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Phytochemical Screening of *Aglaia edulis* (Meliaceae) and its Toxicity Assessment and Anti-Microbial Potential against Fish Pathogens

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Abstract: This study investigated the antimicrobial potential of *Aglaia edulis*, a traditionally used medicinal plant from the Meliaceae family, against fourteen predominant fish pathogens. Phytochemical screening of leaf and bark extracts revealed the presence of bioactive compounds including alkaloids, terpenoids, phenols, and flavonoids. GC-MS analysis identified 34 compounds across the extracts, with Sesquiterpenes, terpenoid, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (23.67%) being predominant in leaf extract. Antimicrobial activity was assessed using agar well diffusion and minimum inhibitory concentration (MIC) methods. Ethanol extracts demonstrated superior antimicrobial properties compared to ethyl acetate extracts, with leaf extracts showing greater potency than bark extracts. Maximum inhibition was observed against *Staphylococcus aureus* (17±1.73 mm), and *Aeromonas hydrophila* (17±1mm) with leaf ethanol extracts. MIC values ranged from 72.3 – 86.2% against *Enterococcus faecalis* and *S. aureus*, respectively. Toxicity evaluated using brine shrimp lethality assays demonstrated dose-dependent toxicity values from 350.5-388 ppm. This first scientific report on the antimicrobial properties of *A. edulis* demonstrates its significant potential as a sustainable alternative for aquaculture disease management, offering a promising approach to combat antimicrobial resistance in fish pathogens.

Keywords: *Aglaia edulis*, Antimicrobial resistance, Aquaculture pathogens, Phytochemical screening, Sustainable disease management

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Introduction

The increasing prevalence of antimicrobial resistance in aquaculture pathogens threatens global fish production and food security, creating an urgent need for sustainable alternatives to conventional antibiotics in an industry (Luthman *et al.*, 2024). Aquaculture is growing rapidly

worldwide, but diseases remain a major challenge while technology enhances production, high fish densities can increase stress and promote bacterial infections. (Kowalska *et al.*, 2020). The global surge in antimicrobial resistance (AMR) has become one of the most pressing challenges in modern healthcare, with the World Health Organization declaring it a top ten global public health threat (Bertagnolio *et al.*, 2024). The proliferation of antimicrobial-resistant fish pathogens explores plant-based therapeutic alternatives, offering a sustainable and environmentally friendly approach to combating pathogens like *Aeromonas hydrophila*, *Streptococcus iniae*, and *Vibrio* species (Abdella *et al.*, 2024; Juárez-Cortés *et al.*, 2024). This crisis has intensified the search for natural novel antimicrobial compounds. Traditionally used plants from the Meliaceae family have emerged as promising candidates due to their rich phytochemical diversity and documented medicinal properties. This tropical tree species, widely distributed across Southeast Asia, has been traditionally used by indigenous communities in Malaysia, Indonesia, and the Philippines to treat various ailments including skin infections, typhoid, and gastrointestinal disorders (Adeniran *et al.*, 2024).

Recent phytochemical investigations of *A. edulis* have revealed the presence of several bioactive compounds, including rocaglamides, silvestrols, and bisamides (Agarwal *et al.*, 2021). These findings have stimulated interest in exploring the plant's potential as a source of novel antimicrobial agents. This plant's various parts, including leaves, bark, and fruits, have been documented in traditional medicine systems for treating infections, suggesting their potential application in aquaculture disease management. The research evaluates the plant extract's efficacy against both drug-sensitive and resistant bacterial strains while investigating toxicity mechanisms using *Artemia salina* larvae, a valuable monitoring tool for natural product toxicity assessment (Sunaryo *et al.*, 2024). The bio-compounds from some plant extracts may contain active compounds that can be toxic to human and fish health. This study aims to investigate the antimicrobial activity of various extracts from *A. edulis* against prevalent fish pathogens, characterize the bioactive compounds responsible for such activity, and evaluate their potential application in aquaculture disease management. Understanding the antimicrobial properties of *A. edulis* could contribute to the development of novel, sustainable solutions for aquaculture health management while potentially reducing reliance on conventional antibiotics.

Materials and Methods

Collection of plant materials

Leaves and bark of *Aglaia edulis* were collected during the month of March, 2024 from the Palappilly, Thrissur, India and identified from the Xavier Research Foundation, Palayamkottai.

Preparation of plant crude extracts

The collected leaves and bark of *Aglaia edulis* were powdered using an electrical blender plant materials were extracted using organic solvents. The extraction process was carried out using the Soxhlet extraction method, where 50 g of powdered materials used series of extraction solvents, including Hexane, Petroleum ether, Ethyl acetate, and Ethanol (95%). Solvent was removed using a rotary vacuum evaporator at 60°C in a water bath. The obtained concentrated extract was then frozen until further use.

Test microorganisms and growth media

The following bacteria (Gram-positive and Gram-negative) and fungus was used for antimicrobial activity: bacteria included *Aeromonas hydrophila*, *Aeromonas veronii*, *Aeromonas caviae*, *Staphylococcus aureus*, *Staphylococcus albus*, and *Enterococcus faecalis*; whereas fungus included *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum*, and *Candida albicans*. The bacterial strains were grown in Muller Hinton Agar (MHA) plates at 37°C, whereas the fungus was grown in Potato Dextrose Agar (PDA) media at 30°C. Then stock cultures were maintained at 4°C.

Phytochemical screening

The presence of major secondary metabolites such as Alkaloids Flavonoids, Tannins, and Phenols were analyzed using standard procedure (Edeoga *et al.*, 2005).

Gas chromatography–mass spectrometry (GC-MS) analysis of Aglaia edulis leaf and bark extract

GC-MS analysis was carried out in a combined 7890A Gas Chromatograph System (Agilent 19091-433HP, USA) and Mass Spectrophotometer, fitted with a HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0 m × 250 µm, film thickness 0.25 µm), interfaced with 5675C Inert MSD with Triple-Axis detector. Helium gas was used as carrier gas and was adjusted to column velocity flow of 1.0 ml/min (Oliva *et al.*, 2023).

Preliminary antimicrobial screening: Aglaia edulis different solvent extract

Preliminary antimicrobial screening was conducted using hexane, petroleum ether, ethyl acetate, and ethanol. Notably, hexane and petroleum ether extracts showed no inhibitory activity against the tested pathogenic strains, indicated by the absence of zones of inhibition in different concentrations. In contrast, both ethyl acetate and ethanol extracts showed significant antimicrobial potential, this differential activity pattern suggests that the bioactive compounds responsible for the antimicrobial properties are preferentially extracted in more polar solvents (ethyl acetate and ethanol) compared to non-polar solvents (hexane and petroleum ether). Based on these preliminary findings, ethyl acetate and ethanol extracts were selected for further detailed studies.

Evaluation of antimicrobial activity of Aglaia edulis leaf and bark extract

The plant extract was tested for antibacterial and antifungal activities by the well diffusion method (Schumacher *et al.*, 2018). The MHA plates surface of the plate was inoculated by swabbing the bacterial culture (1 × 10^6 CFU/ml) and (2× 10^5 CFU/ml) fungal culture on PDA plates, respectively. After solidification, wells were made using a sterile corkborer (6 mm in diameter) into agar plates containing inoculums. Then, 100 µl of each extract (stock culture at 5, 10, 50, 150 mg/ml) was added to respective wells. The well loaded with different concentrations (0.5 mg, 1 mg, 5 mg, 15 mg) of leaf and bark crude extract of *A. edulis* were prepared by the addition of DMSO. The DMSO served as the negative control, while streptomycin was used as the positive control. The plates were incubated at 35 ± 2 °C for 24 h, and the entire process was conducted in triplicate. The diameters of the inhibition zones were measured in millimetres.

Minimum Inhibitory Concentraion (MIC) of Aglaia edulis ethyl acetate and ethanol extract

The determination of the MIC for *Aglaia edulis* was carried out using the micro dilution method (Lachumy *et al.*, 2010) with small modification. For antibacterial MIC assessment, the plant extracts were serially diluted in nutrient broth to achieve concentrations ranging from 1500 to 2.9 µg/0.1 ml. Nutrient broth used for bacteria at 1x10^6 CFU/ml and PDB for fungi at 2x10^5 CFU/ml. Following 24 h incubation at 25±2 °C, MTT was added to detect metabolic activity, with bacterial growth indicated by colour change from yellow to purple-pink and measured bacterial growth was detected by reading absorbance at 500 nm using an ELISA reader. MIC inhibition percentage calculated as [(OD Control - OD Test)/OD Control] ×100%, using streptomycin as positive control and DMSO with bacterial suspension as negative control (Eloff).

Toxicity bioassay

Brine shrimp lethality assay was performed to evaluate the toxicity of plant extracts following the method described by Joshi and Karna (2013) and Solis *et al.* (1992) with slight modifications. Artificial seawater was prepared by dissolving sea salt (38 g/l) in water, and the pH was adjusted to 8.5 using 1N NaOH. Brine shrimp eggs were hatched for 48 h in a well aerated conical flask containing 300 ml of seawater. The brine shrimp toxicity assay was conducted using plant extracts (ethyl acetate and ethanol) dissolved in DMSO at

Table 1: Phytochemical analysis of *A. edulis* plant extracts

Phytochemical tests	Leaf extracts		Bark extracts	
	Ethyl acetate	Ethanol	Ethyl acetate	Ethanol
Test for Alkaloids	++	++	-	+
Test for steroids	-	++	+	++
Test for tannins	-	-	+	+
Test for saponins	–	–	-	-
Test for flavonoids	-	++	-	+
Test for quinones	-	+	-	-
Test for carotenoids	+	++	+	++
Test for cardiac glycosides		+	-	-
Test for terpenoids	++	++	-	++
Test for phenols	--	++	+	++

concentrations of 50, 75, 125, 250, 500 and 1000 ppm, with 30 *Artemia nauplii* per concentration tested in triplicate, using potassium dichromate as a positive control and recording mortality after 24 h. The mortality was noted, and the concentration that killed 50% of the nauplii LC_{50} values were determined using the statistical method of probit analysis (Bastos *et al.*, 2009).

Statistical analysis

Results are expressed as the mean ± SD of mean (SEM). The data generated from antibacterial and antifungal activity were subjected to ANOVA using Statistix version 8.1. Comparison among mean values was made by Least Significant Difference (LSD) to test significant differences at $P < 0.05$. Probit regression analysis was used to calculate LC_{50} values.

Results and Discussion

Phytochemical screening

The phytochemical analysis of leaf and bark extracts (ethyl acetate and ethanol solvents) of *Aglaia edulis* showed the presence of major bioactive secondary metabolites. Ethyl acetate extract (leaf) tested indicate the presence of active constituents such as alkaloids, terpenoids, saponins, phenolic compounds and flavonoids. Ethanol extract (leaf) showed the presence of alkaloids, steroids, flavonoids, quinones, carotenoids, cardiac glycosides, terpenoids, phenols and absence of tannins and saponins (Table 1). Additionally, phytochemical analysis of solvent extract of bark indicated the existence of various types of phytocomponents. In bark extracts, both ethyl acetate and ethanol extracts showed positive results for alkaloids, flavonoids, phenols, and tannins. However, the intensity of reactions varied, with ethanolic extract showing more intense reactions for phenols and tannins compared to ethyl acetate extract. Plant based products display antimicrobial potential properties are interesting and promising compounds to control pathogenic infection in

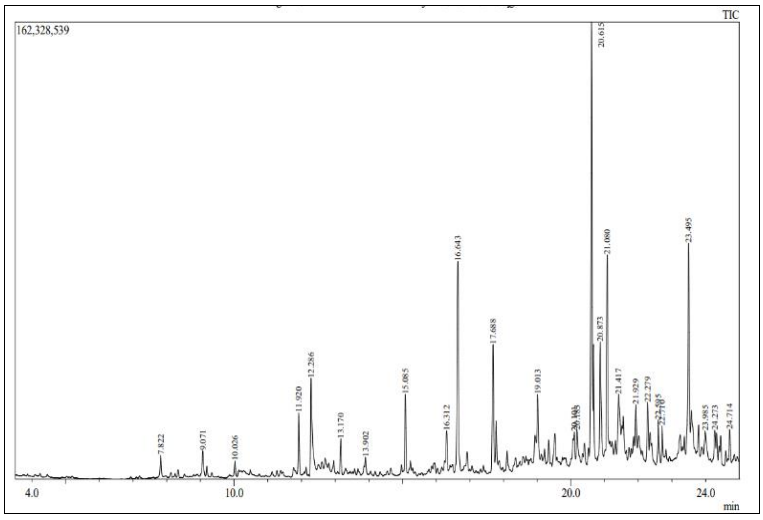

Fig. 1: GC-MS spectrum of ethyl acetate extract of leaf of *Aglaia edulis.*

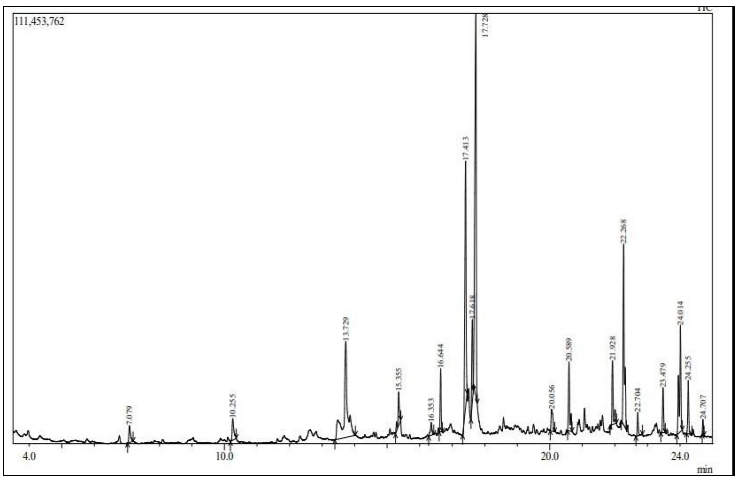

Fig. 2: GC-MSspectrumofethanol extractof *Aglaia edulis leaf.*

aquaculture production. The present investigation provides potential of *Aglaia edulis* plant leaf and bark extracts. Phytochemical screening exposed a wide range of bioactive compounds like alkaloids, steroids, flavonoids, phenols and terpenoids particularly in ethanol extracts, which is in agreement with findings by Archana (2020). Flavonoids and phenolic compounds are naturally occurring compounds which plays a vital role in health promotion and disease prevention (Boziaris *et al.*, 2011), and antioxidant and antimicrobial activities (Shi *et al.*, 2022; Akl *et al.*, 2022).

GC-MS analysis of Aglaia edulis leaf extract

In GC/MS analysis *Aglaia edulis* extracts showed total of 34 compounds which were detected from the ethyl acetate and ethanol leaf extracts (Figs. 1, 2) and ten compounds from bark ethanol extracts (Fig. 3). Compounds were grouped into various phytoconstituent classes like alkaloids, phenols, terpenoids, steroids, flavonoids, esters, fatty acids, alcohols etc. GC/MS analysis of the ethyl acetate leaf extract of *A. edulis* revealed 20 compounds along with their mass spectra (Fig. 1). The majority of the compounds belonged to the classes like sesquiterpenes, triterpenes, organic compounds, alkaloids, steroids, esters etc. A terpenoid compound 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (23.67%) was found in highest amount. This was followed by phytol (9.78%) and Neophytadiene (7.12%). *A. edulis* leaf ethanol extract revealed 14 compounds along with their

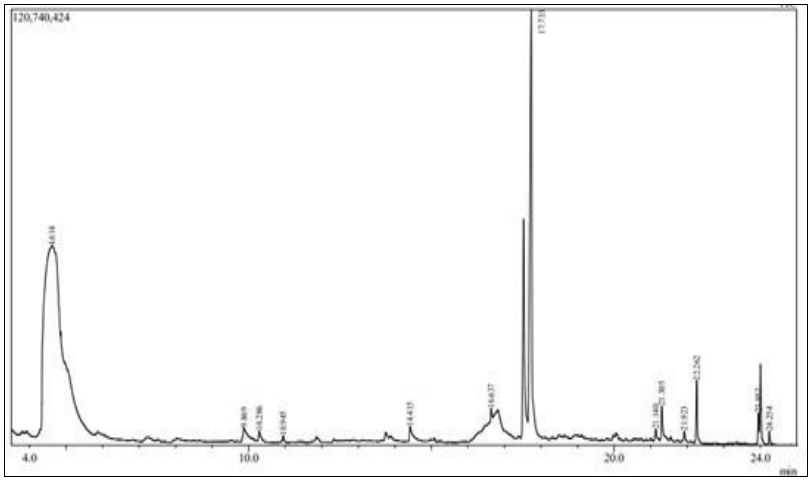

Fig. 3: GC-MS spectrum of ethanol extract of *Aglaia edulis* bark.

mass spectra (Fig. 2). Sesquiterpenes were represented by 5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0] heptan-2-ol (2.21%), Diethyl2-(p-tolyl) malonate (17.72%) and 1,4-Benzenedicarboxylicacid,diethylester (17.41%) in considerable amounts as well as Hexadecanoic acid, ethyl ester (22.26%), Ethyl Oleate (7.41%), and 2,4-Di-tert-butylphenol (Phenol - 2.74%). Bark ethanol extract of *A. edulis* revealed 10 compounds along with their mass spectra (Fig. 3). The phenolic compounds identified in the extract were 2,4-Di-tert-butylphenol (0.24%), Dimethylsulfoxonium formylmethylide (68.90%) and Diethyl2-(p-tolyl)malonate (25.90%). Compounds with fatty acids like Hexadecanoic acid, ethyl ester (1.30%), Octadecanoic acid, ethyl ester (0.28) were detected in considerable amounts. The GC–MS based analysis of the chemical composition of *A. edulis* extracts revealed the presence of Sesquiterpenes represented by 5-Isopropenyl-2-methyl-7-oxabicyclo [4.1.0] heptan-2-ol, and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (23.67%) being the predominant compound in leaf extracts as these compounds have been previously associated with antimicrobial properties in *Aglaia minahassae* (Kurniasih *et al.*, 2018).

Evaluation of antimicrobial activity of Aglaia edulis

The antimicrobial potential of *Aglaia edulis* leaf and bark extracts was evaluated against ten predominant fish pathogens using agar well diffusion method with concentrations of 0.5 mg, 1 mg, 5 mg, and 15 mg dissolved in DMSO, with streptomycin and DMSO used as the positive and negative controls, respectively which shows both the zone of inhibition (mm) and relative percentage of inhibition to the positive control (Tables 2, 3). Antibacterial activity increased with extract concentration, with ethanol extracts demonstrating superior antimicrobial properties compared to ethyl acetate extracts, and leaf extracts showing greater antimicrobial potency than bark extracts.

Maximum inhibition was observed in leaf ethanol extracts against *Staphylococcus aureus* (17±1.73 mm, 56.05%) and *Aeromonas hydrophila* (17±1 mm, 77.27%), while *S. albus* and *E. faecalis* were most susceptible to leaf ethyl acetate extract. Conversely, *Aeromonas caviae* showed relatively lower susceptibility in leaf and bark extract. The antimicrobial activity of various *Aglaia* species against gram-positive and gram-negative bacteria with the ethanolic extract of *Aglaia lawii* and *Aglaia odorata* leaves and bark germacrene D is an antibacterial bioactive compound (Joycharat *et al.*, 2014; Dong *et al.*, 2023). In a previous study, methanol extracts of *Aglaia* sp. showed significant antimicrobial activity against *E. coli* and *S. aureus*, likely due to the presence of alkaloid compounds that are recognized for their antibacterial

Table 2: Antibacterial activity and % zone of Inhibition of *A. edulis* leaf ethyl acetate and ethanol extracts against fish pathogens

Pathogens	Concentration	Zone of Inhibition		% Zone of Inhibition relation to P	
		Ethyl Acetate	Ethanol	Ethyl Acetate	Ethanol
Aeromonas hydrophila	0.5 mg	0	0	0 %	0 %
	1 mg	10.33 ± 1.52	12.33 ± 1.52	46.9%	56.0%
	5 mg	13.33 ± 0.57	12±2	60.5%	54.5%
	15 mg	13.66 ± 0.57	17± 1	60.5%	77.2%
	P	22 ± 1	22 ± 1	100%	100%
	N	0	0	0%	0 %
Aeromonas veronii	0.5 mg	0	0	0%	0 %
	1 mg	10.66 ± 1.15	11 ± 1	55.9%	55.9%
	5 mg	11 ± 1	13.33 ± 1.52	55.9%	67.8%
	15 mg	11.66 ± 0.57	16.33 ± 1.15	59.3%	83.0%
	P	19.66 ± 1.52	19.66 ± 1.52	100%	100%
	N	0	0	0 %	0 %
Aeromonas caviae	0.5 mg	0	0	0%	0%
	1 mg	9.666 ± 1.15	9.333 ± 0.57	39.7%	38.3%
	5 mg	10 ± 1	12.33 ± 1.15	41.1%	50.6%
	15 mg	12.66 ± 1.15	14 ± 1	52.0%	57.5%
	P	24.33 ± 2.08	24.33 ± 2.08	100%	100%
	N	0	0	0%	0%
Staphylococcus aureus	0.5 mg	0	0	0 %	0%
	1 mg	11.66 ± 1.154	10.33 ± 1.527	38.44%	32.97%
	5 mg	13.33 ± 1.154	13 ± 1	43.94%	42.86%
	15 mg	16.66 ± 1.527	17 ± 1.732	53.84%	56.05%
	P	30.33 ± 1.527	30.33 ± 1.527	100 %	100 %
	N	0	0	0 %	0 %
Staphylococcus albus	0.5 mg	0	0	0 %	0 %
	1 mg	12.33 ± 0.577	11.33 ± 0.577	45.66%	41.96
	5 mg	14 ± 1	11.33 ± 1.527	51.85%	41.96
	15 mg	16 ± 1	15.66 ± 1.154	59.25	58%
	P	27 ± 7.937	27 ± 7.937	100 %	100 %
	N	0	0	0 %	0 %
Enterococcus faecalis	0.5 mg	0	0	0 %	0 %
	1 mg	9.333 ± 1.154	11 ± 1.732	38.87%	45.83%
	5 mg	12.66 ± 1.154	11.66 ± 1.527	52.75%	48.58%
	15 mg	15.33 ± 1.154	14.66 ± 1.527	63.87%	61.08
	P	24 ± 1	24 ± 1	100 %	100 %
	N	0	0	0 %	0 %

properties (Hadrian *et al.*, 2023). The present study revealed that plant extracts exhibited antimicrobial activity against a broad spectrum of microorganisms, with particularly higher effectiveness against Gram-positive bacteria, especially *S. aureus* (Khan *et al.*, 2024). This differential sensitivity may be attributed to the exposed peptidoglycan layer in Gram-positive bacteria (Routh *et al.*, 2024), while antimicrobial action against Gram-negative bacteria occurs through electrostatic binding to lipopolysaccharides, leading to membrane disruption and cell death (Stephani *et al.*, 2024). Among fungi, ethanol extracts showed highest activity against *Fusarium oxysporum* (15.66±1.15 mm) and *Candida albicans* (15.66±0.57 mm). When compared to positive controls, ethanol extracts at 15 mg reached up to 83.06% inhibition against *A. veronii* and 61.02% against *F. oxysporum*. Moreover, *A. argentea* species contain

Table 3: Antifungal activity and % zone of Inhibition of *A. edulis* in leaf ethyl acetate and ethanol extracts against fish pathogens

Pathogens	Concentration	Zone of Inhibition		% Zone of Inhibition relation to P	
		Ethyl Acetate	Ethanol	Ethyl Acetate	Ethanol
Aspergillus niger	0.5 mg	0	0	0 %	0%
	1 mg	10.66 ± 1.15	10.33 ± 0.57	35.9%	34.8%
	5 mg	12.33 ± 0.57	12 ± 1	41.57%	40.45%
	15 mg	14.66 ± 0.57	15.33 ± 0.57	49.4%	51.6%
	P	29.66 ± 0.57	29.66 ± 0.57	100%	100%
	N	0	0	0%	0 %
Aspergillus flavus	0.5 mg	0	0	0%	0 %
	1 mg	0	8.666 ± 1.15	0%	30.5%
	5 mg	9.33 ± 1.15	13 ± 1	32.9%	45.8%
	15mg	11 ± 1	13.66 ± 0.57	38.8%	48.2%
	P	28.33 ± 1.52	28.33 ± 1.52	100 %	100 %
	N	0	0	0 %	0 %
Fusarium oxysporum	0.5 mg	0	0	0%	0%
	1 mg	12.33 ± 0.57	10 ± 1	48.0%	38.9%
	5 mg	12.66 ± 0.57	11.33 ± 1.15	49.3%	44.1%
	15 mg	14 ± 1	15.66 ± 1.15	54.5%	61.0%
	P	25.6 ± 1.15	25.66 ± 1.15	100%	100 %
	N	0	0	0%	0%
Candida albicans	0.5 mg	0	0	0%	0 %
	1 mg	10.66 ± 0.57	12.66 ± 1.15	37.6%	44.6%
	5 mg	12.33 ± 0.57	13.66 ± 0.57	43.52%	48.21
	15mg	14.66 ± 1.52	15.66 ± 0.57	51.7%	55.2%
	P	28.33 ± 1.52	28.33 ± 1.52	100 %	100 %
	N	0	0	0%	0 %

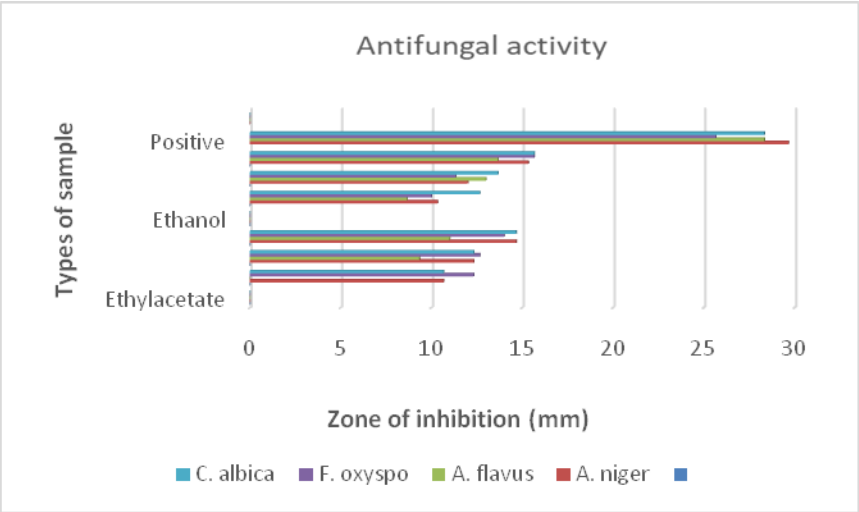

Fig. 4: Antifungal activity of *A. edulis* leaf ethyl acetate and ethanol extracts against pathogens.

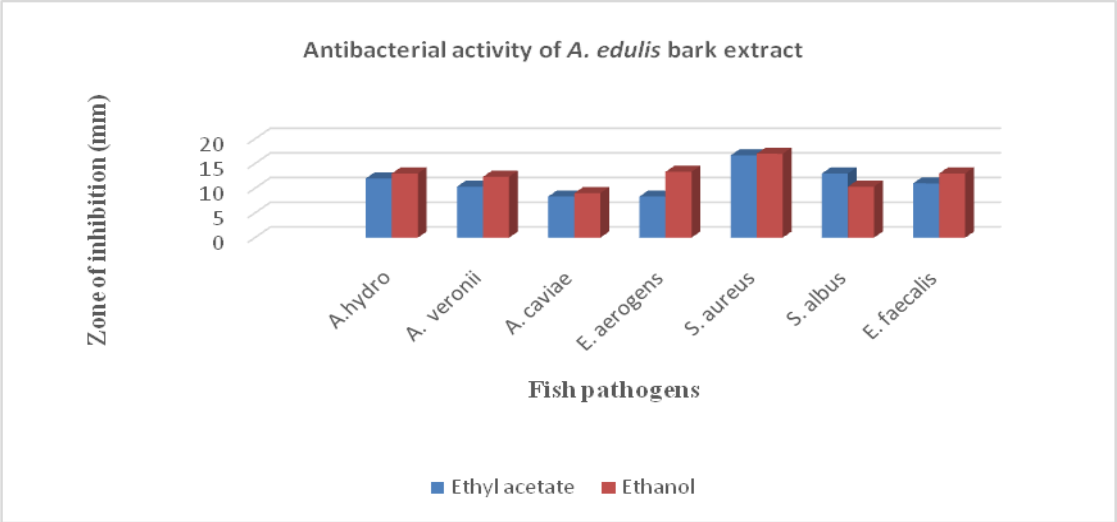

Fig. 5: Antibacterial activity of *A. edulis* bark ethyl acetate and ethanol extracts against fish pathogens.

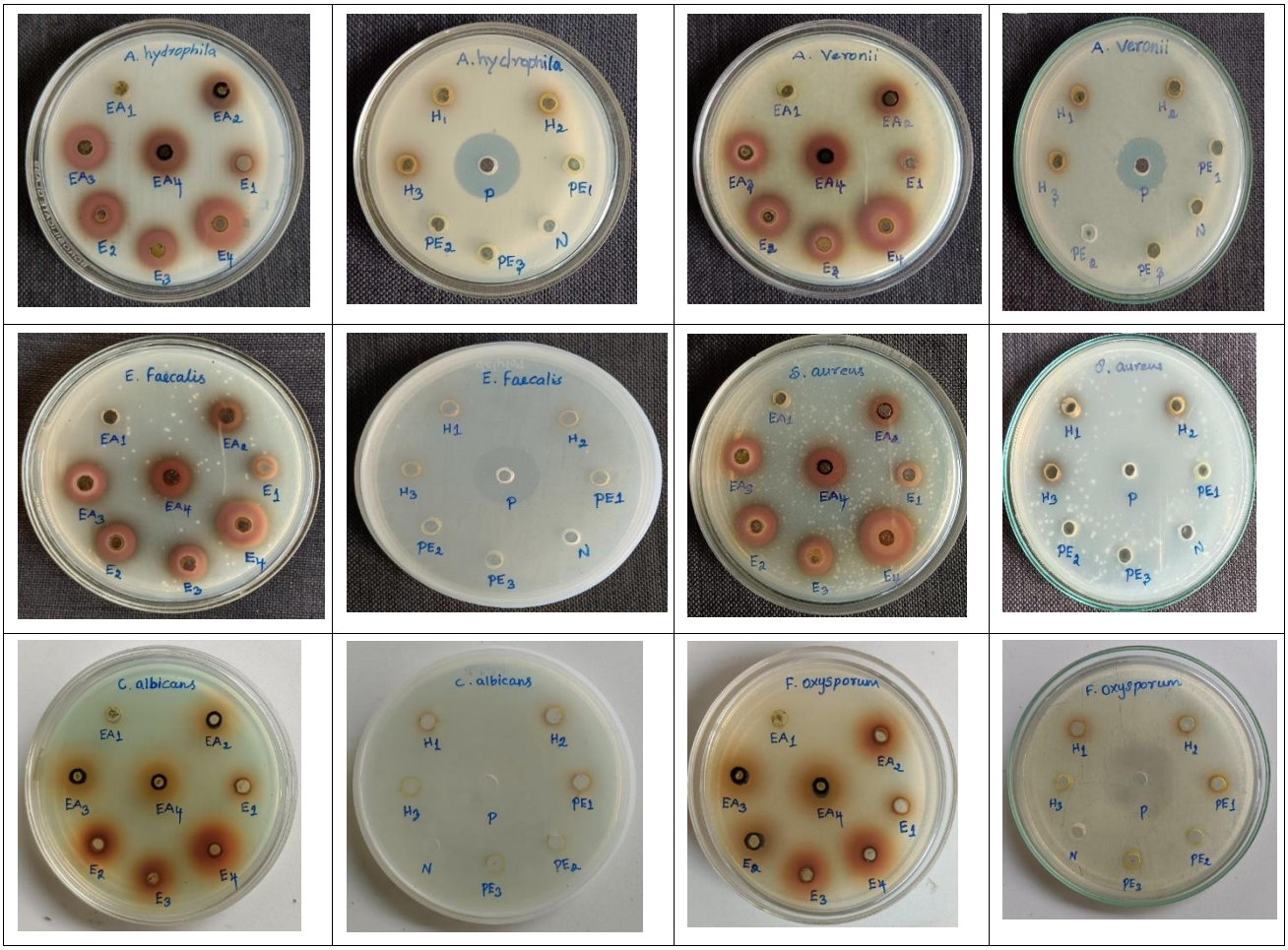

Fig. 6: Determination of the antimicrobial activity of *Aglaia edulis* extract against pathogens. $EA_{1,2,3,4}$ -ethyl acetate extract, $E_{1,2,3,4}$ -ethanol extract 0.5 mg, 1 mg, 5 mg and 15 mg concentration.

alkaloid compounds that also have antifungal activities (Praptiwi *et al.*, 2009) and Rocaglamide compound exhibited clear antifungal activity against various fungi (Engelmeier *et al.*, 2000).

Bark ethanol extract showed maximum inhibition in *Aeromonas hydrophila*13±1.7. 15 mg concentration of leaf extracts showed zone of inhibition ranging from 7.6-13.4 mm across pathogens. Bark ethyl acetate extract showed maximum inhibition in *S. aureus* (13.33 mm). This first scientific report on antimicrobial properties of *A. edulis* demonstrated significant potential against diverse fish pathogens. Both *A. edulis* leaf and bark extracts demonstrated concentration-dependent antimicrobial activity, with the largest inhibition zones recorded for leaf extract (19.6 mm) and bark extract (13.3 mm) at 150 mg/ml. These findings align with previous research by Parekh and Chanda (2007). The broad-spectrum cytotoxic activity of flavaglines (Kim *et al.*, 2006) and bisamides in *A. edulis* supports its traditional medicinal applications for cancer treatment and *Aglaia eximia* showed significant antioxidant activity of kaempferol compounds the type of flavonoid isolated by Sianturi (2016).

The potential of Meliaceae family plants in aquaculture disease management has been previously established, with *Azadirachta indica* (neem) leaf extract effectively controlling *A. hydrophila* infections in Nile tilapia (Khalifa *et al.*, 2024). Chemical constituents and antimicrobial properties of *Melia dubia* plant extract exhibit linolenic acid which effectively control fish pathogens (Goswami *et al.*, 2020). While *A. edulis* remains less explored in this context, previous investigations have demonstrated its promising properties, including flavaglines with insecticidal properties and antiviral activity against Herpes simplex virus (Bacher *et al.*, 1999; Saifah *et al.*, 1999), suggesting broader antimicrobial applications. Pharmacognostic features, nutritional value, significant bioactivities of limonoids from the fruits of *Aglaia edulis* also demonstrated promising antimicrobial sources for health maintenance and disease control (Sun *et al.*, 2022).

Minimum inhibitory concentration of A. edulis leaf in ethyl acetate and ethanol extracts against fish pathogens

Minimal inhibitory concentration (MIC) values were used to determine the concentration of bacteria to drugs and also to assess the activity of new antimicrobial agents (Table 5). Minimum inhibitory concentration (MIC) tests confirmed ethanol's greater performance, with effectiveness ranging from 72.3% in *E. faecalis* (1.87 mg/ml) to 86.2% in *S. aureus* (1.87 mg/ml) against bacteria. Ethyl acetate showed MIC ranging from 61.7% in *A. veronii* (3.75 mg/ml) to 76.6% (*S. aureus*) and from 69.2% (1.87 mg/ml) in *A. niger* to 77.9% (3.75 mg/ml) against *C. albicans* fungi. Overall, both extracts showed dose-dependent activity but ethanol extracts reliably demonstrated better antimicrobial properties, particularly against gram-positive bacteria. Similarly, ethanol extracts from *A. edulis* demonstrated superior anti-microbial activity against *Aeromonas hydrophila* and *Staphylococcus aureus* with 84.4% MIC effectiveness at 1.5 mg/ml concentration, with β-stigmasterol glucoside as a key active compound disrupting bacterial membrane integrity function, thereby inhibiting bacterial growth and proliferation (Dhey *et al.*, 2023).

Toxicity study

The result of the brine shrimp lethality bioassay showed that the leaf ethyl acetate extract (LC_{50}: 388 ppm at 24 h), ethanol extract (LC_{50}: 350.5 ppm at 24 h) and bark ethyl acetate extract and ethanol extract (392.2; 337 ppm at 24 h) exhibited moderate toxicity toward *A. salina.* Potassium dichromate served as the positive control, with an LC_{50} value of 44.61 ppm (Fig. 8). The toxicity assessment of *Aglaia edulis* extracts using brine shrimp (*Artemia salina*) revealed differential toxicity profiles, with leaf extracts lethal concentration categorized as low toxic compared to bark extracts after 24 h exposure. The enhanced toxicity of ethanol extracts is attributed to

Table 5: MIC of *A. edulis* leaf ethyl acetate and ethanol extracts against pathogens

Pathogens	Leaf extracts	
	Ethyl acetate	Ethanol
Aeromonas hydrophila	69.5%	84.4%
Aeromonas veronii	61.7%	81.9%
Aeromonas caviae	63.1%	72.5%
S. aureus	76.6%	86.2%
S. albus	75.1%	74.2%
E. faecalis	74.8%	72.3%
A. niger	62.8%	69.2%
A. flavus	60.4%	71.03%
F. oxysporum	66%	77%
C. albicans	68.1%	77.9%

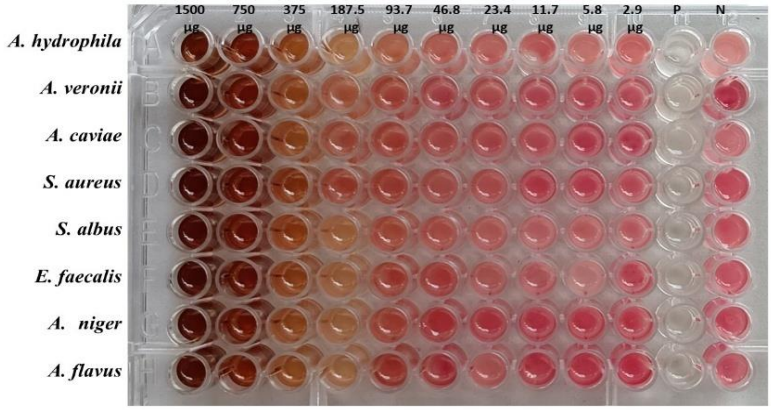

Fig. 7: Determination of the MIC values of *Aglaia edulis* leaf extract against pathogens.

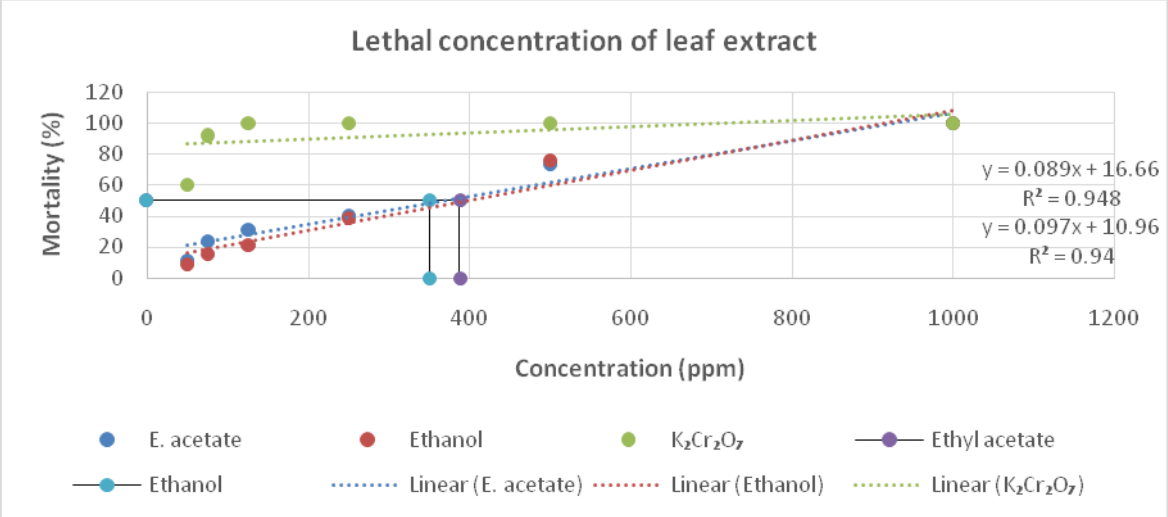

Fig. 8: Probit analysis to determine the LC_{50} value of *Aglaia edulis* leaf extracts on *Artemia salina.*

alkaloids, flavonoids, terpenes, and phenolic compounds that readily penetrate the cytoplasmic membrane of *Artemia*, with silvestrol known to possess cytotoxic and anticancer effects in *A. edulis* (Kim *et al.*, 2006). Previous research demonstrated that flavaglines from *Aglaia* species exhibit selective toxicity to normal cells (Greger *et al.*, 2022). Similarly, methanol extract of *Aglaia argentea* containing rocaglate showed significant biological activity with an IC_{50} value of 15.5 mg/ml, indicating potent toxic components warranting further investigation (Hidayat *et al.*, 2017). Comparative studies revealed that *A. edulis* leaf and bark extracts were toxic with an LC_{50} of 68.57 ppm, consistent with the presence of cyclopenta[b]benzofurans cytotoxic compounds in *Aglaia foveolata*, suggesting potential safe-level use as antimicrobial agents (Greger *et al.*, 2020). In contrast, *Aglaia roxburghiana* extracts demonstrated significant toxicity, particularly the methanol extract (Islam *et al.*, 2009), while other Meliaceae plant extracts showed high toxicity against brine shrimp larvae (LC_{50}<500 µg/ml), suggesting caution for medicinal applications. Conversely, neem extracts were found non-toxic to brine shrimp larvae (Madesh *et al.*, 2024). These findings collectively suggest that *A. edulis* extracts represent promising natural alternatives to conventional antibiotics for controlling fish pathogens in aquaculture sector.

Conclusion

This study highlights the significant antimicrobial potential of *Aglaia edulis* plant extracts against common fish pathogens, with ethanol extracts demonstrating superior activity. Phytochemical bioactive compounds, including alkaloids, flavonoids, phenols, and terpenoids, with sesquiterpene derivatives exhibited concentration-dependent antimicrobial effects, particularly against *Staphylococcus aureus* and *Aeromonas hydrophila*, with ethanol extracts showing higher efficacy and moderate toxicity. These findings support the potential of *A. edulis* extracts as a safe and effective natural alternative to antibiotics in aquaculture.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Zoology, St. Xavier's College (Autonomous, Palayamkottai, 627002, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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