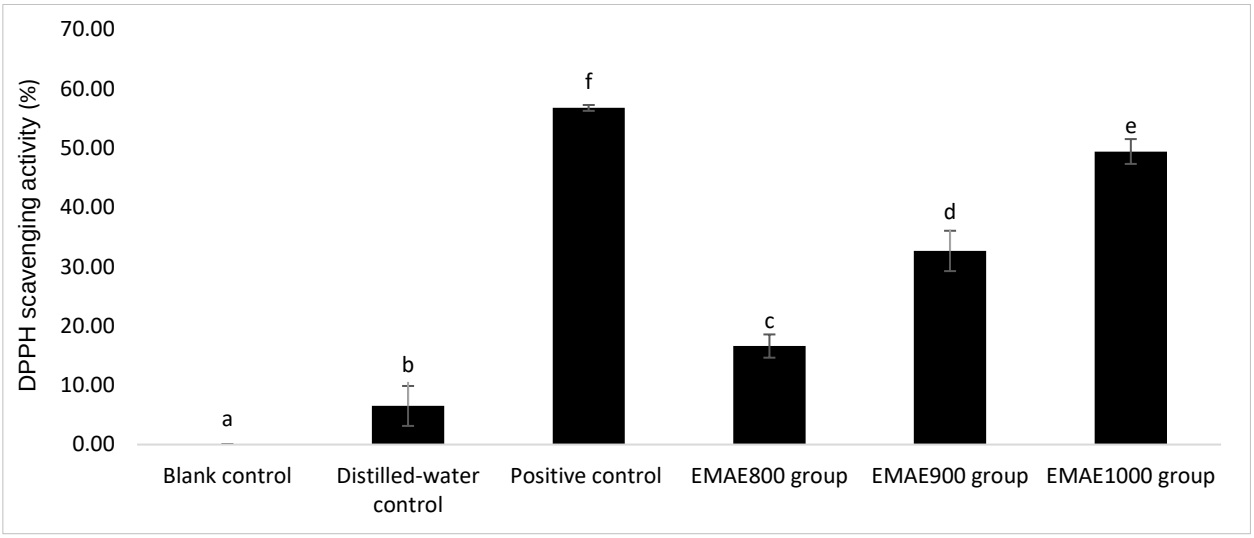

Figure 1. DPPH and FRAP radical scavenging activities of the ethanol extract of Melia azedarach leaves (EMAE) compared to ascorbic acid. Note: Bars represent Mean±SD; different letters indicate significant differences at $P<0.05$.

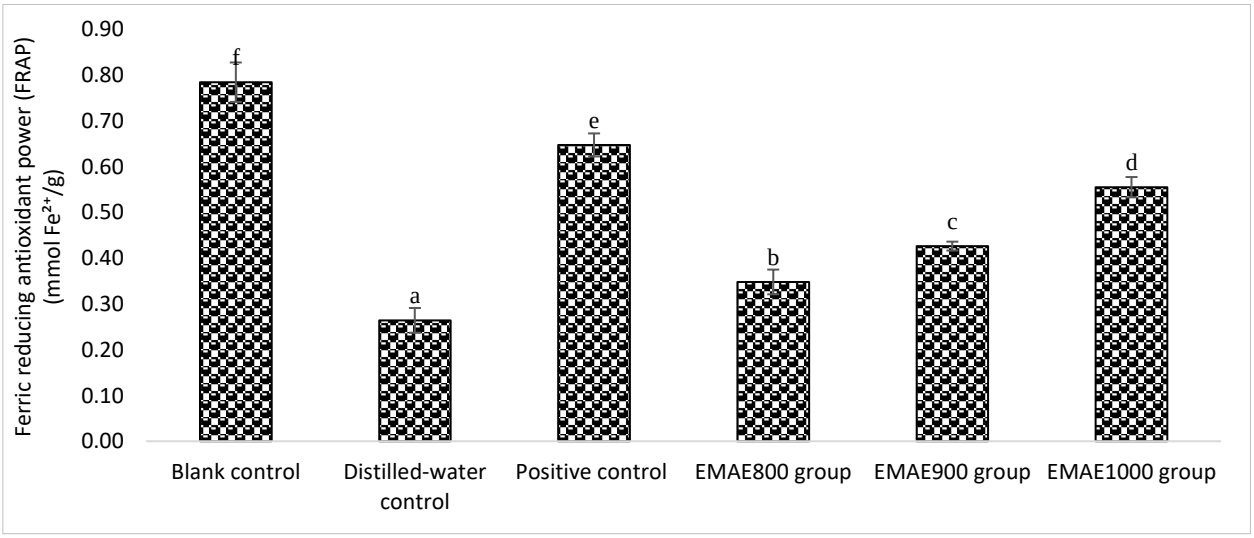

Figure 2. In vitro growth inhibition effect of EMAE and formalin against Trichodina nigra over 24, 48, and 72 hours. Note: Bars represent Mean±SD; different letters indicate significant differences at $P<0.05$.

Table 2. In vitro effectiveness of growth inhibition assay and microscopic examination against *Trichodina nigra*

Groups	Growth inhibition (%) 24h	Growth inhibition (%) 48h	Growth inhibition (%) 72h	Parasite count 24h	Parasite count 48h	Parasite count 72h
Blank control	0.13±0.06[a]	1.49±0.03[a]	2.02±0.03[a]	45.67±2.08[e]	41.33±1.53[e]	39.33±1.53[e]
Distilled-water control	1.53±0.06[a]	1.81±0.03[a]	2.24±0.03[a]	43.67±1.53[e]	39.33±2.08[e]	37.33±1.53[e]
Positive control	12.22±1.81[c]	15.37±0.67[e]	22.67±0.67[d]	18.33±1.53[a]	13.33±2.08[a]	9.67±2.08[a]
EMAE800 group	7.67±1.04[b]	9.75±0.11[b]	12.18±0.43[b]	35.33±1.53[d]	29.67±2.52[d]	25.33±1.53[d]
EMAE900 group	8.65±1.02[b]	10.95±0.12[c]	12.68±0.52[b]	30.67±2.08[c]	24.67±1.15[c]	21.33±1.53[c]
EMAE1000 group	10.97±0.50[c]	14.23±0.72[d]	20.98±0.31[c]	22.33±1.53[b]	17.33±1.53[b]	13.33±1.53[b]

Values are mean±SD; in vitro n= 3 wells per group per time point; in vivo n= 10 fish per group. Units growth inhibition (%) and parasites per field of view.

Table 3. Effect of *Melia azedarach* leaf extract on growth performance in fish

Group	BWG (%)	DWG (g/day)	SGR (%/day)	FCR	PER
Normal control	60.02±0.97[f]	0.95±0.01[f]	4.27±0.06[f]	2.11±0.07[f]	2.55±0.06[f]
Disease control	-39.02±0.23[a]	-0.61±0.01[a]	-1.52±0.02[a]	0.75±0.03[a]	0.91±0.01[a]
Positive control	46.94±0.97[e]	0.74±0.00[e]	4.18±0.05[e]	1.76±0.05[e]	2.13±0.05[e]
EMAE800 group	1.51±0.09[b]	0.02±0.00[b]	0.14±0.02[b]	0.96±0.03[b]	1.16±0.02[b]
EMAE900 group	13.15±0.03[c]	0.21±0.00[c]	0.58±0.02[c]	1.17±0.04[c]	1.42±0.03[c]
EMAE1000 group	37.86±0.71[d]	0.59±0.01[d]	1.24±0.05[d]	1.51±0.04[d]	1.82±0.04[d]

Values are mean±SD; in vitro n= 3 wells per group per time point; in vivo n= 10 fish per group. Units BWG (%), DWG (g day^{-1}), SGR (% day^{-1}), FCR (unitless), PER (g g^{-1}). Negative BWG/DWG/SGR values in the Disease control reflect net weight loss due to infection/anorexia during the trial; by definition, these indices become negative when final body mass is lower than initial. FCR and PER for net-loss intervals are not biologically interpretable; therefore, values were recalculated for positive-gain windows or marked 'NA' where appropriate.

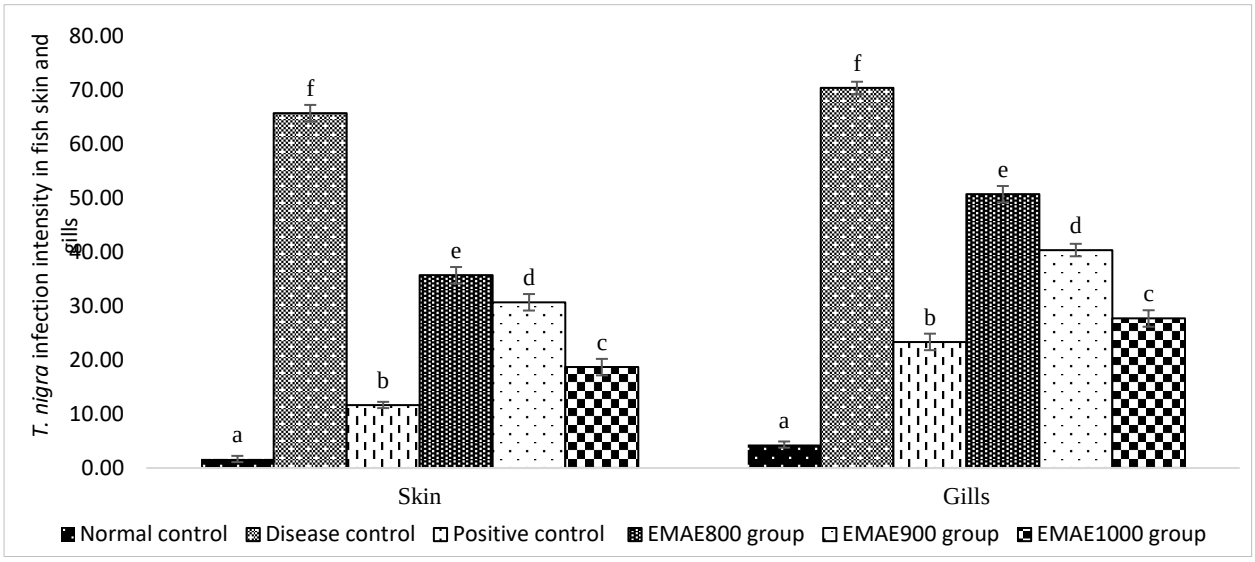

Figure 3. Effect of EMAE treatments on nitric oxide (NO) levels in gill, skin, and liver tissues of infected fish. Note: Bars represent Mean±SD; different letters indicate significant differences at P<0.05.

fall in parasite load and improvement in growth suggest that EMAE-mediated control translates into meaningful performance gains, although efficacy at 1000 ppm remains below the positive control, indicating scope for dose optimization or combinations.

In Vivo Reduction of Oxidative Stress Biomarkers

Table 4 presents nitric oxide (NO) and superoxide anion (O_2^-) levels across gills, skin, and liver tissues. Untreated fish exhibited significantly elevated NO and O_2^- levels (P<0.05), indicating severe oxidative stress. EMAE administration resulted in dose-dependent reductions in both markers. EMAE1000 achieved the greatest decrease, closely approaching the efficacy of formalin. These downward shifts toward control values indicate attenuation of infection-induced oxidative stress across multiple tissues and mirror the observed antiparasitic trend. In parallel, fish growth performance improved with dose. EMAE1000 showed the highest BWG and SGR, while the disease control remained lowest, and the positive control performed best overall, linking reduced oxidative stress to better growth outcomes.

Table 5 and Figures 4 and 5 detail the effects of EMAE on paraoxonase (PON), thioredoxin reductase (TrxR), glutathione disulfide (GSSG), and peroxidase (POD). Untreated fish showed markedly reduced antioxidant enzyme activities ($P<0.05$). EMAE administration increased these biomarkers in a concentration-dependent manner, with EMAE1000 producing the highest elevations ($P<0.05$). Restoration of enzymatic defenses (PON, TrxR, POD) and normalization of the GSSG profile indicate re-engagement of endogenous antioxidant systems and improved redox homeostasis, aligning with reduced ROS markers and parasite loads. These enzymatic improvements paralleled the gains in growth (BWG/SGR), supporting a mechanistic link between antioxidant restoration and performance in treated fish.

Discussion

The ethanol extract of *Melia azedarach* leaves (EMAE) showed dual antioxidant and antiparasitic activities that are coherent across our phytochemistry and bioassays. The high polyphenol and flavonoid contents paralleled concentration-dependent gains in DPPH/FRAP, indicating efficient radical quenching and electron-donating capacity. Interpreted within the contemporary redox framework, oxidative stress as an oxidant/antioxidant imbalance, these results support EMAE's role in re-balancing redox tone (Sies, 2020). Consistent with a three-line antioxidant defense model, improvements observed here map onto first-line enzymatic defenses (SOD/CAT/GPx) and related systems reported to constrain ROS damage (Jomova et al., 2023). These interpretations are also aligned with prior work emphasizing polyphenol-rich botanicals as anti-oxidative supports in aquatic species (Hasin et al., 2024; Arpita et al., 2022).

At the tissue level, EMAE lowered NO and O_2^- while elevating PON, TrxR, POD, and normalizing GSSG, an overall pattern indicative of redox homeostasis restoration. Mechanistically, *M. azedarach* limonoids can down-regulate iNOS and NF-κB-linked signaling and suppress ROS production, offering a plausible molecular basis for the biomarker shifts we observed (Cao et al., 2024).

Beyond redox effects, EMAE reduced *Trichodina nigra* burdens in vitro and in vivo in a clear dose-responsive manner, although formalin remained the most potent. This trade-off must be weighed against chemotherapeutic constraints: formalin efficacy can be offset by toxicity risks and context-dependent mortality in sensitive life stages (Fetherman et al., 2023). Our findings add to growing evidence that plant-derived products can contribute to parasite control in aquaculture while supporting sustainability goals (Buchmann, 2022). Notably, *M. azedarach* has documented antimicrobial activity with high selectivity indices, reinforcing its therapeutic promise (Hemdan et al., 2023).

Functionally, the decrease in *T. nigra* on skin and gills coincided with better growth (BWG, SGR), suggesting that parasite control and redox relief together translate into performance benefits. Similar polyphenol-linked improvements in growth and antioxidant capacity have been reported in cultured fish, supporting this interpretation (Jayaseelan et al., 2022). Given that trichodinids are associated with morbidity and mortality in cultured species, reducing their loads is likely to yield tangible production gains (Khetsha et al., 2024).

This study has limitations. We did not isolate the specific activities driving antiparasitic effects; targeted fractionation/structure–activity studies are warranted. Safety margins under commercial conditions,

Table 4. Effect of *Melia azedarach* leaf extract on nitric oxide (NO) and superoxide anion (O_2^-) levels in fish

Groups	NO (Gills)	NO (Skin)	NO (Liver)	O_2^- (Gills)	O_2^- (Skin)	O_2^- (Liver)
Normal control	0.28±0.02^a	0.13±0.03^a	0.31±0.03^a	0.49±0.02^a	0.24±0.03^a	0.37±0.02^a
Disease control	0.78±0.02^f	0.36±0.02^e	0.87±0.03^f	1.37±0.05^f	0.67±0.02^e	1.04±0.03^f
Positive control	0.34±0.04^b	0.16±0.04^{ab}	0.37±0.03^b	0.59±0.05^b	0.29±0.03^{ab}	0.44±0.05^b
EMAE800 group	0.62±0.02^e	0.29±0.01^d	0.68±0.03^e	1.08±0.05^e	0.53±0.05^d	0.81±0.05^e
EMAE900 group	0.53±0.02^c	0.25±0.04^d	0.59±0.02^c	0.93±0.04^c	0.46±0.04^c	0.68±0.04^c
EMAE1000 group	0.39±0.02^d	0.18±0.03^c	0.43±0.04^d	0.69±0.04^d	0.34±0.07^b	0.52±0.04^d

Values are mean±SD; in vitro n= 3 wells per group per time point; in vivo n= 10 fish per group. Units NO (µmol nitrite eq mg^{-1} protein), O_2^- (ΔA_{560} min^{-1} mg^{-1} protein).

Table 5. Effect of *Melia azedarach* leaf extract on paraoxonase (PON) and thioredoxin reductase (TrxR) levels in fish

Groups	PON (Gills)	PON (Skin)	PON (Liver)	TrxR (Gills)	TrxR (Skin)	TrxR (Liver)
Normal control	2.96±0.07^f	1.75±0.07^f	9.55±0.09^f	5.94±0.02^f	2.99±0.07^f	12.55±0.08^f
Disease control	1.18±0.05^a	0.63±0.07^a	3.41±0.09^a	2.12±0.04^a	1.07±0.02^a	4.48±0.02^a
Positive control	2.46±0.05^e	1.46±0.06e	7.96±0.05^e	4.95±0.04^e	2.49±0.02^e	10.48±0.12^e
EMAE800 group	1.35±0.11^b	0.76±0.05^b	4.34±0.09^b	2.58±0.07^b	1.38±0.02^b	5.48±0.05^b
EMAE900 group	1.55±0.11^c	0.92±0.05^c	5.03±0.02^c	3.13±0.01^c	1.57±0.07^c	6.61±0.04^c
EMAE1000 group	2.11±0.05^d	1.25±0.01^d	6.82±0.08^d	4.24±0.01^d	2.14±0.01^d	8.96±0.03^d

Values are mean±SD; in vitro n= 3 wells per group per time point; in vivo n= 10 fish per group. Units PON and TrxR (ΔA min^{-1} mg^{-1} protein).

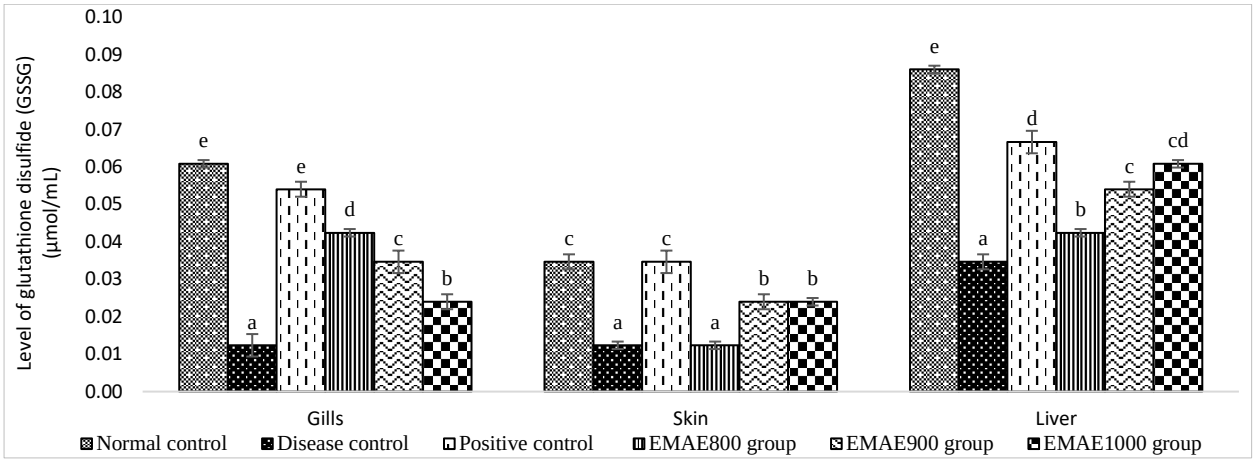

Figure 4. Effect of EMAE treatments on superoxide anion (O_2^-) levels in gill, skin, and liver tissues of infected fish. Note: Bars represent Mean±SD; different letters indicate significant differences at P<0.05.

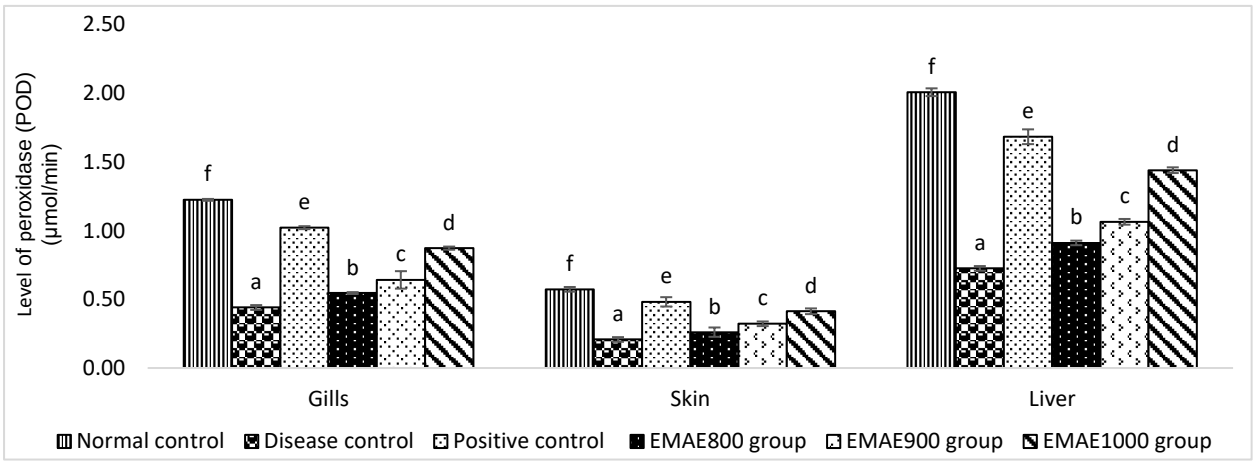

Figure 5. Changes in antioxidant enzyme activities (PON and TrxR) in gill, skin, and liver tissues after EMAE treatment. Note: Bars represent Mean±SD; different letters indicate significant differences at P<0.05.

pharmacokinetics, and potential residue issues require assessment, and immune-gene endpoints (e.g., cytokines/AMPs) should complement redox biomarkers in future work. Optimization of treatment regimens (dose, exposure time, repeated pulses) or combination strategies may narrow the efficacy gap with formalin while preserving ecological compatibility.

In summary, EMAE provides a coherent package of redox modulation and antiparasitic activity that improves fish health indicators while offering a more environmentally responsible alternative to conventional chemotherapeutics. These findings, together with literature on plant-based interventions in aquaculture, justify further translational studies toward scalable, sustainable parasite management.

Conclusions

The ethanol extract from *Melia azedarach* leaves (EMAE) demonstrates significant antioxidant and antiparasitic effects. DPPH and FRAP assays confirm its potent free radical scavenging and reducing capabilities, with the EMAE1000 dosage showing the highest activity. Additionally, EMAE reduces *the intensity of T. nigra infection* and enhances fish growth in a dose-dependent manner. It also lowers nitric oxide (NO) and superoxide anion (O_2^-) levels while increasing GSSG, PON, TrxR, and POD activities, strengthening the oxidative defense system. These results indicate that EMAE is a promising therapeutic agent for conditions associated with oxidative stress and parasitic infections.

Ethical Statement

All animal experimental procedures were conducted by ethical guidelines and were approved by the Animal Research Ethics Committee at Industrial University of Ho Chi Minh City (Approval number: 205/HD-DHCN).

Funding Information

The authors received no specific funding for this work.

Author Contribution

Thi Phuong Nhung Tran: Writing – review & editing, Conceptualization; Thi Hoan Vu: Writing – review & editing, Supervision.

Conflict of Interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to thank the Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, for technical support and research facilities.

References

Adak M., Kumar P. (2022). Herbal anthelmintic agents: a narrative review. J Tradit Chin Med., 42(4), 641–651. https://doi.org/10.19852/j.cnki.jtcm.2022.04.007

Arpita R., Ariba K., Irfan A., Saad A., Bodour S. R., Ahmad O. B., Mohammad Y. A., Saiful I., Rabiul I. (2022). Flavonoids: A bioactive compound from medicinal plants and its therapeutic applications. Biomed Research International, 2022, 5445291. https://doi.org/10.1155/2022/5445291

Aurora B. (2022). Good Anesthesia Practice for Fish and Other Aquatics. Biology, 11(9), 1355; https://doi.org/10.3390/biology11091355

Bahaa A. H., Ahmed M. D., Marwa M. E., Amal M. E. F., Tsvetelina P. K., Stoyanka S., Mohamed A. E. L., Gamila E. E. T., Mobarak A. (2023). Bioactive Azadirachta indica and Melia azedarach leaves extracts with anti-SARS-CoV2 and antibacterial activities. PLoS One, 18(3), e0282729. https://doi.org/10.1371/journal.pone.0282729

Bernd S., Milen N. (2022). Effects of multiple stressors in fish: How parasites and contaminants interact. Parasitology, 149(14), 1822–1828. https://doi.org/10.1017/S0031182022001172

Buchmann K. (2022). Control of parasitic diseases in aquaculture. Parasitology, 149(14),1985–1997. https://doi.org/10.1017/S0031182022001093

Burgess T. I., Dang Q. N., Le B. V., Pham N. Q., White D., Pham T. Q. (2020). Phytophthora acaciivora sp. nov. associated with dying Acacia mangium in Vietnam. Fungal Syst Evol., 6, 243–252. https://doi.org/10.3114/fuse.2020.06.11

Cao F., Jing C., Lin Z.T., Lin H.Y., Bin L., Chen, Z.W., Ma X.H., Zhang Y. H. (2024). Chemical Constituents from the Fruit of Melia azedarach and Their Anti-Inflammatory Activity. Antioxidants (Basel), 13(11), 1338. https://doi.org/10.3390/antiox13111338

Changyou S., Cunxin S., Bo L., Pao X. (2023). Oxidative stress in aquatic organisms. Antioxidants (Basel), 12(6), 1223. https://doi.org/10.3390/antiox12061223

Claudia D. G., Giuseppe A. M., Barbara T., Simone B., Rosaria A. (2023). Natural compounds and glutathione: Beyond mere antioxidants. Antioxidants (Basel), 12(7), 1445. https://doi.org/10.3390/antiox12071445

Faranak D., Josef V., Eliska Z. (2023). An update about beneficial effects of medicinal plants in aquaculture: A review. Vet Med (Praha), 68(12), 449–463. https://doi.org/10.17221/96/2023-VETMED

Fetherman E. R., Brad N., Chris P., Tracy D. (2023). Concentration, Life Stage, Feeding, Density, Flow, and Strain Effects on Formalin Sensitivity in Rainbow Trout (Oncorhynchus mykiss). Animals (Basel), 13(15), 2425. https://doi.org/10.3390/ani13152425

Gholami P., Khataee A., Bhatnagar A., Vahid B. (2021). Synthesis of N-Doped Magnetic WO3-x@Mesoporous Carbon Using a Diatom Template and Plasma Modification: Visible-Light-Driven Photocatalytic Activities. ACS Applied Materials & Interfaces, 13(11), 13072–13086. https://doi.org/10.1021/acsami.0c21076

Government of India. (2021). Animal Husbandry and Dairying, Guidelines of CPCSEA for Experimentation on Fishes. Committee for Control and Supervision of Experiments on Animals (CPCSEA).

Hasin H., Suriya A. S., Mirazul I., Safaet A., Fahmida T. R., Nazim U. E., Sania A., Nazim U. A., Nafees R. C., Nour F., Sakhawat H., Avoy G., Firoj A. (2024). Flavonoids: A treasure house of prospective pharmacological potentials. Heliyon, 10(6), e27533. https://doi.org/10.1016/j.heliyon.2024.e27533

Hemdan B. A., Ahmed M., Marwa M. E., Amal M. El-F., Tsvetelina P. K., Stoyanka S., Mohamed A. El-L., Gamila E. El-T., Mobarak A. M. (2023). Bioactive Azadirachta indica and Melia azedarach leaves extracts with anti-SARS-CoV-2 and antibacterial activities. PLoS One., 18(3), e0282729. https://doi.org/10.1371/journal.pone.0282729

Hoda S. M. A. G., Magdy M. F., Mai M. A., Sobhy A. S., Rabab M. E. K., Essam M. H. (2019). In vitro acaricidal effect of Melia azedarach and Artemisia herba-alba extracts on Hyalomma dromedarii (Acari: Ixodidae): Embryonated eggs and engorged nymphs. Journal of Parasitic Diseases, 43(4), 696–710. https://doi.org/10.1007/s12639-019-01149-9

Jayaseelan M., Anand P. K., Srinivasa G. R., Katia I., Stephen H., John P. H., Yara M., Saranya A., Wireko A. A., Ruiz A. M. J., Nanono S., Esther P. N., Bruno T., Paola R., Natalia R. C., Javier M. M., Rohul A., Ballamoole K. K., Abishek K., Abdul R. T., Thaint N. Z., Oluwatosin O. A., Maneesh P. S., Maarten B. M. D. (2022). Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics. Antibiotics (Basel), 11(2), 200. https://doi.org/10.3390/antibiotics11020200

Jomova K., Renata R., Suliman Y. A., Saleh H. A., Eugenie N., Kamil K., Marian V. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol. 2023, 97(10), 2499–2574. https://doi.org/10.1007/s00204-023-03562-9

Khetsha Z., Van der Watt E., Masowa M., Legodi L., Satshi S., Sadiki L., Moyo K. (2024). Phytohormone-based biostimulants as an alternative mitigating strategy for horticultural plants grown under adverse multi-stress conditions: Common South African stress factors. Caraka Tani: Journal of Sustainable Agriculture, 39(1), 167–193. https://doi.org/10.20961/carakatani.v39i1.80530

Liuhong S., Hao L., Lei F., Xinyu T., Anguo T., Xiaofeng W., Ke D., Zhetong S. (2024). Potential immunoregulatory mechanism of plant saponins: A review. Molecules, 29(1), 113. https://doi.org/10.3390/molecules29010113

Luise G., Eva L., Fabian C. H., Verena S. (2022). Condensed tannins act as anthelmintics by increasing the rigidity of

the nematode cuticle. Scientific Reports, 12, 18850. https://doi.org/10.1038/s41598-022-23566-2

Mahmoud A.O. D., Abdel-Tawwab M., Hany M.R. A. L. (2020). Lycopene reduces the impacts of aquatic environmental pollutants and physical stressors in fish. Reviews in Aquaculture, 12(4), 2511-2526. https://doi.org/10.1111/raq.12455

Maria C. D., Diana C. G. A. P., Maria C., Márcia A., Conceição S., Artur M. S. S. (2022). Phytochemical and antioxidant profile of the medicinal plant Melia azedarach subjected to water deficit conditions. International Journal of Molecular Sciences, 23(21), 13611. https://doi.org/10.3390/ijms232113611

Maria G. S., Gabriele S., Federico D. A., Roger R. G., Federica C., Elena D., Ela M. C. M., Augusto O. (2020). Oxidative stress and new pathogenetic mechanisms in endothelial dysfunction: Potential diagnostic biomarkers and therapeutic targets. Journal of Clinical Medicine, 9, 1995–2034. https://doi.org/10.3390/jcm9061995

Marta P., Celestyna M. K., Jan S., Alina W. (2024). Oxidative stress in parasitic diseases—Reactive oxygen species as mediators of interactions between the host and the parasites. Antioxidants, 13(1), 38–49. https://doi.org/10.3390/antiox13010038

Martha E., Hadi P., Retno W., Katsuyoshi M., Sukardiman. (2020). Bio-selective hormonal breast cancer cytotoxic and antioxidant potencies of Melia azedarach L. wild type leaves. Biotechnology Reports (Amsterdam), 25, e00437. https://doi.org/10.1016/j.btre.2020.e00437

Marzie K., Jafar V., Hamid R. S., Javad M. (2024). Phytochemical profiles and antioxidant activity of Iranian Melia azedarach L. Journal of Medicinal Plants and By-products, 1, 51–56. https://doi.org/10.22092/jmpb.2022.358593.1472

Meihong L., Xiaoyang B., Lijuan Z., Jiguang H. (2022). Insecticidal triterpenes in Meliaceae: Plant species, molecules, and activities: Part II (Cipadessa, Melia). International Journal of Molecular Sciences, 23(10), 5329. https://doi.org/10.3390/ijms23105329

Melba G. B. R., Brett M., Iddya K., Sophie F., Victoria A. S., Edgar B., Marc L. G., Aihua L., Win S., Indrani K., Bin H., Andrea D. O., Ruggero U., Andrea C. (2023). Review of alternatives to antibiotic use in aquaculture. Reviews in Aquaculture, 15, 1421–1451. https://doi.org/10.1111/raq.12786

Nathalie P. du S., Viki H., Amrita A., Sabina A., Marc T. A., Monya B., William J. B., Alejandra C., Innes C. C., Ulrich D., Michael E., Paul G., Stephen T.H., David W.H., Natasha A.K., Stanley E.L., Katie L., Catriona J. M.C., Malcolm M., Esther J.P., Ole H.P., Frances R., Penny R., Kieron R., Emily S.S., Shai D. S., Thomas S., Han W. (2020). The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. Br J Pharmacol., 177(16), 3617–3624. https://doi.org/10.1111/bph.15193

Natividad C., Antonio S., Juan C. A. (2020). Quantification of the antioxidant activity of plant extracts: Analysis of sensitivity and hierarchization based on the method used. Antioxidants (Basel), 9(1), 76. https://doi.org/10.3390/antiox9010076

Nhung T. P. T., Buu G. T. (2023). Application of bead tree leaf extract (Melia azedarach) as a green and natural anti-parasitic drug to control trichodinosis in whitespotted freshwater catfish (Clarias fuscus) farming. Chemical Engineering Transactions, 106, 187–192. https://doi.org/10.3303/CET23106032

Nhung TTP (2025). Correlation-Driven Analysis of Synergistic Effects of Dual Medicinal Mushroom Extracts in a DMBA-Induced Murine Breast Cancer Model Trop J Nat Prod Res., 9(8): 3496 – 3504 https://doi.org/10.26538/tjnpr/v9i8.7

Nhung T. P. T., Trang T. N., Buu G. T. (2023). Anti-arthritis effect of ethanol extract of Sacha inchi (Plukenetia volubilis L.) leaves against complete Freund's adjuvant-induced arthritis model in mice. Tropical Life Sciences Research, 34(3), 237–257. https://doi.org/10.21315/tlsr2023.34.3.13

Nils O., Jenny W., Matthias S., Lars L., Stefanie B., Céline H. (2024). Assessment of the effect of tricaine (MS-222)-induced anesthesia on brain-wide neuronal activity of zebrafish (*Danio rerio*) larvae. Front. Neurosci., 18, 1-11. https://doi.org/10.3389/fnins.2024.1456322

Nisa S., Mahroze F., Syed Z. H. S., Noor K., Beenish A., Wazir A., Maryam, Saba S., Amber F. (2024). Evaluation of growth, antioxidant status, hepatic enzymes, and immunity of Nanoselenium-Fed Cirrhinus mrigala. PLoS One., 19(8), e0308761. https://doi.org/10.1371/journal.pone.0308761

Ramírez C., Cardozo M., Gastón M. L., Galdeano E., Collavino M. M. (2024). Plant growth-promoting activities of endophytic bacteria from Melia azedarach (Meliaceae) and their influence on plant growth under gnotobiotic conditions. Heliyon, 10(15), e35814. https://doi.org/10.1016/j.heliyon.2024.e35814

Samar A. E. G., Rawia S. A., Viola H. Z., Eman Z. (2023). Host-pathogen interaction unveiled by immune, oxidative stress, and cytokine expression analysis to experimental Saprolegnia parasitica infection in Nile tilapia. Scientific Reports, 13, 9888. https://doi.org/10.1038/s41598-023-36892-w

Sies H. (2020). Oxidative Stress: Concept and Some Practical Aspects. Antioxidants (Basel)., 9(9), 852. https://doi.org/10.3390/antiox9090852

Tran T. P. N., Le P. T. Q. (2025). Acute Toxicity Evaluation of Ethanol Leaf Extract of Bead Tree (*Melia azedarach* Linne) on White-Spotted Freshwater Catfish (*Clarias fuscus*). Malaysian Journal of Biochemistry & Molecular Biology, 1, 8-20.

Velusamy S., Roy A., Sundaram S., Mallick T.K. (2021). A Review on Heavy Metal Ions and Containing Dyes Removal Through Graphene Oxide-Based Adsorption Strategies for Textile Wastewater Treatment. The Chemical Record, 21(7), 1570–1610. https://doi.org/10.1002/tcr.202000153