

Antibacterial activity and GC–MS profiling of *Artemisia annua* extract against *Aeromonas hydrophila* isolated from African catfish

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Abstract

Aeromonas hydrophila is an important bacterial pathogen of cultured fish and is associated with motile *Aeromonas septicemia* and economic losses in aquaculture. The increasing concern regarding antimicrobial resistance has encouraged the evaluation of plant-derived alternatives. This study evaluated the *in vitro* antibacterial activity and chemical profile of an ethanolic extract of *Artemisia annua* against *A. hydrophila* isolated from *Clarias gariepinus* in Dutsin-Ma, Katsina State, Nigeria. *Clarias gariepinus* showing clinical signs compatible with bacterial disease were collected from selected fish farms and markets in Dutsin-Ma and processed under aseptic conditions. The bacterial isolate was identified by cultural morphology, Gram staining, and biochemical tests including oxidase, catalase, indole, motility, urease, and Triple Sugar Iron reactions. *A. annua* leaves were authenticated, air-dried, powdered, and extracted with 99% ethanol at a 1:5 (weight/volume) ratio using a rotary evaporator. Gas chromatography–mass spectrometry (GC–MS) was used to characterize compounds detected in the extract. GC–MS profiling identified 28 library-assigned compounds. The most abundant detected compounds were 3-dibenzofuranamine (24.0%), 4-dibenzofuranamine (19.2%), a limonene isomer (9.44%), and 3,5-heptanedione, 2,2,6,6-tetramethyl- (10.1%). Antibacterial activity was assessed by agar well diffusion, while minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution and subculture, respectively. The isolate displayed the expected phenotypic profile of *A. hydrophila*: Gram-negative rod, oxidase-positive, catalase-positive, indole-positive, motile, TSI-positive, and urease-negative. The extract inhibited *A. hydrophila* at the highest concentration tested, with an MIC of 100 mg/mL; no MBC was recorded within the tested range. Thus, the extract was demonstrated to have a bacteriostatic rather than a demonstrably bactericidal effect under the conditions used. Ethanolic *A. annua* extract showed potential as a plant-derived antibacterial candidate against *A. hydrophila*. The findings support further standardized phytochemical confirmation, dose optimization, toxicity assessment, and *in vivo* evaluation before practical application in aquaculture.

Keywords: *Artemisia annua*, *Aeromonas hydrophila*, *Clarias gariepinus*, antibacterial activity

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INTRODUCTION

Aquaculture is an increasingly important contributor to global animal protein production and has an important role in food security, income generation, and employment. In Nigeria, the African catfish (*Clarias gariepinus*) is widely cultured because of its fast growth, tolerance of relatively harsh culture conditions, omnivorous feeding habit, and strong market acceptance. However, intensive production can increase stress and disease pressure, thereby constraining sustainable fish production (FAO, 2024; El-Sayed *et al.*, 2021). Bacterial diseases are among the major health problems in aquaculture. *Aeromonas hydrophila* is a Gram-negative, facultatively anaerobic aquatic bacterium associated with motile *Aeromonas septicemia* in fish. Clinical manifestations may include hemorrhages, ulceration, fin erosion, abdominal swelling, septicemia, and mortality. Its pathogenicity is associated with virulence determinants such as hemolysins, aerolysin, proteases, lipases, and enterotoxins (Semwal *et al.*, 2023).

Control of bacterial disease in aquaculture has traditionally relied heavily on antibiotics and other chemotherapeutic agents. However, indiscriminate or excessive use

may contribute to antimicrobial resistance, environmental contamination, and drug residues in fish products (FAO, 2024; Defoirdt, 2020; Hossain *et al.*, 2020). These concerns have increased interest in medicinal plants as alternative or complementary disease-control resources. *Artemisia annua* (sweet wormwood), a member of the Asteraceae family, is widely recognized for its artemisinin content and medicinal importance. In addition to artemisinin, *A. annua* contains diverse secondary metabolites that may contribute to antimicrobial and antioxidant effects (Efferth, 2017; Salehi *et al.*, 2020).

Previous studies have reported activity of *Artemisia*-derived preparations against fish pathogens, including *A. hydrophila* and other bacterial species (Juteau *et al.*, 2002; Soares *et al.*, 2020; Sakhaie *et al.*, 2025). Despite these reports, information on the activity of *A. annua* ethanolic extract against *A. hydrophila* isolated from *C. gariepinus* in Dutsin-Ma, Katsina State, Nigeria, remains limited. Therefore, this study evaluated the *in vitro* antibacterial activity of an ethanolic extract of *A. annua* against *A. hydrophila* isolated from *C. gariepinus* and characterized compounds detected in the extract using GC–MS.

MATERIALS AND METHODS

Study area

The study was conducted in Dutsin-Ma, Katsina State, Nigeria, with laboratory work carried out in the Department of Microbiology, Federal University Dutsin-Ma. Federal University Dutsin-Ma, Take-off Campus, Katsina State, Nigeria. The FUDMA take-off campus in Dutsin-Ma Local Government Area of Katsina State, Nigeria, lies between Latitude 12.47275°N and Longitude 7.48582°E (Muhammad *et al.*, 2025).

Collection and processing of fish samples

Live and freshly harvested *C. gariepinus* showing clinical signs such as skin lesions, hemorrhages, and fin erosion were collected from selected fish farms and markets in Dutsin-Ma. Samples were transported in sterile containers under aseptic conditions to the microbiology laboratory for immediate processing (Austin and Austin, 2021).

Isolation and identification of *Aeromonas hydrophila*

Bacterial isolates were characterized by colony morphology and Gram staining. Identification was supported by oxidase, catalase, indole, motility, urease, and Triple Sugar Iron (TSI) tests following the procedures described in the supplied methodology (Buller, 2020; Benson, 2021; Cheesbrough, 2023; CLSI, 2023).

Collection and preparation of *Artemisia annua*

Fresh *A. annua* leaves were collected from the Department of Plant Science and Biotechnology botanical garden, Federal University Dutsin-Ma, and authenticated by the same department. The leaves were washed, air-dried for 7–10 days, pulverized using a sterile blender, and stored in an airtight container until extraction.

Ethanol extraction

One hundred grams of powdered *A. annua* leaves were soaked in 99% absolute ethanol at a 1:5 (w/v) ratio for 48–72 h with intermittent shaking. The mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator at 40 °C, and the crude extract was stored at 4 °C until use.

Preparation of test concentrations

The supplied document describes initial test concentrations of 100, 50, 25, and 12.5 mg/mL and also describes preparation of a 500 mg/mL stock followed by two-fold serial dilution, producing 125, 62.5, 31.25, and 15.63 mg/mL. The latter concentrations were used in the agar-well assay according to the stated procedure. Sterile distilled water and 5% DMSO were used as diluent/solvent as described in the source document.

Agar well diffusion assay

Mueller–Hinton agar was prepared and sterilized. The bacterial suspension was standardized to 0.5 McFarland (approximately 1.5×10^8 CFU/mL). Six-millimetre wells were made using a sterile cork borer, and the prepared extract concentrations were introduced into the wells. Tetracycline served as the positive control. Plates were incubated at 28–30 °C for 24 h and zones of inhibition were measured in millimetres (Balouiri *et al.*, 2021; CLSI, 2023).

Determination of minimum inhibitory concentration

MIC was determined by broth dilution. Serial concentrations of the extract were prepared in nutrient broth, inoculated with a standardized bacterial suspension, and incubated at 28–30 °C for 24 h. The lowest concentration showing no visible turbidity was recorded as the MIC (CLSI, 2023).

Determination of minimum bactericidal concentration

Samples from MIC tubes showing no visible growth were subcultured onto fresh agar and incubated. No concentration in the tested range produced complete absence of bacterial growth after subculture; consequently, an MBC was not recorded.

GC–MS analysis

The GC–MS chromatogram and a library-based spectral profile of the ethanolic *A. annua* extract. Twenty-eight compounds were assigned from the chromatographic data. Molecular formulas, molecular weights, retention times, percentage areas, and similarity indices were reported where available.

RESULTS

Identification of *Aeromonas hydrophila*

The bacterial isolate recovered from the fish sample was identified phenotypically as *A. hydrophila* (Table 1). It was a rod-shaped, Gram-negative bacterium and was positive for oxidase, catalase, indole, motility, and TSI reactions, while urease was negative.

GC–MS profile of *Artemisia annua* extract

GC–MS analysis resulted in 28 library-assigned peaks/compounds (Table 2). The profile included phenolic compounds, hydrocarbons, terpenoid-related compounds, esters, aromatic nitrogen-containing compounds, quinoline derivatives, and siloxane-related compounds. The largest reported peak areas were for 3-dibenzofuranamine (23.9568%), 4-dibenzofuranamine (19.253%), 3,5-heptanedione, 2,2,6,6-tetramethyl- (10.1191%), and the limonene-isomer compound cyclohexane, 1-methylene-4-(1-methylethenyl)- (9.4431%).

Table 1: Biochemical characteristics of the bacterial isolate identified as *Aeromonas hydrophila*

Sample	Shape	Gram	Oxidase	Catalase	Indole	Motility	Urease	TSI	Identification
1	Rod	–	+	+	+	+	–	+	<i>A. hydrophila</i>

Minimum inhibitory and bactericidal concentrations

The extract produced inhibition of *A. hydrophila* at the highest concentration tested (Table 3). The result showed MIC as 100 mg/mL and indicates that no MBC was recorded because viable bacterial growth remained after subculture from the non-turbid MIC tube.

DISCUSSION

The phenotypic profile of the isolate was consistent with *A. hydrophila*. The organism was a Gram-negative rod and showed positive oxidase, catalase, indole, motility, and TSI reactions, with a negative urease reaction. The positive oxidase reaction is useful in differentiating *Aeromonas* from many members of the Enterobacteriaceae, which are generally oxidase-negative. The observed profile agrees with established descriptions of *A. hydrophila* and supports the identification (Janda and Abbott, 2010; Austin and Austin, 2021). However, because

identification in this study was phenotypic, molecular confirmation would strengthen species-level identification in future work.

The GC-MS profile indicated substantial chemical diversity in the ethanolic *A. annua* extract. Twenty-eight library-assigned compounds were reported, including phenolics, terpenoid-related compounds, hydrocarbons, esters, and nitrogen-containing aromatic compounds. Such chemical diversity is consistent with the general phytochemical complexity reported for *Artemisia* species, whose biological properties may arise from multiple constituents rather than a single compound (Nigam *et al.*, 2019; Salehi *et al.*, 2020). Among the most abundant library assignment compounds were 3-dibenzofuranamine and 4-dibenzofuranamine, which together accounted for approximately 43.2% of the reported chromatographic area. A limonene-isomer assignment accounted for 9.44% of the reported area. Monoterpenes such as limonene have been associated with antimicrobial activity and can af-

Table 2: GC-MS spectral profile and library-assigned compounds detected in the ethanolic *Artemisia annua* extract

Peak	RT (min)	Compound	Formula	MW (g/mol)	Area (%)	SI
1	8.430	Phenol, 2,6-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206.32	1.0205	87
2	9.324	9-Octadecene, (E)-	C ₁₈ H ₃₆	252.48	1.1948	90
3	10.208	2,5-Dihydroxybenzoic acid, 3TMS derivative	C ₁₆ H ₃₀ O ₄ Si ₃	370.61	0.4895	37
4	10.620	7-Octen-2-one	C ₈ H ₁₄ O	126.20	1.3591	38
5	11.070	Isobutyl tetradecyl ether	C ₁₈ H ₃₈ O	270.49	1.1824	49
6	11.613	5-Eicosene, (E)-	C ₂₀ H ₄₀	280.54	0.9715	87
7	11.681	Oxalic acid, cyclobutyl hexadecyl ester	C ₂₂ H ₄₀ O ₄	368.55	1.6001	58
8	12.030	Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	C ₁₃ H ₄₂ O ₅ Si ₆	458.99	0.5589	35
9	13.263	1-Hentetracontanol	C ₄₁ H ₈₄ O	592.11	0.5370	18
10	13.913	Phthalic acid, undec-2-en-1-yl undecyl ester	C ₃₀ H ₄₈ O ₄	472.70	1.3283	78
11	14.035	Phthalic acid, isobutyl 2,4,4-trimethylpentyl ester	C ₁₉ H ₂₈ O ₄	320.42	0.7139	47
12	14.228	3-Eicosene, (E)-	C ₂₀ H ₄₀	280.54	0.4824	60
13	15.885	Triacetyl pentafluoropropionate	C ₃₃ H ₆₁ F ₅ O ₂	580.83	0.6356	38
14	19.741	4-(Dimethylamino)benzaldehyde dinitrophenylhydrazone	C ₁₅ H ₁₅ N ₅ O ₄	329.31	6.7485	38
15	20.405	Cyclohexane, 1-methylene-4-(1-methylethenyl)- (limonene isomer)	C ₁₀ H ₁₆	136.24	9.4431	62
16	20.681	Trifluoroacetyl-epiisoborneol	C ₁₂ H ₁₇ F ₃ O ₂	250.26	3.2074	60
17	22.058	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-ethyl-]	C ₂₉ H ₄₄ O ₂	424.66	1.1712	95
18	22.591	Phthalic acid, butyl tetradecyl ester	C ₂₆ H ₄₂ O ₄	418.61	1.3359	50
19	26.484	Squalene	C ₃₀ H ₅₀	410.72	1.0198	64
20	30.819	Silicic acid, diethyl bis(trimethylsilyl) ester	C ₁₀ H ₂₈ O ₄ Si ₃	296.58	1.4883	37
21	31.038	Cyclotrisiloxane, hexamethyl-	C ₆ H ₁₈ O ₃ Si ₃	222.46	2.6937	27
22	31.402	7-Chloro-4-methoxy-3-methylquinoline	C ₁₁ H ₁₀ ClNO	207.66	1.0038	32
23	32.441	Cyclotrisiloxane, hexamethyl-	C ₆ H ₁₈ O ₃ Si ₃	222.46	0.6201	35
24	33.033	3,5-Heptanedione, 2,2,6,6-tetramethyl-	C ₁₁ H ₂₀ O ₂	184.28	10.1191	38
25	33.439	Indole-2-one derivative	C ₁₁ H ₁₃ NO ₃	207.23	2.3589	43
26	35.636	3-Dibenzofuranamine	C ₁₂ H ₉ NO	183.21	23.9568	30
27	35.762	4-Dibenzofuranamine	C ₁₂ H ₉ NO	183.21	19.2532	42
28	37.152	Benzenesulfonyl chloride derivative	C ₁₀ H ₁₀ ClNO ₂ S	291.71	3.5064	10

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration of ethanolic *Artemisia annua* extract against *Aeromonas hydrophila*

Test organism	Extract	Solvent	MIC	MBC
<i>Aeromonas hydrophila</i>	<i>Artemisia annua</i>	Ethanol	100 mg/mL	Not recorded

fect bacterial membranes and membrane permeability (Bakkali *et al.*, 2008; Dhifi *et al.*, 2016; Han *et al.*, 2019). The detection of a limonene-related compound therefore provides a plausible chemical basis for part of the inhibitory activity observed in the present study. Squalene was detected at 1.02%. Squalene is a naturally occurring triterpene that has been discussed in relation to antioxidant and membrane-associated biological functions (Popa *et al.*, 2015). Its relatively low abundance in this extract suggests that it is more appropriately considered a possible minor contributor rather than the principal determinant of antibacterial activity. Phenolic compounds were also detected. Phenolics can exert antibacterial effects through several mechanisms, including interactions with microbial membranes, proteins, and enzymes (Cushnie and Lamb, 2011). The presence of phenolic constituents, together with terpenoid-related compounds, supports the possibility that the observed activity arose from combined effects of multiple constituents. The MIC result indicates that the ethanolic extract inhibited visible growth of *A. hydrophila* at the highest concentration tested, while an MBC was not obtained within the tested range. MIC and MBC represent different endpoints: MIC identifies the lowest concentration preventing visible growth, whereas MBC is based on a defined reduction in viable bacterial cells after subculture (Ishak *et al.*, 2025). The absence of an MBC in the tested range therefore indicates that the extract demonstrated inhibition; therefore, the extract is said to be bacteriostatic rather than bactericidal. The inhibitory rather than bactericidal outcome is biologically plausible for a complex plant extract. Several constituents may exert sublethal pressure on bacterial membranes, metabolic processes, or other cellular targets without achieving complete killing at the tested concentrations. Similar interest in phytochemicals as antibacterial agents against *Aeromonas* species has been reported, supporting continued investigation of plant-derived products in aquaculture (Kot *et al.*, 2019; Semwal *et al.*, 2023).

From an aquaculture perspective, the findings indicate potential for *A. annua* as a candidate phytobiotic, but the present data do not justify claiming that the extract can cure established *A. hydrophila* infections.

CONCLUSION

The study demonstrated that an ethanolic extract of *Artemisia annua* inhibited *Aeromonas hydrophila* isolated from *Clarias gariepinus* under the *in vitro* conditions investigated. Phenotypic tests supported identification of the isolate as *A. hydrophila*, while GC-MS profiling revealed a chemically diverse extract containing several compound classes potentially relevant to antimicrobial activity. The reported MIC was 100 mg/mL, whereas no MBC was obtained within the tested range, indicating an inhibitory rather than demonstrably bactericidal effect. The extract therefore represents a promising candidate for further investigation as a plant-derived aquaculture phytobiotic, but additional analytical confirmation, safety studies, dose optimization, and *in vivo* efficacy trials are required.

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