ASIAN JOURNAL OF AGRICULTURE
Volume 9, Number 2, December 2025 E-ISSN: 2580-4537
Pages: 500-508 DOI: 10.13057/asianjagric/g090216

Eco-friendly control of Trichodinid infestation in Nile tilapia *Oreochromis niloticus* using *Psidium guajava* leaf extract

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Manuscript received: 5 July 2025. Revision accepted: 13 September 2025.

Abstract. *Sulistiawati A, Lamata G, Sriwulan. 2025. Eco-friendly control of Trichodinid infestation in Nile tilapia* Oreochromis niloticus *using* Psidium guajava *leaf extract. Asian J Agric 9: 498-506.* Trichodinid ectoparasites are among the most detrimental pathogens in Nile tilapia (*Oreochromis niloticus*) aquaculture, causing tissue damage, growth suppression, and high mortality, particularly in small-scale systems. Chemical treatments such as formalin remain common but raise concerns regarding toxicity, environmental contamination, and pathogen resistance. Developing eco-friendly, plant-based alternatives is therefore crucial for sustainable aquaculture. This study evaluated the antiparasitic efficacy, safety, and histopathological impacts of ethanol extract of guava (*Psidium guajava*) leaves against Trichodinid infections in Nile tilapia. Juvenile tilapia (n=120) were naturally infected through cohabitation and randomly assigned to four treatments (0, 10, 20, and 30 ppm extract; three replicates each). Acute toxicity tests determined a 24 h LC_{50} value of 43.65 ppm. Extract efficacy was assessed through prevalence, infection intensity, survival rate, water quality, and histopathology, analyzed by one-way ANOVA with Tukey's post hoc test (α=0.05). The 30 ppm treatment significantly reduced Trichodinid prevalence from 70.0% to 43.3% and infection intensity from 5.4 to 3.2 parasites per fish, representing 38.1% and 41.3% reductions, respectively ($p<0.05$). The highest survival rate occurred at 10 ppm (93.3%), compared to 86.7% at 20 ppm, 80.0% at 30 ppm, and 66.7% in the control. Water quality (temperature, dissolved oxygen, pH) remained within optimal culture ranges. Histopathological observations confirmed milder epithelial erosion, necrosis, and gill lamella fusion in treated groups relative to controls. These findings demonstrate that guava leaf ethanol extract, particularly at 10-30 ppm, is an effective, safe, and environmentally friendly antiparasitic agent. Its integration into aquaculture health management could reduce reliance on synthetic chemicals, promote fish welfare, and support the sustainability of tilapia farming systems.

Keywords: Antiparasitic treatment, guava leaf extract, Nile tilapia, phytotherapy, Trichodinid

INTRODUCTION

Nile tilapia *Oreochromis niloticus* Linnaeus, 1758 is among the most economically important aquaculture species in Indonesia due to its rapid growth, high feed conversion efficiency, and adaptability to a wide range of environmental conditions (Hasan et al. 2019). In South Sulawesi, for example, tilapia production increased from 8,169 tons in 2018 to 11,550 tons in 2021 (Ministry of Marine Affairs and Fisheries, 2024). However, the intensification of tilapia farming has been increasingly hampered by parasitic outbreaks, particularly those caused by ectoparasites such as Trichodinids, which result in significant economic losses due to impaired fish quality, reduced growth, increased susceptibility to secondary infections, and elevated mortality rates (Abd-Elrahman et al. 2023).

Trichodinid species are ciliated protozoans characterized by a denticular ring that allows them to attach to and scrape host epithelial tissues, leading to lesions, impaired respiration, and increased vulnerability to secondary pathogens (Younis et al. 2021; Islas-Ortega et al. 2022). These parasites have a direct life cycle and are highly influenced by environmental factors such as water quality and stocking density, which explains their high prevalence in intensive aquaculture systems (Adly et al. 2015; Lieke et al. 2020). Trichodinids are the etiological agents of trichodiniasis and primarily invade the skin, gills, and fins, especially under crowded conditions. Clinical manifestations include excess mucus production, dermal erosion, anorexia, lethargy, and mortality (Collymore et al. 2013). Histopathological observations frequently reveal epithelial hyperplasia, necrosis, and lamellar fusion in the gill tissues of infected fish (Adly et al. 2015).

Surveys in Indonesia have reported Trichodinid prevalence between 55.5% and 88.88% in tilapia, with intensities reaching 22 parasites per fish (Rozik and Djauhari 2022). Such infections reduce growth and survival, particularly in small-scale farms, where losses from mortality and reduced product quality may exceed 30% (Verdegem et al. 2023). This challenge has increased interest in botanical alternatives for parasite control. Compounds from garlic (*Allium sativum*), neem (*Azadirachta indica*), and betel leaf (*Piper betle*) have shown antiparasitic activity against protozoan and helminth infections in fish (Sahandi et al. 2023; Ukwa et al. 2023). These phytotherapeutics are valued for low toxicity and reduced environmental impact compared with synthetic chemicals.

Chemical treatments such as formalin and potassium permanganate remain common but raise concerns regarding toxicity, tissue residues, environmental contamination, and pathogen resistance (Abou-Okada et al. 2021). By contrast, plant-based therapies offer multiple benefits, including antimicrobial, anti-inflammatory, antioxidant, and growth-promoting effects in aquaculture species (Emara et al. 2020; Zhu 2020; Kurian et al. 2021). This aligns with global trends toward safer, sustainable, and cost-effective disease management.

Among botanicals, guava (*Psidium guajava*) leaves are abundant, inexpensive, and rich in bioactive compounds such as tannins, flavonoids, saponins, and terpenoids. These phytochemicals act through diverse mechanisms: tannins precipitate parasite proteins and damage membranes, flavonoids function as antioxidants and immunomodulators, saponins destabilize protozoan cell membranes, and terpenoids exert antiparasitic and antimicrobial effects (Morais-Braga et al. 2016; Toutou et al. 2024; Daniel 2024). The synergistic effects enhance host resilience and parasite suppression. For instance, Santrianda and Aji (2021) demonstrated reduced Trichodinid prevalence in *Clarias batrachus* treated with guava leaf infusion. Compared with other botanicals, guava leaves are widely accessible in tropical regions like Indonesia, offering practical advantages for aquaculture.

The choice of ethanol as an extraction solvent also strengthens the practical application of guava leaves. Ethanol is safer than methanol or chloroform and is capable of extracting both polar and semi-polar compounds, resulting in higher recovery of bioactive phytochemicals, including flavonoids, terpenoids, and tannins (Desiyana et al. 2016; Margareta and Wonoraharjo 2023). Ethanol-based extracts of guava leaves have been reported to show stronger antimicrobial and antiparasitic effects compared with aqueous extracts. Globally, tilapia farming has surpassed 6 million tons annually, making it one of the most cultured fish species (FAO 2022). Yet parasitic infections remain a key bottleneck to sustainable production. Eco-friendly and effective control strategies are urgently needed to sustain both national and international aquaculture industries.

Therefore, this study aimed to evaluate the antiparasitic efficacy of ethanol-extracted guava leaves against Trichodinid infections in Nile tilapia under controlled conditions. It also examined toxicity profiles, effects on fish health, and potential histological benefits. By integrating ecological and economic perspectives, this research contributes to phytotherapy-based disease management strategies that are effective, safe, and environmentally sustainable.

MATERIALS AND METHODS

Study area and ethical approval

The experiment was conducted in November 2024 at the PT. Tiran Group hatchery, Makassar, South Sulawesi, Indonesia. Preparation of guava leaf extract was performed at the Marine Microbiology Laboratory, Universitas Hasanuddin, while GC-MS analysis was carried out at the Chemistry Laboratory, Politeknik Negeri Ujung Pandang, and histopathological examinations at the Maros Veterinary Center. All procedures involving live fish were reviewed and approved by the Universitas Hasanuddin Animal Ethics Committee, in accordance with national guidelines for the care and use of experimental animals.

Experimental fish and design

Juvenile Nile tilapia (8-10 cm, total n=120) were acclimatized prior to the trial and randomly distributed into 12 aquaria (60×40×40 cm; 50 L working volume) with 10 fish each. The study applied a completely randomized design (CRD) with four treatments and three replicates: A: Control (0 ppm), B: Guava leaf extract 10 ppm, C: Guava leaf extract 20 ppm, D: Guava leaf extract 30 ppm. Treatment concentrations were selected based on the 24 h LC_{50} value (43.65 ppm), ensuring sublethal exposure levels.

Preparation of guava leaf extract

Fresh guava leaves were washed, shade-dried, and ground into powder. A total of 500 g was macerated in 2 L of 96% ethanol for 48 h, then filtered and concentrated using a rotary evaporator at 60°C and 50 rpm. The crude extract was stored in dark glass bottles under refrigeration until use.

GC-MS (Gas Chromatography-Mass Spectrophotometry) analysis

Chemical composition of the extract was analyzed using a Shimadzu GC-MS-QP 2010 Ultra system with helium as the carrier gas (1 mL min^{-1}). Compounds were identified based on retention time and spectral comparison with the NIST 20 MS library.

Acute toxicity (LC_{50}) test

For the 24 h LC_{50} (Lethal Concentration 50%) test, five groups of tilapia (10 fish each) were exposed to 0, 10, 20, 30, 35, and 40 ppm of guava leaf extract. Mortality was recorded, and LC_{50} was determined by probit analysis (Aris and Adriana 2022).

Parasite infection and identification

Trichodinid-infected tilapia were obtained from the Taretta hatchery (Bone Regency) and cohabitated with healthy tilapia in a 1:6 ratio (18 infected with 102 healthy fish) for 48 h to establish infection. Infection was confirmed by mucus and gill scraping. Wet mounts were examined under a compound light microscope at 100× and 400× magnification. Identification of Trichodinid was based on denticle morphology and size using standard taxonomic keys (Kabata 1985).

Treatment application

Fish were exposed by immersion in guava leaf extract solutions for 24 h at assigned concentrations. Immersion was selected over oral administration or injection for its greater effectiveness against external parasites (Santrianda and Aji 2021).

Prevalence and intensity counts

For each treatment, 10 fish per replicate (n = 30 per treatment) were sampled before and after treatment. Mucus smears and gill filaments were observed microscopically, and parasite counts recorded. Prevalence and intensity were calculated using the Kabata (1985) formula:

Prevalence (%) = (Number of infected fish / Total fish) × 100

Intensity = (Total parasites counted / Number of infected fish)

Survival rate

Survival was monitored daily. At the end of the experiment, the survival rate was calculated as (Effendie 1993):

SR (%) = (Nt / N0) × 100

Where Nt: Number of surviving fish; N0: Initial fish count.

Water quality monitoring

Water temperature, dissolved oxygen (DO), and pH were measured twice daily (morning and afternoon). Temperature was measured with a calibrated mercury thermometer, DO with a digital DO meter (YSI Pro20), and pH with a digital pH meter (Hanna Instruments). All values were compared with the Indonesian National Standard for Nile tilapia culture (SNI 7550:2009).

Histopathological examination

At the end of the experiment, gill and skin tissues were collected and fixed in 10% neutral buffered formalin, dehydrated in graded ethanol, cleared in xylene, and embedded in paraffin. Sections (5 µm) were stained with hematoxylin and eosin (H&E) and examined under a compound microscope for epithelial erosion, necrosis, hyperplasia, and lamellar fusion (Attia et al. 2021).

Statistical analysis

Prevalence, intensity, and survival data were analyzed by one-way ANOVA (Analysis of Variance) followed by Tukey's HSD test at α=0.05 (SPSS v29.0). LC_{50} values were estimated by probit regression. Water quality and histopathological results were analyzed descriptively.

RESULTS AND DISCUSSION

GC-MS analysis

GC-MS analysis identified 28 compounds in the ethanol extract of guava leaves (Figure 1; Table 1). The most abundant compounds were humulene (6.73%), caryophyllene (5.21%), vitamin E (4.88%), and 10,10-dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5-ol (3.96%). These compounds are known for their bioactivity, including antiparasitic, antioxidant, and antimicrobial properties, suggesting a potential mechanistic basis for the observed antiparasitic effects. Other notable constituents included phytol, squalene, and α-pinene, which have been reported in the literature to contribute to protozoan parasite suppression.

Acute toxicity (LC_{50}-24h)

The 24 h LC_{50} of guava leaf extract against Nile tilapia was estimated at 43.65 ppm (Table 2). No mortality occurred in the 10 ppm group, while 100% mortality was recorded at 50 ppm, confirming the suitability of sublethal concentrations (10-30 ppm) for the efficacy trial.

Prevalence and intensity of Trichodinid infection

Prior to treatment, all groups exhibited similar prevalence (66.7-70%) and intensity levels (5.3-5.7 parasites/fish) (p>0.05). Post-treatment analysis revealed significant reductions in prevalence and intensity, especially at 30 ppm (p<0.05; Tukey HSD). Treatment D (30 ppm) reduced prevalence from 70.0% to 43.3% and intensity from 5.4 to 3.2 parasites/fish, demonstrating superior antiparasitic efficacy (Table 3).

Survival rate

The survival rate of Nile tilapia over 24 hours confirmed the extract's safety at lower concentrations (Figure 2). Treatment B (10 ppm) had the highest survival (93%), followed by C (86.7%), D (80%), and the control (67%). The ANOVA showed significant differences (Sig. 0.028<0.05), with treatment B considered optimal. Thus, 10 ppm is seen as balancing efficacy and safety.

Water quality

Water temperature (26.8-28.6°C), dissolved oxygen (3.4-5.2 ppm), and pH (6.7-7.7) remained within optimal ranges for tilapia culture (SNI 7550:2009). No significant differences were observed among treatments (Table 4).

Histopathological observations

Skin and gill tissues of infected control fish displayed epithelial erosion, necrosis, and severe secondary lamella fusion (Figure 3.A and 3.C). In contrast, fish treated with guava leaf extract exhibited milder lesions. At 30 ppm, epithelial structure was largely preserved with only slight hyperplasia, moderate protective effects were evident, with reduced necrosis and partial lamella repair (Figure 3.B and 3.D). These findings support the role of phytochemicals in mitigating tissue damage induced by Trichodinid infestation.

Table 1. The results of the GC-MS analysis are dominant in guava leaf extract

Compound name	Retention Time (min)	Area	Area (%)	A/H ratio
Caryophyllene	14.336	45,368,132	5.87	3.30
Humulene	14.835	51,985,991	6.73	3.77
10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	17.266	37,098,593	4.80	5.05
Vitamin E (α-Tocopherol)	43.965	34,605,754	4.48	6.50

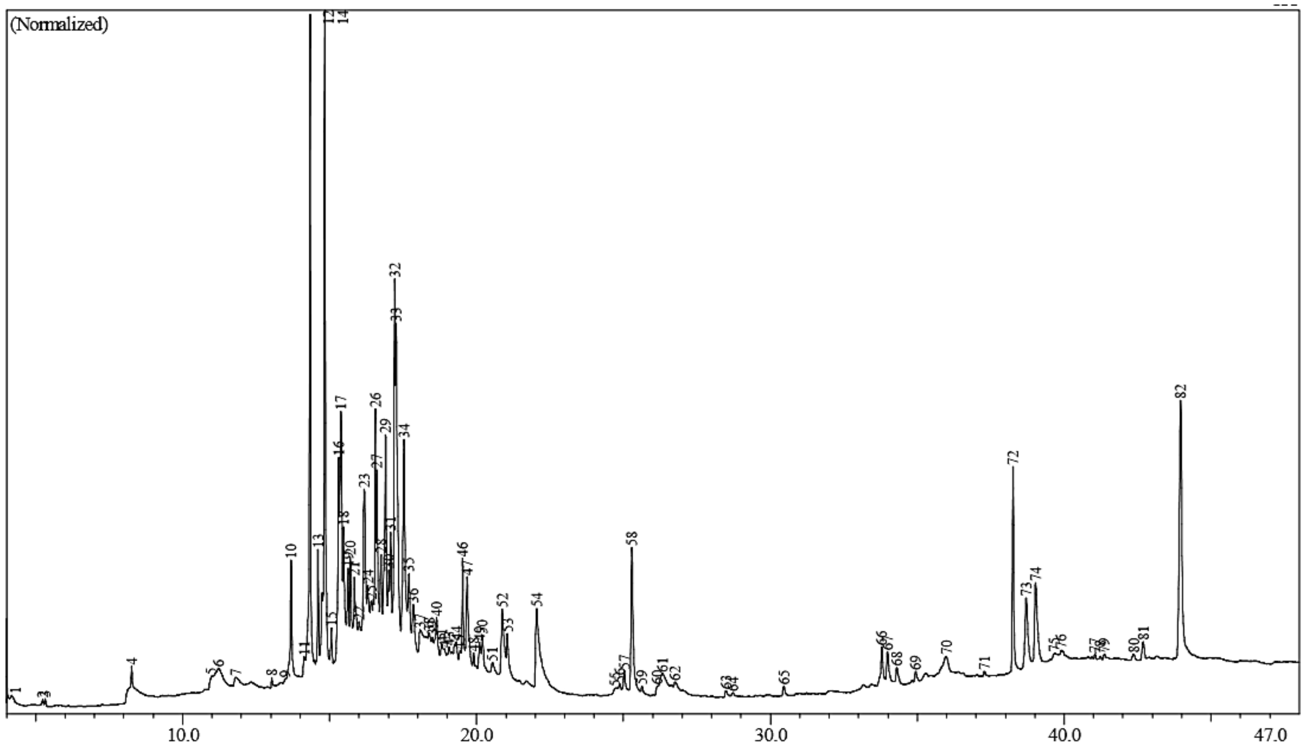

Figure 1. GC-MS analysis results of guava leaf extract chromatogram

Table 2. 24-hour LC_{50} toxicity test results of guava leaf extract on Nile tilapia

Concentration (ppm)	Mean fish mortality	Mortality (%)
10	0	0
15	1	10
30	3	30
35	4	40
40	7	70

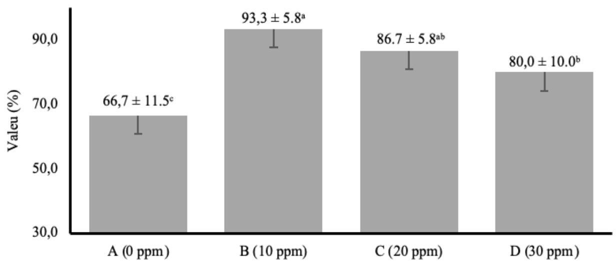

Figure 2. Survival rate (%) of nile tilapia by treatment

Discussion

This study demonstrated that ethanol-extracted guava leaves effectively suppressed Trichodinid infections in *O. niloticus* by significantly reducing both prevalence and intensity of parasitism. At the highest sublethal concentration tested (30 ppm), prevalence was reduced from 70.0% to 43.3% and infection intensity decreased by 41.3%. These findings highlight the potential of guava leaf extract as an eco-friendly antiparasitic agent suitable for sustainable aquaculture practices in Indonesia and other tropical aquaculture regions.

GC-MS analysis revealed that humulene ($C_{15}H_{24}$) was the most abundant compound in the guava leaf extract, comprising 6.73% of the total chromatographic area. As a sesquiterpenoid, humulene exhibits various biological activities, including anti-inflammatory, antioxidant, and immunostimulatory effects that support angiogenesis and tissue regeneration in aquatic organisms (Hartsel et al. 2016; Giri et al. 2020). Salam et al. (2022) further emphasized its pharmacological relevance, citing its anticancer, antimicrobial, and local anesthetic properties particularly beneficial for treating Trichodinid infections that irritate external fish tissues (Abu-Elala et al. 2021). Faridha Begum et al. (2016) also confirmed its anticancer, anti-inflammatory, antifungal, and antibacterial potentials. Another major compound identified was caryophyllene (5.87%), a sesquiterpene hydrocarbon with strong anti-inflammatory properties. It is under active investigation in pharmaceutical applications, including as a component in chemotherapeutic agents (Nursanty et al. 2023; Dadras et al. 2023). Caryophyllene also demonstrates antibacterial activity against *Staphylococcus aureus*, antifungal properties, potent antioxidant effects, and antiproliferative mechanisms that inhibit cell migration, invasion, and spheroid formation in colon cancer models (Khan et al. 2021). Both humulene and caryophyllene have been previously detected in ethanol extracts of *Psidium guajava* leaves (Santhosh and Sarojini 2024). The third most abundant compound, 10,10-dimethyl-2,6-dimethylenebicyclo [7.2.0]undecan-5β-ol (4.80%), is a terpenoid alcohol with documented antifungal, antibacterial, and antiviral effects

(Somnuek et al. 2025). Vitamin E (4.48%) was also present in the extract and is recognized as a lipophilic antioxidant that enhances immune function and mitigates oxidative stress in parasitized fish (Rahimnejad et al. 2021). The structural form of the 4 compounds can be seen in Figure 4.

Numerous pharmacological benefits have been attributed to guava's secondary metabolites, particularly those found in its leaves and fruits (Pimpley and Murthy 2021; Toutou et al. 2024). These include phenols, flavonoids, carotenoids, terpenoids, and triterpenes, all of which possess notable bioactivities (Kumar et al. 2021; Daniel 2024). Guava leaves have long been used in traditional medicine for their antioxidant, antimicrobial, antihypertensive, antiallergic, antitoxic, anti-inflammatory, antineoplastic, cytotoxic, hepatoprotective, antispasmodic, cardioprotective, and anti-ulcer properties (Ceballos-Francisco et al. 2020; Giri et al. 2020; Nhu et al. 2020).

The antiparasitic effects of guava leaf extract observed in this study are comparable to other plant-based treatments reported in aquaculture. Garlic (*Allium sativum*) extract reduced *Ichthyophthirius multifiliis* infections in guppies and tilapia (Sahandi et al. 2023; Ukwa et al. 2023), while neem (*Azadirachta indica*) demonstrated efficacy against monogeneans in catfish (El-Demerdash et al. 2020). Chamomile and ginger extracts have also shown protozoan inhibition in freshwater fish (Emara et al. 2020). However, guava leaves present distinct advantages: they are abundantly available year-round in tropical countries, inexpensive, and culturally familiar as traditional medicine. These characteristics make guava particularly suitable for adoption by smallholder farmers in Indonesia.

Toxicity testing of the extract yielded a 24-hour LC_{50} value of 43.65 ppm. Although this places the extract in the "toxic" category based on Meyer et al. (1982), concentrations below this threshold are considered safe for therapeutic use. This biphasic nature therapeutic at low doses and toxic at high concentrations is common among plant-derived compounds (Nofita et al. 2021). Therefore, precise dose selection is essential for balancing safety and efficacy in aquaculture settings (Zhu 2020; El-Zayat et al. 2024).

The antiparasitic efficacy of the guava leaf ethanol extract was reflected in its ability to significantly reduce both the prevalence and intensity of Trichodinid infections. In the control group (0 ppm), prevalence increased from 66.7% to 80.0%, whereas treatment with 30 ppm extract reduced prevalence from 70.0% to 43.3%. A similar trend was observed for infection intensity, which in the control group rose from 5.4 to 6.2 ind./fish, while in the 30 ppm treatment it decreased from 5.4 to 3.2 ind./fish, representing a 41.3% reduction. These results are in line with Koniyo et al (2020), who reported that Trichodinids proliferate rapidly in juvenile fish with underdeveloped immune systems (Koniyo et al. 2020). Such infections impair gill function through excessive mucus production, which can block lamellae, disrupt gas exchange, and increase mortality risk (Ihsan and Sitinjak 2023). The observed antiparasitic effect is likely attributable to bioactive compounds identified in the GC-MS analysis such as humulene, caryophyllene, tannins, flavonoids, saponins, and terpenoids which are known to inhibit parasite activity, stimulate immune responses, and facilitate epithelial tissue recovery (Morais-Braga et al. 2016; Rahimi et al. 2022; Daniel 2024).

Survival rate data further confirmed the extract's protective efficacy. The lowest survival rate (67%) was recorded in the untreated group, falling below the SNI-recommended minimum of 70%. In contrast, the 10 ppm treatment yielded the highest survival rate (93.3%), while higher concentrations showed a decreasing trend, suggesting dose-dependent toxicity (Giri et al 2020). Maintaining an optimal dose is thus critical to maximizing therapeutic benefits while minimizing adverse effects.

Histopathological examination of Nile tilapia infected with Trichodinid ectoparasites revealed distinct tissue morphology between the untreated (control) and treated groups exposed to 30 ppm ethanol extract of guava leaves for 24 hours. Trichodinid parasites actively traverse the host's skin, fins, and gills using cilia, feeding on sloughed epithelial cells and mucus via an abrasive, tooth-like feeding apparatus (Van as and Basson 1989; Younis et al. 2021). These parasitic activities induce mechanical irritation and chronic tissue damage, manifesting in histological lesions such as epithelial erosion, lamellar fusion, and necrosis. In the control group (Figure 3.A), skin samples exhibited pronounced tissue damage, including dermal epithelial erosion and epidermal necrosis. Displacement of epithelial cells and widening of intercellular spaces were also evident (Triastuti et al. 2024). In contrast, skin sections from treated fish (Figure 3.B) still showed signs of epithelial erosion and localized necrosis but to a much lesser extent, suggesting that the extract helped preserve tissue integrity and mitigated damage severity. While full regeneration was not observed, these findings indicate a protective effect of the guava leaf extract.

Table 3. Prevalence and intensity data are shown as mean ± SD from 3 replicate aquaria (n=10 fish/aquarium)

Treatment	∑sample fish	Prevalence (%)		Intensity (Ind/fish)	
		Before	After	Before	After
A (0 ppm)	30	$66.7±5.8^{a}$	$80.0±10.0^{a}$	$5.4±0.6^{a}$	$6.2±0.8^{a}$
B (10 ppm)	30	$70.0±0.0^{a}$	$53.3±15.3^{b}$	$5.3±0.5^{a}$	$4.0±1.0^{ab}$
C (20 ppm)	30	$66.7±11.5^{a}$	$46.7±5.8^{bc}$	$5.7±1.1^{a}$	$3.6±1.1^{ab}$
D (30 ppm)	30	$70.0±10.0^{a}$	$43.3±15.3^{c}$	$5.4±0.8^{a}$	$3.2±1.1^{c}$

Table 4. Water quality before and after treatment

Parameters	Measurement results							
	A		B		C		D	
	Before	After	Before	After	Before	After	Before	After
Suhu (°C)	27.3-27.6	28.3-28.8	27-27.1	28.3-28.5	27	28.4-28.6	26.8-27.6	28.6-28.8
DO (ppm)	4.3-5.0	4.2-5.1	3.4-4.5	3.8-5.2	4.0-4.1	4.0-4.7	3.5-5.2	3.5-4.8
pH	7.0-7.4	7.7-7.8	7.0-7.6	7.5-7.7	6.7-6.9	7.5-7.6	6.9-7.1	7.6-7.6

Note: A: Control (0 ppm), B: Guava leaf extract 10 ppm, C: Guava leaf extract 20 ppm, D: Guava leaf extract 30 ppm

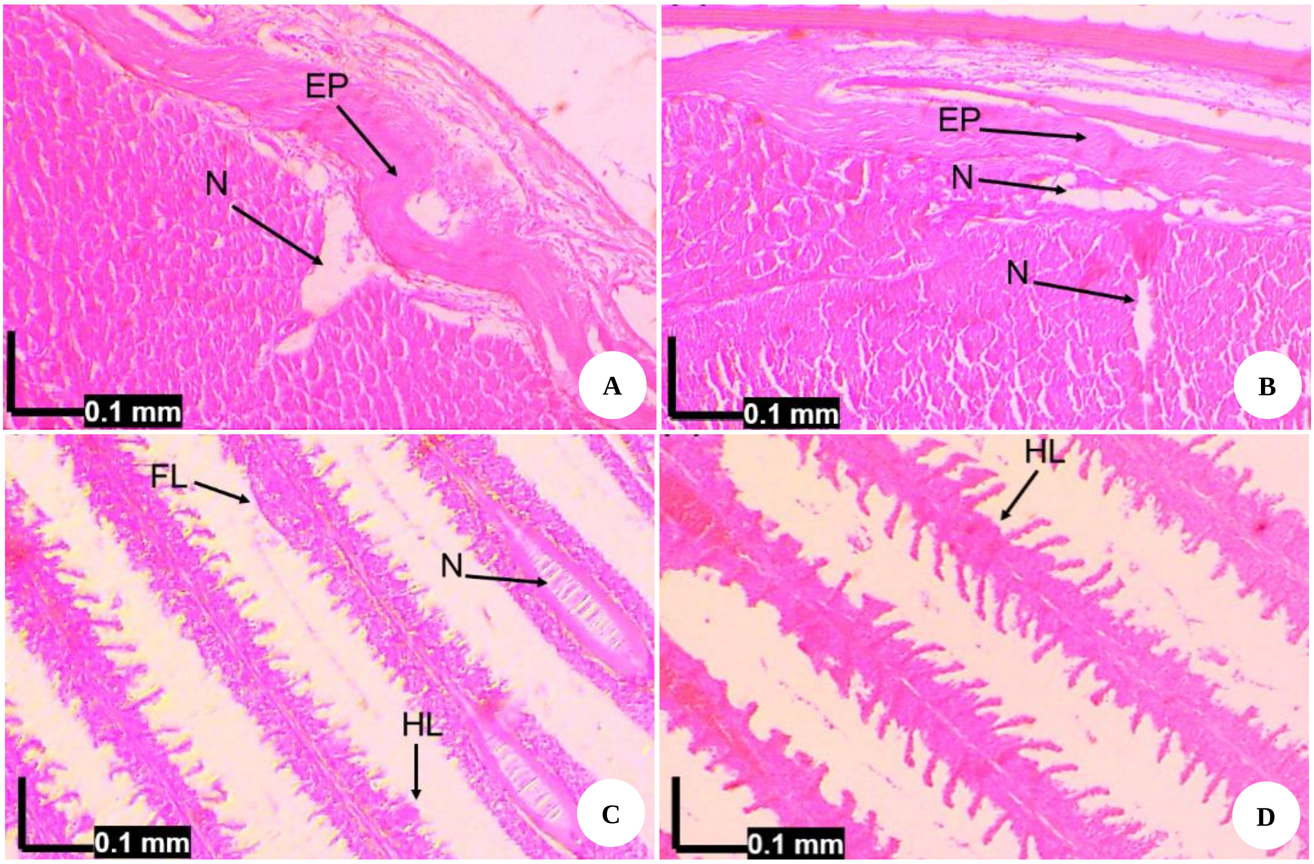

Figure 3. Histological sections of Nile tilapia skin and gill tissues (H&E stain, scale bar: 0.1 mm). A. Skin of infected fish (control group), EP: Epithelial erosion in dermal tissue and N: Epidermal necrosis; B. Skin of infected fish treated with 30 ppm ethanol extract of guava leaves, EP: Localized epithelial erosion and N: Necrosis with reduced severity; C. Gills of infected fish (control), FL: Secondary lamellar fusion, HL: Epithelial hyperplasia, and N: Necrosis in the primary lamellae; D. Gills of treated fish, HL: Remaining hyperplasia with lower severity

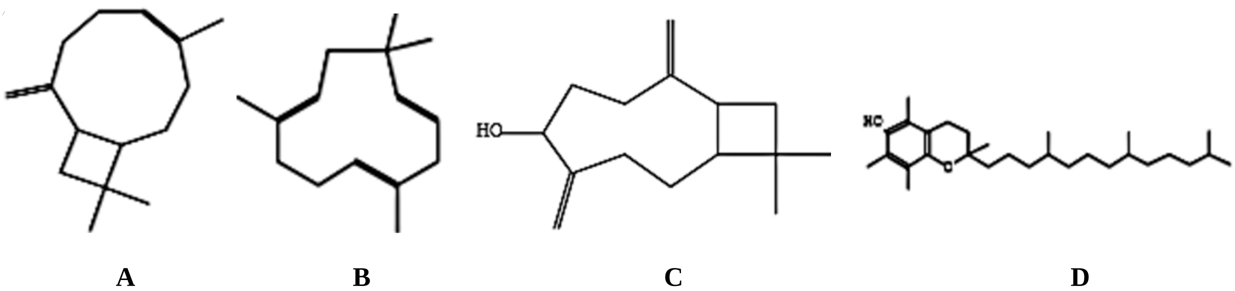

Figure 4. Structure of A. Humulene, B. Caryophyllene, C. 10,10-Dimetil-2,6dimetilenbisiklo[7.2.0]undecan-5β-ol and D. Vitamin E

Gill tissues followed a similar pattern. Control fish gills (Figure 3.C) displayed severe secondary lamellar fusion, epithelial hyperplasia, and necrosis of primary lamellae, typical of chronic irritation from parasitic infestation (Steckert et al. 2019). These morphological changes likely result from immune responses that increase mucus production and epithelial thickening, narrowing the interlamellar spaces and impeding respiration. If left unchecked, such conditions can lead to hypoxia, anemia, and mortality (da Cruz et al. 2022; O'Halloran et al. 2022). Gills of treated fish (Figure 3.D) showed milder degrees of epithelial hyperplasia, with the absence of lamellar fusion and necrosis, indicating that the extract reduced the extent of histological disruption. According to Adly et al. (2015) and Steckert et al. (2018), hyperplasia and lamellar fusion are typical adaptive responses to persistent parasitic irritation. Without intervention, these may progress to necrosis, characterized by vacuole formation and poorly stained areas under hematoxylin-eosin staining (Sachet et al. 2017; Schuermans et al. 2024).

The observed differences in tissue damage between control and treatment groups highlight the potential of guava leaf ethanol extract as a protective agent against parasitic damage. Although full histological healing was not apparent within the short observation window, the presence of bioactive compounds such as flavonoids, tannins, saponins, and terpenoids, as identified by GC-MS analysis, is believed to stabilize tissues and limit further deterioration (Giri et al. 2020; Pudota et al. 2025; Sravya et al. 2025). This is further supported by Toutou et al. (2024), who found that guava leaf extract modulates immune enzymes like protease, antiprotease, and peroxidase in the skin mucus of Nile tilapia. In summary, while histological regeneration remained incomplete, guava leaf extract provided early-stage protection against tissue degeneration caused by Trichodinid infection. This supports its application in phytotherapeutic strategies for managing fish health in sustainable aquaculture systems.

Water quality parameters remained stable across all treatments, with temperature (27-28.7°C), dissolved oxygen (4.0-4.7 ppm), and pH (6.9-7.7) all within SNI-recommended thresholds (SNI 2009). These environmental conditions support both fish health and Trichodinid viability, underscoring the importance of integrated water management (FAO 1985; Koniyo et al. 2020; Lieke et al. 2020). The antiparasitic efficacy of guava leaf extract holds strong practical relevance for tilapia aquaculture. Trichodinids remain a major constraint in Indonesia, with prevalence rates of 55-89% (Rozik and Djauhari 2022), impairing respiration, growth, and resistance to secondary infections (Collymore et al. 2013). While chemicals such as formalin are still used, their toxicity, residues, and environmental risks (Abou-Okada et al. 2021) highlight the need for safer alternatives (Keck and Blanc 2002; Elgendy et al. 2024). Guava extract is biodegradable, locally available, and compatible with immersion methods already familiar to farmers, making adoption feasible with minimal adaptation. Beyond antiparasitic effects, its antioxidant and immunostimulatory properties may enhance overall fish health.

Nonetheless, this study was conducted under controlled conditions; farm environments present greater variability in water quality and microbial interactions. Long-term impacts of repeated treatments, and the practicality of ethanol versus aqueous extraction, remain uncertain. Future research should focus on field-scale validation, cost-benefit analyses, and evaluation of ecotoxicity. Isolating key compounds such as humulene and caryophyllene, or combining guava extract with probiotics and other botanicals, may further optimize efficacy.

In conclusion, guava leaf extract offers a promising eco-friendly tool for Trichodinid control. By reducing parasite burdens, preserving tissue health, and supporting high survival rates at safe concentrations, it provides a low-cost, sustainable option for small-scale farmers while contributing to reduced chemical use in aquaculture.

ACKNOWLEDGEMENTS

The author thanks PT. Tiran Group Makassar, the Veterinary Hall of Maros, and the Faculty of Marine and Fisheries Sciences, Universitas Hasanuddin, Indonesia, for technical and laboratory support. Special thanks are extended to the supervisors for their guidance during the research and manuscript preparation. The author also expresses heartfelt gratitude to her parents for their unwavering support, encouragement, and prayers throughout the course of this study. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

Abd-Elrahman SM, Gareh A, Mohamed HI, Alrashdi BM, Dyab AK, El-Khadragy MF, Elbarbary NK, Fouad AM, El-Gohary FA, Elmahallawy EK, Mohamed SA. 2023. Prevalence and morphological investigation of parasitic infection in freshwater fish (Nile tilapia) from Upper Egypt. Animals 13 (6): 1088. DOI: 10.3390/ani13061088.

Abou-Okada M, AbuBakr HO, Hassan A, Abdel-Radi S, Aljuaydi SH, Abdelsalam M, Taha E, Younis NA, Abdel-Moneam DA. 2021. Efficacy of acriflavine for controlling parasitic diseases in farmed Nile tilapia with emphasis on fish health, gene expression analysis, oxidative stress, and histopathological alterations. Aquaculture 541: 736791. DOI: 10.1016/j.aquaculture.2021.736791.

Abu-Elala NM, Attia MM, Abd-Elsalam RM, Gamal A, Younis NA. 2021. Treatment of *Ichthyophthirius multifiliis* and trichodinid infections in Nile tilapia using peracetic acid reduces *Aeromonas hydrophila* infection and improves survival in Nile tilapia (*Oreochromis niloticus*). Aquaculture 538: 736591. DOI: 10.1016/j.aquaculture.2021.736591.

Adly MA, Abd El-Galil MAA, Soliman FM, Ahmed FAA. 2015. Histopathological studies on trichodinosis of farmed *Oreochromis niloticus*. Am J Life Sci 3: 30-37. DOI: 10.11648/j.ajls.s.2015030601.15.

Aris M, Adriana AN. 2022. Uji LC_{50} ekstrak daun mentimun (*Cucumis sativus* L) terhadap larva udang renik air asin (*Artemia salina* Leach) dengan menggunakan metode BSLT. Fito Medicine: J Pharm Sci 14 (1): 36-42. DOI: 10.47650/fito.v14i1.493. [Indonesian]

Attia MM, Abdelsalam M, Korany R, Mahdy OA. 2021. Characterization of digenetic trematodes infecting African catfish (*Clarias gariepinus*) based on integrated morphological, molecular, histopathological, and immunological examination. Parasitol Res 120: 3149-3162. DOI: 10.1007/s00436-021-07257-x.

Ceballos F, Kannan S, Kramer B. 2020. Impacts of a national lockdown on smallholder farmers' income and food security: Empirical evidence from two states in India. World Dev 136: 105069. DOI: 10.1016/j.worlddev.2020.105069.

Collymore C, White JR, Lieggi C. 2013. *Trichodinid xenopodus*, a ciliated protozoan, in a laboratory-maintained *Xenopus laevis*. Comp Med 63 (4): 310-312.

da Cruz MG, Jerônimo GT, dos Santos Torres G, de Matos LV, dos Santos DKM, Serra BNV, Gonçalves LU. 2022. Acute toxicity of trichlorfon and histological changes in the gills of *Arapaima gigas*, a neotropical fish from Amazon. *Aquaculture Reports* 25: 101229. DOI: 10.1016/j.aqrep.2022.101229.

Dadras F, Velisek J, Zuskova E. 2023. An update about beneficial effects of medicinal plants in aquaculture: A review. Vet Med 68 (12): 449. DOI: 10.17221/105/2023-VETMED.

Daniel E. 2024. Use of medicinal plants in the control of fish parasites and problems related to their use in ethnoveterinary treatment: A review. J Istanbul Vet Sci 8 (3): 247–272. DOI: 10.30704/http-www-jivs-net.1459423.

Desiyana LS, Husni MA, Zhafira S. 2016. Effectiveness test of ethyl acetate fraction gel of guava leaves (*Psidium guajava* Linn) on open wound healing in mice (*Mus musculus*). J Nat 16 (2): 23.

Effendie MI. 1993. Biologi Perikanan. Fakultas Perikanan, Institut Pertanian Bogor, Bogor. [Indonesian]

El-Demerdash AE, El-Sayed E, Elamin A, Yusuf M. 2020. Impacts of fish meal replacement with fermented animal by-products in African catfish (*Clarias gariepinus*) diets on growth, body composition, and fish quality. Assiut Vet Med J 66 (166): 153-165. DOI: 10.21608/avmj.2020.166373.

Elgendy MY, Ali SE, Dayem AA, Khalil RH, Moustafa MM, Abdelsalam M. 2024. Alternative therapies recently applied in controlling farmed fish diseases: Mechanisms, challenges, and prospects. Aquac Intl 32 (7): 9017-9078. DOI: 10.1007/s10499-024-01603-3.

El-Zayat SA, Abdel-Motaal FF, Mohamed SH, Hamouda AH. 2024. Efficiency of *Persicaria salicifolia* as a natural alternative antifungal against *Aspergillus flavus* infection in *Oreochromis niloticus* from Aswan Governorate, Egypt. Aquac Intl 32 (4): 5085-5105. DOI: 10.1007/s10499-024-01601-y.

Emara M, ElSawy H, Bauch G. 2020. A spatiotemporal model for peak AoI in uplink IoT networks: Time versus event-triggered traffic. IEEE Internet Things J 7: 6762-6777. DOI: 10.1109/JIOT.2020.2983129.

FAO [Food and Agriculture Organization]. 1985. Training Manual: Integrated Fish Farming in China. Food and Agriculture Organization of the United Nations, Rome.

FAO [Food and Agriculture Organization]. 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. FAO, Rome, Italy. DOI: 10.4060/cc0461en.

Faridha Begum I, Mohankumar R, Jeevan M, Ramani K. 2016. GC-MS analysis of bioactive molecules derived from *Paracoccus pantotrophus* FMR19 and the antimicrobial activity against bacterial pathogens and MDROs. Indian J Microbiol 56 (4): 426-432. DOI: 10.1007/s12088-016-0609-1.

Giri SS, Kim HJ, Kim SG, Kim SW, Kwon J, Lee SB, Park SC. 2020. Effectiveness of the guava leaf extracts against lipopolysaccharide-induced oxidative stress and immune responses in *Cyprinus carpio*. Fish Shellfish Immunol 105: 164-176. DOI: 10.1016/j.fsi.2020.06.004.

Hartsel JA, Eades J, Hickory B, Makriyannis A. 2016. *Cannabis sativa* and Hemp. In: Gupta RC (eds). Nutraceuticals: Efficacy, Safety and Toxicity. Academic Press, London. DOI: 10.1016/B978-0-12-802147-7.00053-X.

Hasan V, Mukti AT, Putranto TWC. 2019. Range expansion of the invasive Nile tilapia *Oreochromis niloticus* (Perciformes: Cichlidae) in Java Sea and first record for Kangean Island, Madura, East Java, Indonesia. Ecol Environ Conserv 25: S187-S189. DOI: 10.1088/1755-1315/718/1/012096.

Ihsan B, Sitinjak RS. 2023. Prevalence of ectoparasites in tilapia (*Oreochromis niloticus*) in Tarakan. Jurnal Medik Veteriner 6 (2): 185-190. DOI: 10.20473/jmv.vol6.iss2.2023.185-190.

Islas-Ortega AG, Marcotegui PS, Basson L, de Jager GP, Aguilar-Aguilar R. 2022. Morphological plasticity of *Trichodinid centrostrigeata*, an ectoparasite from gills of tilapia (*Oreochromis niloticus* and *O. mossambicus*). Folia Parasitol 69: 2022.022. DOI: 10.14411/fp.2022.022.

Kabata Z. 1985. Parasites and diseases of fish cultured in the tropics. Taylor and Francis, London.

Keck N, Blanc G. 2002. Effects of formalin chemotherapeutic treatments on biofilter efficiency in a marine recirculating fish farming system. Aquat Living Resour 15: 361-370. DOI: 10.1016/S0990-7440(02)01198-1.

Khan F, Magaji MG, Abdu-Aguye I, Hussaini IM, Hamza A, Olorukooba AB, Maje IM. 2021. Phytochemical profiling of the bioactive principles of *Alysicarpus glumaceus* (Vahl) DC. aerial parts. İstanbul J Pharm 51 (2): 228-238. DOI: 10.26650/IstanbulJPharm.2020.007.

Koniyo Y, Juliana, Pasisingi N, Kalalu D. 2020. Parasitic infection level and growth of red tilapia (*Oreochromis* sp.) fed vegetable fern flour (*Diplazium esculentum*). AACL Bioflux 13 (5): 2421-2430.

Kumar M, Tomar M, Amarowicz R, Saurabh V, Nair MS, Maheshwari C, Sasi M, Prajapati U, Hasan M, Singh S, Changan S, Prajapat RK, Berwal MK, Satankar V. 2021. Guava (*Psidium guajava* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods 10 (4): 752. DOI: 10.3390/foods10040752.

Kurian A, Lakshmi S, Fawole FJ, Faggio C, Elumalai P. 2021. Combined effects of *Leucas aspera*, oxy-cyclodextrin and bentonite on the growth, serum biochemistry, and the expression of immune-related gene in Nile tilapia (*Oreochromis niloticus*). Turkish J Fish Aquat Sci 21 (3): 147-158. DOI: 10.4194/1303-2712-v21_3_01.

Lieke T, Meinelt T, Hoseinifar SH, Pan B, Straus DL, Steinberg CE. 2020. Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. Rev Aquac 12 (2): 943-965. DOI: 10.1111/raq.12348.

Margareta MAH, Wonorahardjo S. 2023. Optimization of eugenol compound determination method in clove oil using GC-MS with injection temperature variation. J Sci Educ Sci 6 (2): 95-103. DOI: 10.24246/juses.v6i2p95-103.

Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DEJ, McLaughlin JL. 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 45 (5): 31-34. DOI: 10.1055/s-2007-971236.

Ministry of Marine Affairs and Fisheries. 2024. Fisheries production. https://portaldata.kkp.go.id/portals/data-statistik/prod-ikan/summary. [26 September 2024] [Indonesian]

Morais-Braga MFB, Carneiro JNP, Machado AJT, Dos Santos ATL, Sales DL, Lima LF, Coutinho HDM. 2016. *Psidium guajava* L.: From ethnobiology to scientific evaluation of bioactivity against pathogenic microorganisms. J Ethnopharmacol 194: 1140-1152. DOI: 10.1016/j.jep.2016.11.017.

Nhu VH, Shirzadi A, Shahabi H, Singh SK, Al-Ansari N, Clague JJ, Jaafari A, Chen W, Miraki S, Dou J, Luu C, Górski K, Pham BT, Nguyen HD, Ahmad BB. 2020. Shallow landslide susceptibility mapping: A comparison between logistic model tree, logistic regression, naïve Bayes tree, artificial neural network, and support vector machine algorithms. Intl J Environ Res Public Health 17 (8): 2749. DOI: 10.3390/ijerph17082749.

Nofita N, Ulfa AM, Delima M. 2021. Toxicity test of Australian guava leaf ethanolic extract (*Psidium guajava* L.) using BSLT method. J Farm Lampung 9 (1): 10-17. DOI: 10.37090/jfl.v9i1.326.

Nursanty R, Padzil KNBM, Ramli NIAB, Mahyudin NA, Jaafar AHB, Rukayadi Y. 2023. Phytochemical analysis of ethanolic *Psidium guajava* leaves extract using GC-MS and LC-MS. Biodiversitas 24 (5): 2723-2732. DOI: 10.13057/biodiv/d240526.

O'Halloran E, Mooney R, Rodgers K, Henriquez FL. 2022. Microbial interactions that contribute to gill disease in aquaculture. Parasitologia 2 (4): 266-291. DOI: 10.3390/parasitologia2040018.

Pimpley VA, Murthy PS. 2021. Influence of green extraction techniques on green coffee: Nutraceutical compositions, antioxidant potential and in vitro bioaccessibility of phenolics. Food Biosci 43: 101284. DOI: 10.1016/j.fbio.2021.101284.

Pudota BA, Tambireddy N, Chennu R, Chethurajupalli L, Shaik H, Nadella RK, Paturi AP. 2025. Exploring the perspectives of phytobiotics and their role in aquaculture: Present status and future trends. Microbe 8: 100496. DOI: 10.1016/j.microb.2025.100496.

Rahimi NNMN, Natrah I, Loh JY, Ervin Ranzil FK, Gina M, Lim SHE, Lai KS, Chong CM. 2022. Phytocompounds as an alternative antimicrobial approach in aquaculture. Antibiotics 11 (4): 469. DOI: 10.3390/antibiotics11040469.

Rahimnejad S, Dabrowski K, Izquierdo M, Hematyar N, Imentai A, Steinbach C, Policar T. 2021. Effects of vitamin C and E supplementation on growth, fatty acid composition, innate immunity, and antioxidant capacity of rainbow trout (*Oncorhynchus mykiss*) fed oxidized fish oil. Front Mar Sci 8: 760587. DOI: 10.3389/fmars.2021.760587.

Rozik M, Djauhari R. 2022. Prevalence and intensity of Nile tilapia (*Oreochromis niloticus*) ectoparasites in Martapura River, South Kalimantan. AACL Bioflux 15 (6): 2850-2860.

Sachet M, Liang YY, Oehler R. 2017. The immune response to secondary necrotic cells. J Autoimmun 83: 1189-1204. DOI: 10.1016/j.jaut.2017.05.012.

Sahandi J, Lakani FB, Zorriehzahra MJ, Shohreh P. 2023. Effects of garlic (*Allium sativum*) and chamomile (*Matricaria chamomilla*) extracts on *Ichthyophthirius multifiliis* parasite in guppy fish (*Poecilia reticulata*). J Survey Fish Sci 10 (1): 18-28.

Salam DM, Fernandes A, Maharani R. 2022. Profil fitokimia dan GC-MS resin *Dryobalanops keithii*. Jurnal Penelitian Sosial dan Ekonomi Kehutanan 8 (1): 17-34. DOI: 10.20886/jped.2022.8.1.17-34. [Indonesian]

Santhosh SK, Sarojini S. 2024. Comparative analysis of phytochemicals and antioxidant potential of ethanol leaf extracts of *Psidium guajava* and *Syzygium jambos*. J Nat Remed 24 (1): 2173-2187. DOI: 10.18311/jnr/2024/36164.

Santrianda A, Aji OR. 2021. Pengendalian parasit *Trichodina* sp. menggunakan infusa daun jambu biji (*Psidium guajava* L.) pada permukaan kulit ikan lele (*Clarias batrachus* L.). BIOSEL Biol Sci Educ J 10 (1): 25-33. DOI: 10.33477/bs.v10i1.1308. [Indonesian]

Schuermans S, Kestens C, Marques PE. 2024. Systemic mechanisms of necrotic cell debris clearance. Cell Death Dis 15 (8): 557. DOI: 10.1038/s41419-024-06947-5.

SNI [Standar Nasional Indonesia]. 2009. SNI 7550:2009 - Produksi ikan nila (*Oreochromis niloticus* Bleeker) kelas pembesaran di kolam air tenang. Badan Standardisasi Nasional, Jakarta. [Indonesian]

Somnuek S, Thipmanee K, Jaenaksorn T. 2025. Antifungal activity and phytochemical analysis of Myrtaceae leaf extracts in lettuce against *Alternaria* leaf spot. Hortic Sci Technol 43 (1): 33-60. DOI: 10.7235/HORT.20250009.

Sravya MVN, Sandeep TR, Beulah G, Sampath Kumar NS, Simhachalam G. 2025. Biopotency of *Avicennia marina* leaf extracts against pathogenic bacteria in carp culture. AMB Express 15 (1): 2. DOI: 10.1186/s13568-024-01791-4.

Steckert LD, Cardoso L, Tancredo KR, Martins ML, Jeronimo GT. 2019. *Dermocystidium* sp. in the gills of farmed *Oreochromis niloticus* in Brazil. An Acad Bras Cienc 91 (3): e20180959. DOI: 10.1590/0001-3765201920180959.

Toutou MM, Abd allah Zaki MA, Abd EL-Monem AIM, Elmaghraby AM, Abdel-Rahman SM, Abd Rabou EMN. 2024. Effect of different levels of the dietary *Psidium guajava* L. leaves meal and extract on growth performance, feed utilization, body composition, immune-related genes expression, and health status of the thinlip grey mullet (*Liza ramada*) fish. Egypt J Aquat Biol Fish 28 (3): 931-949. DOI: 10.21608/ejabf.2024.361014.

Triastuti J, Luthfiyah L, Satriya MGRI. 2024. Histology of skin, gill, liver, and kidney of tilapia (*Oreochromis niloticus*) exposed to acid red 88 azo dyes. IOP Conf Ser Earth Environ Sci 1392 (1): 012017. DOI: 10.1088/1755-1315/1392/1/012017.

Ukwa UD, Saliu JK, Akinsanya B. 2023. Phytochemical profiling and anthelmintic potential of extracts of selected tropical plants on parasites of fishes in Epe Lagoon. Sci Rep 13 (1): 22727. DOI: 10.1038/s41598-023-48164-8.

Van As JG, Basson L. 1989. A further contribution to the taxonomy of the Trichodinidae (Ciliophora: Peritrichia) and a review of the taxonomic status of some fish ectoparasitic trichodinids. Syst Parasitol 14 (3): 157-179. DOI: 10.1007/BF02187051.

Younis NA, Attia MM, Saleh NM. 2021. Analysis of TNF alpha and Interleukin-1β genes in *Oreochromis niloticus*: Inflammatory responses induced by *Myxobolus* spp. and *Trichodina* spp. Iran J Ichthyol 8 (1): 30-40. DOI: 10.22034/iji.v8i1.450.

Zhu F. 2020. A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture 526: 735422. DOI: 10.1016/j.aquaculture.2020.735422.