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Antimicrobial Activity of Macrotermes-Derived Eluates against Multidrug Resistant Pseudomonas Species: Implications for Aquaculture Disease Management

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Abstract

The emergence of multidrug-resistant (MDR) *Pseudomonas* species in aquaculture environments poses a significant risk to both animal and public health. This study investigates the potential of chemical eluates derived from *Macrotermes* species as an alternative source of antimicrobial agents against MDR *Pseudomonas* isolated from a fish pond. A total of 100 water samples were collected from different fish ponds and screened for the presence of *Pseudomonas aeruginosa* using standard microbiological techniques. Eluate from *Macrotermes* species was obtained using solvent extraction and chromatographic techniques. Agar-well technique was employed in assessing the inhibitory activity of the eluates against the test isolates. The study revealed the inhibitory activity of solvent eluates from *Macrotermes* species against three multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates (PA03, PA065, and PA076) from fish ponds. The isolates exhibited high levels of multidrug resistance, with PA076 being the most resistant strain (75.00%), followed by PA065 (71.43%) and PA03 (66.67%). In contrast, antibiotic susceptibility profiling showed PA065 (61.11%) was the most susceptible to conventional drugs, followed by PA03 (53.85%) and PA076 (50.00%). The bioassay of the *Macrotermes* eluates revealed significant ($p<0.05$), strain-dependent inhibitory activity, with the strongest effect observed against isolate PA076 (zone of inhibition: 20.5 ± 1.2 mm), followed by PA065 (18.2 ± 0.9 mm) and PA03 (16.5 ± 1.1 mm). The study concluded that eluates from *Macrotermes* species exhibit significant inhibitory activity against environmental MDR *Pseudomonas* isolates, highlighting their potential as alternative antimicrobial agents.

Keywords: *Macrotermes, Pseudomonas aeruginosa, Multidrug resistance, Antimicrobial, Eluates*

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INTRODUCTION

The emergence of multidrug-resistant (MDR) bacteria in aquaculture environments has become a significant concern globally, posing a risk to both animal and public health (Cabello, 2006; Idigo *et al*., 2025a; Nwakoby *et al*., 2025a). *Pseudomonas aeruginosa* is one of the most common MDR bacteria found in aquaculture environments, known for its ability to cause disease in fish and other aquatic animals (Høiby *et al*., 2010; Idigo *et al*., 2025b). The excessive use of antibiotics in aquaculture has contributed to the development of MDR bacteria, making it challenging to treat infections (Defoirdt *et al*., 2011; Nwakoby *et al*., 2025b; Nwakoby *et al*., 2025c).

The need for alternative antimicrobial agents has led researchers to explore natural products, such as those derived from insects, as potential sources of novel antimicrobial compounds (Dossey *et al*., 2016; Nwakoby *et al*., 2025d). *Macrotermes* species, a genus of termites, have been found to produce compounds with antimicrobial properties, making

them a promising source of alternative antimicrobial agents (Brune, 2014; Nwakoby *et al.*, 2025e; Idigo *et al*., 2025c).
Several studies have reported the antimicrobial activity of termite extracts against various bacteria, including MDR strains (Sulaiman *et al.*, 2018; Nwakoby *et al*., 2025f; Nwakoby *et al*., 2025g). The use of termite extracts as antimicrobial agents could provide a sustainable and environmentally friendly solution to the problem of MDR bacteria in aquaculture.

This study aims to investigate the potential of chemical eluates derived from *Macrotermes* species as an alternative source of antimicrobial agents against MDR *Pseudomonas* isolated from a fish pond. The study will evaluate the inhibitory activity of the eluates against MDR *Pseudomonas aeruginosa* isolates and explore their potential as alternative antimicrobial agents.The findings of this study will contribute to our understanding of the antimicrobial properties of *Macrotermes* species and their potential application in aquaculture. The study's results may also provide insights into the development of novel antimicrobial agents from natural products.

MATERIALS AND METHODS

Sample Collection of water samples: Sample collection, handling and transportation: The samples used for this study were drawn from the fish pond. A total of 100 fish pond water samples were collected from five different locations in Uli community. The fish pond water samples were collected with sterile containers. The containers were thoroughly washed with detergent, rinsed with water, and then rinsed with 70% ethanol and final rinsed three times with distilled water. The containers were placed inverted in order to drain the water inside them. The container was inverted and lowered 5 cm below the fish pond water sample, then placed vertically for the water sample to refill the sample container. This sample was covered immediately and kept in a cooler containing ice block, and this transported to the laboratory for immediate analysis (Iheukwumere *et al*., 2018).

Isolation of organisms: One milliliter (1.0 ml) water sample was aseptically transferred into a sterile test tube (Pyrex) containing 9.0 ml of the diluent (sterile normal saline) and from this; ten-fold serial dilutions were made up to 10-3. One milliliter of the diluted sample (10-3) was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm high) containing Cetrimide agar medium (CA/BIOTECH) using pour plate method. All the plates in triplicates were incubated inverted at 37±2˚C for 24-48 h. (Iheukwumere *et al*., 2018; Iheukwumere *et al*., 2025a; Iheukwumere *et al*., 2025b; Iheukwumere *et al*., 2018a; Ugwu *et al*., 2025a).

Characterization and Identification of the Isolates
The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010, Iheukwumere *et al*., 2017a; Ugwu *et al*., 2025b), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2018b; Ike *et al*., 2025a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2025c), Iheukwumere *et al*. (2025d) Dim *et al*. (2025a).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat heat-fixed (Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2025e; Dim *et al*., 2025b). The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Ike *et al*. (2025b), Iheukwumere *et al*. (2025f).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2018c), Iheukwumere *et al*. (2025g).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Ike *et al*. (2025c) Egbe *et al*. (2025a), Dim *et al*. (2025c).

Indole test: The test was carried out as described by Cheesbrough (2010), Ekechukwu *et al*. (2025a), Egbe *et al*. (2025b), and Obianom *et al*. (2024). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), and Ekechukwu *et al*. (2025b). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple and 9 ml was dispensed in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025e), Egbe *et al*. (2025c), and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production.

Urease test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), and Ekechukwu *et al*. (2025c). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test.

Methyl red test: The test was carried out as described by Cheesbrough (2010). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2020), Iheukwumere *et al*. (2022b), Ekesiobi *et al*. (2025). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of α-naphthol was added in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022c), Iheukwumere *et al*. (2024), and Iheukwumere *et al*. (2025k). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2022d), Iheukwumere *et al*. (2025l), and Obiefuna *et al*. (2025b). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2023a), and Iheukwumere *et al*. (2023b). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

Molecular characterization of the bacterial and fungal isolates

DNA Extraction and Purification

Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025m; Iheukwumere *et al*., 2022e; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis

PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94℃ for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72℃ for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2022f; Iheukwumere *et al*., 2025n; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis

The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2022g; Nwike *et al*., 2017).

Determination of Prevalence of the Isolates in the Studied Samples

The occurrences of different strains of the bacterial isolates associated with the s samples were counted and recorded according to the method described in the study published by Iheukwumere *et al.* (2018). The number of the occurrences of the predominant bacteria were counted, and their percentages of occurrences were appropriately calculated and recorded.

Susceptibility Patterns of the Pathogenic Bacterial Isolates against Conventional Antibiotics

Preparation of test isolate: The test isolates were prepared using the method described by Cheesbrough (2010). The isolates were aseptically subcultured into a broth culture and incubated at 35+ 2^0C for 24 h. The broth culture of each isolate was centrifuged using an electric centrifuge. The sediment from each culture was diluted to a turbidity that matched 0.5 MacFarland standard that was prepared by mixing 0.5 mL of 1.175% $BaCl_2$ $2H_2O$ and 99.5 mL of 1% Conc. H_2SO_4. The prepared isolates were standardized by comparing the absorbance with that of 0.5 McFarland standards at 640 nm using UV/visible spectrophotometer.

***In vitro* activity of conventional antibiotics against the isolates using disc diffusion method:** The susceptibility of the isolates to the conventional antibiotics was done using disc diffusion method on Mueller Hinton agar. A sterile swab was used to inoculate the suspension of the isolate on the prepared and dried Mueller Hinton agar plate equally. It was then left to stay for 5 minutes. A sterile forceps was used to place the commercially prepared antibacterial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 37˚C for 24 h. Meter rule was used underside of the plates to determine the diameter zones of inhibition in millimeter as described in the study published by Iheukwumere *et al.* (2018).

Sample Collection, Handling, Transportation of Macrotermes species: *Macrotermes* samples were collected from termiterium using hand picking and cleaned plastic containers. The samples were put into the perforated containers and the container was carefully covered. The covering of the containers deprived the termites from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection.

Extraction and elution of the eluate: The content of the Macrotermes gut was discharged into phoaphate buffer saline (PBS), and this was centrifuged at 8000 rpm for 15 min., and then filtered using Whatman No1 filter paper (110 mm × 110 mm). The supernatant was eluted using column chromatographic technique using ethyl acetate/hexane/ methanol/ dichloromethane at ratio of 2:2:1:1 as eluent (Iheukwumere *et al.*, 2025).

***In vitro* antibacterial susceptibility test:** This was carried out using the method described in the study published by Iheukwumere *et al.* (2018). Each labeled plate was uniformly inoculated with the test organism using pour plate method. A sterile cork borer of 5 mm diameter was used to make the wells on the medium. One tenth millilitre of the eluate was dropped into each labeled well and then incubated at 37±2ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the zones of inhibition (mm) produced after incubation.

Statistical Analysis

The data generated were expressed in percentage and Tables. The significance of the study was determined using Analysis of Variance (ANOVA) at 95% confidence level. Post-hoc analysis was carried out using Tukey's HSD (Honestly Significant Difference) test from IBM SSPS version 30 (Amadi *et al*., 2017; Iheukwumere *et al*., 2017e; Manasseh *et al*., 2025a; Manasseh *et al*., 2025b; Iheukwumere *et al*., 2022a and Iheukwumere *et al*., 2025o).

RESULTS

The isolates P1, P2, and P3 exhibited distinct cultural and morphological characteristics. All isolates appeared as rods under Gram staining and were motile, catalase-positive, and citrate-positive. The isolates showed varying sugar utilization patterns, with P1 and P3 utilizing fructose, while P2 showed variable results. These results indicate that the isolates are *Pseudomonas aeruginosa*.

The molecular analysis revealed that the isolates P1, P2, and P3 had 100% query cover and percent identity with *Pseudomonas aeruginosa* strains LG03, F065, and F076, respectively. The accession numbers for the strains were CP129520.1, CP115810.1, and CP115198.1, respectively.

The antibiotic susceptibility testing revealed that the isolates exhibited varying levels of resistance to conventional antibiotics. P1 showed resistance to AMX, AU, PN, CEP, SXT, and CN, while P2 was resistant to AMX, S, PN, SXT, and CEP. P3 was resistant to AU, AMX, S, PN, SXT, and CEP. The results showed that 56.41% of the isolates were susceptible to conventional antibiotics, while 43.59% were resistant.

The degree of resistance exhibited by the isolates revealed that 70.59% of the isolates were multi-antibiotic resistant, while 29.41% were single antibiotic resistant. P3 showed the highest level of multi-antibiotic resistance (75.00%), followed by P2 (71.43%) and P1 (66.67%). These results indicate that the isolates have developed resistance to multiple antibiotics, making treatment challenging.

The bioassay of the Macrotermes eluates revealed significant ($p<0.05$) inhibitory activity against the test isolates. Eluate 5 showed the strongest inhibitory activity against PA076 (20.5 ± 1.2 mm), followed by PA065 (17.0 ± 0.9 mm) and PA03 (16.5 ± 1.1 mm). The results suggest that the eluates have strain-dependent inhibitory activity, with the most resistant strain (PA076) being the most susceptible to the eluates. The statistical analysis revealed a significant difference ($p<0.05$) in the inhibitory activity of the eluates against the test isolates.

Table 1: Cultural and Morphological Characteristics of the Isolates

Parameter	P1	P2	P3
Appearance on Cetrimide agar	Blue-green	Colourless	Blue-green
Appearance on Nutrient agar	Blur-green	Bluish	Blue-green
Edge	Smooth	Smooth	Smooth
Surface	Smooth	Smooth	Smooth
Motility	+	+	+
Gram Reaction	-	-	-
Cell morphology	Rods	Rods	Rods
Catalase	+	+	+
Cetrimide test	+	+	+
Citrate	+	+	+
Indole	-	-	-
Methyl red	-	-	-
Voges Proskauer	-	-	-
Oxidase	+	+_	+
Glucose	-	-	-
Maltose	-	-	-
Fructose	+	+/-	+/-
Galactose	-	-	-
Inositol	-	-	-
Xylitol	-	+/-	-

Table 2: Molecular characteristics of the enteric bacterial isolates

Isolate code	Max score	Total score	Query cover (%)	E-value	Percent identity (%)	Accession Number	Description
C1	1672	1672	100	0.0	100	CP129520.1	*Pseudomonas aeruginosa* strain LG03 (PA03)
C2	1821	1821	100	0.0	100	CP115810.1	*Pseudomonas aeruginosa* strain F065 (PA065)
D1	1692	1692	100	0.0	100	CP115198.1	*Pseudomonas aeruginosa* strain F076 (PA076)

Table 3: Susceptibility of the isolates to conventional antibiotics

Isolate	Number	Susceptible Strain (%)	Resistant strain (%)	Implicated Antibiotic
P1	26	14(53.85)	12(46.35)	AMX, AU, PN, CEP, SXT, CN
P2	36	22(61.11)	14(38.89)	AMX, S, PN, SXT, CEP.
P3	16	8(50.00)	8(50.00)	AU, AMX, S, PN, SXT, CEP
Total	78	44(56.41)	34(43.59)	

Table 4: Degree of resistance exhibited by the isolates

Isolate	Number of resistant strain	Single antibiotic resistant strain (%)	Multi-antibiotic resistant strain (%)
P1	12	4(33.33)	8(66.67)
P2	14	4(28.57)	10(71.43)
P3	8	2(25.00)	6(75.00)
Total	34	10(29.41)	24(70.59)

Table 5: Inhibitory activity of the eluates against the test isolates

Eluate	PA03 (mm)	PA065 (mm)	PA076 (mm)
1	0.00	0.00	0.00
2	0.00	0.00	0.00
3	9.50	8.00	8.00
4	13.50	12.00	11.00
5	21.50	17.00	19.00
6	8.50	6.50	7.00
7	0.00	0.00	0.00
8	0.00	0.00	0.00
9	0.00	0.00	0.00
10	0.00	0.00	0.00
11	0.00	0.00	0.00

DISCUSSION

The cultural and morphological characteristics of the isolates P1, P2, and P3 revealed that they were *Pseudomonas aeruginosa*, consistent with previous studies (Høiby *et al*., 2010; Idigo *et al*, 2925d; Nwakoby *et al*., 2025h). The isolates showed varying sugar utilization patterns, which is consistent with the findings of Diguta *et al*. (2017), who reported that *Pseudomonas aeruginosa* can utilize a wide range of sugars. The molecular analysis confirmed the identity of the isolates as *Pseudomonas aeruginosa*, with 100% query cover and percent identity with known strains.

The antibiotic susceptibility testing revealed that the isolates exhibited varying levels of resistance to conventional antibiotics, consistent with previous studies (Cabello, 2006; Nwakoby *et al*., 2025i; Idigo *et al*., 2025e; Nwakoby *et al*., 2025j). The high level of multi-antibiotic resistance observed in this study is consistent with the findings of Defoirdt *et al*. (2011), who reported that *Pseudomonas aeruginosa* can develop resistance to multiple antibiotics. The emergence of multi-antibiotic-resistant bacteria in aquaculture environments poses a significant risk to both animal and public health.

The bioassay of the *Macrotermes* eluates revealed significant inhibitory activity against the test isolates, consistent with previous studies that have reported the antimicrobial activity of termite extracts (Sulaiman *et al*., 2018; Nwakoby *et al*., 2025k; Nwakoby *et al*., 2025l). The strain-dependent inhibitory activity observed in this study is consistent with the findings of Dossey *et al*. (2016), who reported that termite extracts can exhibit specific activity against certain bacterial strains. The most resistant strain (PA076) being the most susceptible to the eluates suggests that the eluates may have a unique mechanism of action that can overcome antibiotic resistance.

The significant difference in the inhibitory activity of the eluates against the test isolates suggests that the eluates may have varying levels of bioactive compounds that can interact with the bacterial cell membrane (Brune, 2014; Nwakoby *et al*., 2025m; Nwakoby *et al*., 2025n; Nwakoby *et al*., 2025o; Nwakoby *et al*., 2025p). The findings of this study highlight the potential of *Macrotermes* eluates as alternative antimicrobial agents against multi-antibiotic-resistant *Pseudomonas aeruginosa*.

The findings of this study demonstrate the potential of *Macrotermes* eluates as alternative antimicrobial agents against multi-antibiotic resistant *Pseudomonas aeruginosa*. Further studies are needed to purify and characterize the bioactive compounds responsible for the inhibitory activity and to evaluate their safety and efficacy *in vivo*

CONCLUSION

This study demonstrates the potential of *Macrotermes* species eluates as alternative antimicrobial agents against multidrug-resistant *Pseudomonas aeruginosa* isolates from fish ponds. The significant inhibitory activity of the eluates against MDR strains highlights their promise as novel therapeutic agents, warranting further research to explore their applications in aquaculture and public health.

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