Exploration of bioactive metabolites from *Aspergillus austroafricanus* as potential antibacterial agents against *Klebsiella pneumoniae*

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Abstract

Marine-derived fungi are increasingly recognized as promising sources of bioactive metabolites with antimicrobial potential. This study investigated the antibacterial activity, chemical composition, and molecular identity of metabolites produced by *Aspergillus austroafricanus* isolated from marine stone samples collected from Oron River, Nigeria. The fungal isolate was identified using morphological characteristics and confirmed by internal transcribed spacer (ITS) region sequencing. Crude metabolites were extracted using an ethyl acetate–methanol (1:1) solvent system and evaluated against *Klebsiella pneumoniae* (PQ821874) using agar well diffusion, broth dilution, and bactericidal assays. Molecular analysis showed a 100% sequence similarity with *A. austroafricanus*, confirming taxonomic identity. The crude extract demonstrated strong antibacterial activity with inhibition zones ranging from 26–28 mm, outperforming standard antibiotics, including ceftriaxone and amoxicillin–clavulanic acid, against the tested strain. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude extract were 12.5 µL/mL and 50 µL/mL, respectively, indicating potent bacteriostatic and bactericidal effects. The M-fraction and H-fractions showed moderate antibacterial activity relatively comparable to

the crude extract, suggesting synergistic interactions among metabolites in the crude extract. Gas chromatography–mass spectrometry (GC–MS) analysis revealed a diverse array of volatile bioactive compounds, predominantly fatty acids, esters, terpenes, and steroids, with phytol identified as the major constituent (13.447%). These findings highlight potentials of marine-derived *A. austroafricanus* metabolites as a source of anti-drug-resistant *K. pneumonia* treatment.

Keywords: Marine fungi; *Aspergillus austroafricanus*; antibacterial activity; *Klebsiella pneumoniae*; GC–MS analysis; bioactive metabolites

Introduction

Klebsiella pneumoniae, a Gram-negative opportunistic bacterium, is an organism of important public health concern implicated in diverse infections such as pneumonia, urinary tract infections and septicemia.It is frequently isolated from immune-compromised individuals and hospitalized patients (Rahmat *et al.,* 2024). *K. pneumonia* remains one of the major causes of life-threatening infections globally and has contributed significantly to patient morbidity and mortality, especially in intensive care units (Abuh *et al.,* 2026; WHO, 2024).

K. pneumoniae is a public health risk because of its strong capacity to develop and spread antibacterial resistance. The rising incidence of multidrug-resistant (MDR) strains, including carbapenem-resistant strains has drastically reduced available treatment options, thereby resulting in complicated clinical outcomes and increasing healthcare costs (Chen *et al.,* 2024; Abuh and Adeboye 2023). Recently, the emergence of hypervirulent variants that can infect otherwise healthy individuals have further worsened the epidemiological burden posed by this organism (Huy, 2024).

Contaminated equipment, surfaces, and direct human contact are the major sources of transmission of this pathogen which commonly occurs in healthcare settings, enabling outbreaks and sustained colonization. Its ability to carry resistance gene on mobile genetic elements eases rapid dissemination across different populations and geographic regions, accentuating its designation as a high-priority pathogen for global monitoring and novel antimicrobial development (Ntshonga *et al.,* 2024).

Metabolites are low-molecular-weight compounds produced during cellular metabolic processes through enzyme-mediated reactions. These compounds play essential roles in cellular function, including energy generation, structural integrity, signaling, catalysis, defense and ecological interactions. Natural metabolites, particularly those derived from biological sources, have been widely regarded as relatively safe and effective in managing various diseases. Notably, several secondary metabolites of critical therapeutic value, such as penicillin, cephalosporins, cyclosporin, griseofulvin, are of fungal origin (Abuh *et al.,*2026; Gerald& James, 2016).

Despite its contribution to therapeutic armamentarium, fungal-derived metabolites remain comparatively underexplored relative to plant-based bioactive compounds (Fasinu *et al.,* 2022). This study seeks to isolate and

identify secondary metabolites with antibacterial potentials against *Klebsiella pneumoniae* from marine derived fungi.

Methodology

Sampling and isolation of marine fungi

Stone sample collection was carried out at Oron River close to the Atlantic Ocean as reported by Abuh *et al.,* (2026) at the following coordinates "N 04°54'58" and E 08°16'87" into sterile sample bags and transported to the laboratory for analysis.

Isolation and identification of *A. austroafricanus:* Stone samples were aseptically processed by gently brushing their surfaces with a sterile brush into 10 mL of sterile phosphate-buffered saline (PBS) contained in a sterile tube to obtain the stock suspension. The suspension was mixed thoroughly by vortexing. A ten-fold serial dilution was prepared by transferring 1.0 mL of the stock suspension into 9.0 mL of sterile water to obtain a 10^{-1} dilution. This procedure was repeated sequentially to obtain dilutions of 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, ensuring thorough mixing at each step.

From the last three dilutions, 0.1 mL aliquots were aseptically inoculated onto different sterile Czapek-Dox agar plates using the spread plate technique. The inoculum was evenly distributed over the agar surface using a sterile spreader. The inoculated plates were incubated in an inverted position at 25 ± 1 °C in the dark for 7 days (Agbo *et al*., 2025). Distinct Fungal colonies were subcultured onto fresh Czapek-Dox agar plates to obtain pure isolates. The isolates were initially characterized using macroscopic (colony morphology, colour, texture) and microscopic means. Molecular identification of the fungal isolate was performed by amplification and sequencing of the internal transcribed spacer (ITS) region, following the protocol described by Abuh et al. (2026).

Metabolite extraction: Extraction was carried out as reported by (Abuh *et al.,* 2026) with slight modification. Dried mycelia were pulverized and suspended in a mixture of ethyl acetate and methanol in the ratio of 1:1 and agitated in a vortex mixer for 15 minutes at 120 rpm. The mixture was then covered with a foil paper and left to extract for 24h. Using Whatman no 1 filter paper, the mixture was filtered into a pre weighed beaker and the filtrate left on a water bath at 40°C to dry.

Preparation of microbial suspension: The clinical isolate was standardized before use by dissolving a pure colony of the test bacteria in sterile 0.9% (w/v) normal saline. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1×10^{8} CFU/mL, in accordance with established microbiological guidelines (Abuh *et al.,* 2026; CLSI, 2023; Akpanke *et al.,*2023).

***In vitro* activity of crude metabolite against *Klebsiella pneumoniae*:** The antibacterial activity of the crude fungal extractagainst *Klebsiella pneumoniae* was evaluated using the agar well diffusion method. Wells measuring 8 mm were aseptically bored into Mueller–Hinton agar plates previously inoculated with a 0.5 McFarland standard suspension of the test organism. The extract was reconstituted using dimethyl sulfoxide (DMSO) to achieve a concentration of 1 mg/mL. Thereafter, 25 µl of the crude extract

and 10% DMSO (the negative control) were transferred into their respective wells. Standard antibiotic discs of amoxicillin–clavulanic acid (30 µg) and ceftriaxone (30 µg) were applied as positive controls. The plates were incubated at 37 °C for 24 hours, after which the zones of inhibition were measured and recorded in millimeters (Balouiri *et al*., 2016).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

This was determined using the broth dilution method in accordance with standard guidelines with slight modifications (CLSI, 2023). A stock solution of the fungal extract was prepared in 10% DMSO.

A two-fold serial dilution of the extract was carried out in sterile Mueller–Hinton broth to obtain a range of concentrations (e.g., 100, 50, 25, 12.5, 6.25 µg/mL). Each dilution (1 mL) was dispensed into sterile test tubes. A 24-hour culture of *Klebsiella pneumoniae* was adjusted to 0.5 McFarland standard (approximately 1×10^5 CFU/mL), and further diluted to obtain a working inoculum of approximately 1×10^5 CFU/mL. Aliquots (0.1 mL) of the standardized bacterial suspension were inoculated into each tube containing the extract dilutions (Schön *et al.,*2020).

Positive control tubes containing the broth and inoculum without extract, and negative control tubes containing broth only were included. A solvent control (10% DMSO) was also incorporated to rule out solvent effects. All tubes wereincubated at 37°C for 24 hours (Ageevets *et al*., 2024).

After 24 hours of incubation, aliquots from each tube were aseptically subcultured onto Mueller–Hinton agar plates and further incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract showing no visible bacterial growth when compared with the control positive control.

To determine the minimum bactericidal concentration (MBC), aliquots (0.1 mL) from tubes showing no visible growth were aseptically plated onto sterile Mueller–Hinton agar plates and incubated at 37°C for 24 hours. The MBC was recorded as the lowest concentration of the extract that resulted in no visible colony growth on the agar plates, indicating complete bacterial kill.All assays were performed in triplicate, and results were expressed as mean values.

GC-MS analysis of extract: The volatile chemical constituents present in the extracts of *A. austroafricanus* were analyzed using gas chromatography–mass spectrometry (GC–MS) (Agilent Technologies, USA). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The ion source temperature was maintained at 250 °C, while the interface (transfer line) temperature was set at 300 °C. The system operated at a pressure of 16.2 psi with a solvent delay time of 1.5 min. Samples (1 µL) were injected in split mode with the injector temperature maintained at 300 °C. The oven temperature was initially held at 50 °C for 5 min and then increased at a rate of 20 °C/min to 250 °C, where it was maintained for 5 min. Mass spectra-based identification of the components was carried out using the following software: Chromatec Analytic

(Chromatec, Russia); NIST MS Search Program V.2.3 (NIST, USA) and NIST 20 (NIST, Mass Spectra Libraries, USA); Wiley Registry of Mass Spectral Data, 12th ed.

Results

Figure 1 illustrates the antibacterial activity of crude extracts of*Aspergillus austroafricanus* against *Klebsiella pneumoniae* (PQ821874), as measured by inhibition zone diameter (IZD). The results reveal marked differences in antimicrobial efficacy among the crude extract, the M-fraction and H-fractions, and standard antibiotics.

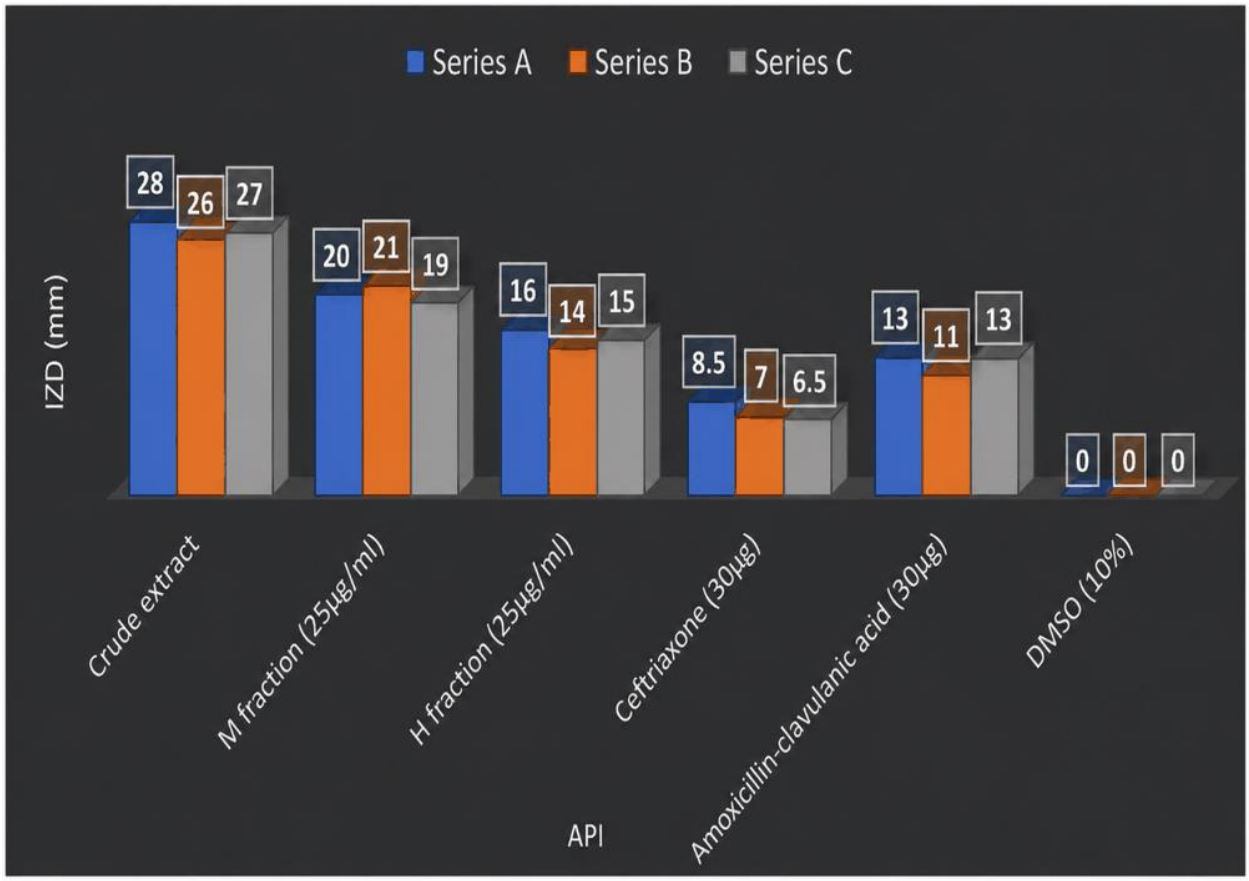

Figure 1: Activity of extract against *Klebsiella pneumoniae*
IZD = Zone of inhibition (mm); DMSO = Dimethyl sulfoxide (Negative control – No zone of inhibition)

Table 1 presents the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the crude extract and its fractions against *Klebsiella pneumoniae* (PQ821874). The results indicate that the crude extract had the highest antibacterial potency, with

the lowest MIC (12.5 µL/mL) and MBC (50 µL/mL), followed by the M-fraction (Methanol fraction) and H-fraction (N-Hexane fraction).

Table 1: MIC and MBC of extract against *Klebsiella pneumoniae* (PQ821874)

Extract	MIC (µL/mL)	MBC(µL/mL)
Crude	12.5	50
H-fraction	25	100
M-fraction	25	50

Figure 2 shows the GC-MS chromatogram of the crude extract sample, the observed trend highlights the heterogeneity of the sample, with a few dominant constituent likely terpenes,

triterpenes, steroids, alcohols, and benzoate derivatives responsible for the primary activity, while other minor constituents may contribute synergistically.

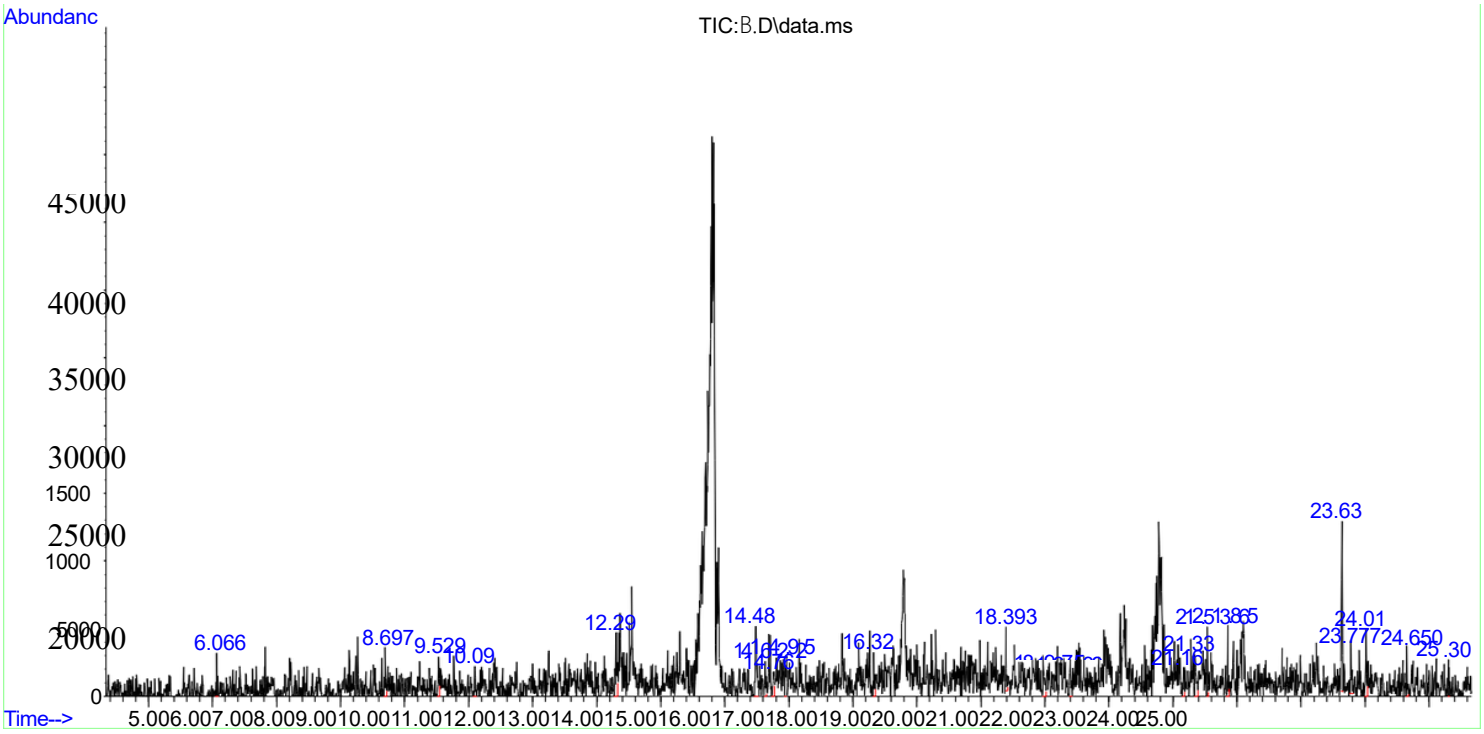

Figure 2:Chromatogram of *Aspergillus austroafricanuss* crude extract

Table 2 presents the GC–MS profile of metabolite extract, revealing a chemically diverse mixture of compounds dominated by fatty acids and their esters, along with terpenes, triterpenes, steroids, alcohols, and benzoatederivatives. This diversity suggests a rich secondary metabolite composition typical of fungal extracts, particularly those from *Aspergillus* species.

TABLE 2. GC-MS RESULT OF SAMPLE B

S/N	Compound	Retention Time	Concentration	Compound Group	Chemical formula
1	Pentanoic acid	6.066	2.800%	Fatty Acid	$C_5H_{10}O_2$
2	Propanedioic acid, propyl-	8.697	3.896%	Carboxylic Acid	$C_6H_{10}O_4$
3	Hexanoic acid, 1,1-dimethylpropyl este	9.529	3.455%	Fatty Acid Esters	$C_{11}H_{22}O_2$
4	Nonanoic acid	10.094	2.875%	% Fatty Acid	$C_9H_{18}O_2$
5	Palmitelaidic acid, trimethylsilyl ester	12.299	6.983%	Fatty Acid Esters	$C_{19}H_{38}O_2Si$
6	Hexadecanoic acid, 9-octadecenyl ester, (Z)	14.482	7.699%	Acid Esters	$C_{34}H_{66}O_2$
7	2,3-Di-O-benzoyl-d,l-glycerononitrile	14.622	3.063%	Benzoate ester	$C_{17}H_{13}NO_4$
8	Tetracosanoic acid	14.767	3.828%	Fatty Acid	$C_{24}H_{48}O_2$
9	Triarachine	14.959	3.944%		C63H122O6
10	Eicosanoic acid, 9-hexadecenyl ester	16.327	3.290%	Fatty Acid Ester	C40H78O3
11	Cholest-2-ene, 4,4-dimethyl-3-[(phenylmethyl)thio]-, (5α)	18.393	3.536%	Steroids	C36H56S
12	9,12,15-Octadecatrienal, dimethyl acetal	18.975	4.781%	Fatty Acid Esters	C20H36O2

13	Methyl eicosa-5,8,11,14,17-pentaenoate	4.025%	4.025%	Fatty Acid Esters	C19H36O
14	9,12,15-Octadecatrienoic acid, 2- phenyl-1,3-dioxan-5-yl ester	21.163	3.891%	Fatty Acid Esters	C28H40O4
15	Hexadeca-2,4,15-trienoic acid, ethyl ester	21.338	5.800%	Fatty Acid Esters	C18H30O2
16	4,8-Decadien-3-ol, 5,9-dimethyl	21.536	2.952%	Alcohol	C12H22O
17	3-Decene, 2,2-dimethyl-, (E)	21.856	4.286%	Fatty Acid Esters	C12H24
18	Phytol	23.637	13.447%	Terpenes	C20H40O
19	Squalane	23.777	4.637%	Triterpenes	C30H62
20	Hexadecane, 2,6,10,14-tetramethyl	5.331%	5.331% s	Terpenes	C20H42
21	9,12,15-Octadecatrienoic acid, 2,2-dimethyl-1,3-dioxolan-4-ylmethyl ester, (Z,Z,Z)	24.650	2.752%	Fatty Acid Esters	C28H42O4

Figure 3 shows the agarose gel electrophoresis profile of PCR-amplified internal transcribed spacer (ITS) regions. Clear bands were detected in lanes 1 and 2 at an approximate size of 650 bp, confirming successful amplification of the ITS fragment from the fungal isolates. The absence of smearing and the presence of single, well-defined bands indicate both good DNA integrity and high specificity of the PCR amplification.

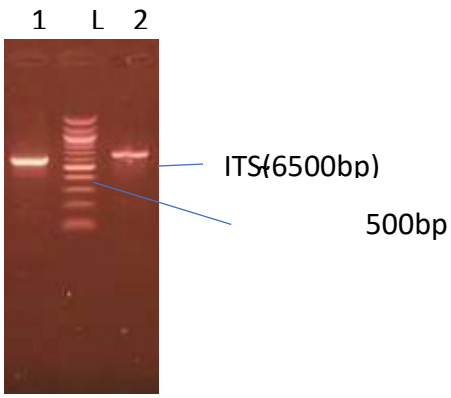

Fig 3: Agarose gel electrophoresis showing the amplified ITS. Lanes 1, 2represent the amplified 650bp while lane L represents the 100bp DNA ladder.

Discussion

The crude extract had the highest antibacterial activity, with inhibition zones ranging from 26 to 28 mm, indicating noticeable efficacy against the test organism. The antibacterial activity recorded may be attributed to the combined action of the fungal secondary metabolites present in the fungal crude extract. Similar findings have been reported for *Aspergilluschevalieri*-derived metabolites, where crude extracts often exhibit greater antimicrobial potency than purified fractions due to combined bioactive effects (Li*et al.,* 2020).

Fractionating the extract led to a reduction in antibacterial activity, though the methanol (M) fraction retained good efficacy (20–21 mm), suggesting that relatively more polar compounds are primarily responsible for the antibacterial activity. In contrast, the n-hexane (H) fraction showed lower inhibition (14–16 mm), indicating that non-polar constituents contribute less to the observed activity. The enhanced antibacterial activity observed in the methanol extract may be attributed to the presence of abroader range of bioactive secondary metabolites, including phenolics, alkaloids, and other polar compounds. These metabolites are widely recognized for their antimicrobial properties. In contrast, hexane, being non-polar, predominantly extracts lipophilic constituents that contribute less to antibacterial activity. This supports previous findings that methanol extracts of fungal metabolites exhibit stronger antibacterial effects due to improved solubility of polar bioactive compounds (Deshmukh *et al.,* 2022).

Notably, the standard antibiotic ceftriaxone (30 µg) exhibited minimal inhibitory activity (6.5–8.5 mm), indicating reduced susceptibility of the tested *Klebsiella pneumoniae* strain. This observation is consistent with reports highlighting the growing prevalence of multidrug-resistant *K. pneumoniae*, often associated with decreased responsiveness to commonly used cephalosporins (Effah et al., 2020; Martin & Bachman, 2018). In contrast, amoxicillin–clavulanic acid demonstrated moderate activity (11–13 mm), suggesting partial restoration of antibacterial efficacy due to the inhibitory effect of clavulanic acid on β-lactamase enzymes; however, its activity remained lower than that observed for the fungal extract. Based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2023), the inhibition zone diameters obtained with the standard antibiotic discs indicate that the test organism is resistant to both antibiotics.

The superior performance of the crude extract relative to conventional antibiotics highlights the therapeutic potential of fungal secondary metabolites as antimicrobial agents. The observed decline in activity following fractionation further supports the hypothesis of synergistic interactions among multiple compounds, a phenomenon widely documented in natural product research (Conrado *et al*., 2022; Atanasov *et al.,* 2021).

These findings are consistent with recent studies demonstrating that *Aspergillus* species produce a wide array of bioactive secondary metabolites, including polyketides, alkaloids, and terpenoids, which exhibit significant activity against drug-resistant pathogens (Zhu *et al*., 2023). Notably, the methanol fraction (20 mm) showed comparable inhibitory activity to the crude extract, indicating that the active constituents are likely concentrated within this fraction. Consequently, further investigations involving compound isolation, structural characterization, and elucidation of the underlying molecular mechanisms are essential to fully harness the antimicrobial potential of *Aspergillus austroafricanus* metabolites.

The relatively low MIC value of the crude extract to its fractions suggests strong bacteriostatic activity, as MIC is defined as the lowest concentration required to inhibit visible bacterial growth (Abuh *et al.,* 2026). The higher MIC values observed for both fractions (25 µL/mL) indicate a reduction in activity after fractionation, implying that the bioactive compounds may act synergistically in the crude extract.

Furthermore, the MBC values reveal that the crude extract and M-fraction (50 µL/mL) possess stronger bactericidal effects compared to the H-fraction (100 µL/mL). The two-fold increase in MBC for the H-fraction suggests a moderately lower bactericidal potential, even though its MIC is comparable to the M-fraction. This aligns with the concept that MIC and MBC are related but distinct parameters, reflecting inhibition versus killing of bacterial cells (Golikova., *et al*. 2023).

Recent studies have reported variable MIC and MBC values for crude plant and microbial extracts against *Klebsiella pneumoniae*, largely influenced by extract composition, extraction solvent, and bacterial strain. For example, crude extracts of selected medicinal plants demonstrated MIC values ranging from 0.78 to 6.25 mg/mL against multidrug-resistant *K. pneumoniae* strains, reflecting moderate antibacterial activity typical of unrefined extracts (Adeosun et al., 2023). Similarly, other plant-based extracts, including herbal formulations and individual species, have shown MIC values commonly within the range of 0.8 to 12.5 mg/mL against *K. pneumoniae*, indicating variability in potency across different crude preparations. These findings highlight that, unlike conventional antibiotics, crude extracts generally exhibit higher MIC and MBC values due to their complex and less concentrated bioactive constituents. Similarly, plazomicin demonstrated MIC values as low as 0.25 mg/L against carbapenem-resistant *K. pneumoniae* isolates (Tan *et al.,* 2021; Jacab *et al*., 2020). Although these values are lower than those obtained in the present study, such

differences are expected because pure antibiotics are more potent than crude natural extracts, which contain a mixture of compounds with varying activity.

In contrast, studies on novel antimicrobial materials or natural products often report higher MIC and MBC values. For example, a 2024 study on a nanocomposite reported MIC and MBC values of 2 mg/mL and 4 mg/mL, respectively, against *K. pneumoniae*, demonstrating that higher concentrations are typically required for non-conventional antimicrobial agents (RSC Advances, 2024). This supports the relevance of the present findings, where moderate MIC and MBC values indicate promising antibacterial potential of the extract.

The *in vitro* antimicrobial activity of the extract, indicates that the bioactive metabolites identified through GC–MS are likely responsible for the observed antimicrobial effects. The predominant compound detected was phytol (13.447%), a diterpene alcohol widely reported to possess antimicrobial, antioxidant, and anti-inflammatory properties. Its relatively high abundance suggests a major contribution to the antibacterial activity of the extract. Other notable terpenoid-related constituents include squalane (4.637%) and 2,6,10,14-tetramethylhexadecane (5.331%), which have been associated with membrane disruption and other biologically active functions.

Fatty acids and their esters constitute the largest chemical group in the sample, including compounds such as hexadecanoic acid derivatives (7.699%), palmitelaidic acid ester(6.983%), and hexadeca-2,4,15-trienoic acid ethyl ester (5.800%). These compounds are widely reported to exhibit antimicrobial activity through disruption of microbial cell membranes and interference with enzyme systems.

The presence of long-chain saturated fatty acids (e.g., tetracosanoic acid and eicosanoic acid esters) further supports the antimicrobial potential of the extract, as such compounds are known to inhibit bacterial growth. Additionally, minor constituents such as cholesterol-derived steroid compounds and aromatic esters may contribute synergistically to the overall bioactivity.

Conclusion

This study demonstrates that metabolites derived from the marine fungal isolate *Aspergillus austroafricanus*, obtained from the Oron River near the Atlantic Ocean, possess notable antibacterial activity against *Klebsiella pneumoniae*, including resistant strains. The extract exhibited appreciable efficacy, with relatively low MIC and MBC values confirming both inhibitory and bactericidal potential.

Molecular identification based on ITS sequencing revealed 100% similarity with *Aspergillus austroafricanus*, supporting the reliability of the isolate characterization. Overall, these findings highlight the promise of marine-derived fungal metabolites as potential alternative antimicrobial agents.

Further research should focus on the purification and characterization of active compounds, elucidation of mechanisms of action, toxicity assessment, and in vivo validation to support future pharmaceutical development.

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Contributions

Samuel Agim Abuh conceived and designed the study, participated in data collection, analysis, and interpretation, and contributed to manuscript drafting and revision. **Malachy C Ugwu** supervised the research, guided the experimental design and assisted in the final proofreading of the manuscript.

IR Iroha: Co-supervised the research work.

Akpanke Justine: Assisted in sample collection and laboratory investigation

Peter Anyigor Edeh: Assisted in manuscript review and organization

Ugochukwu M.Okezie : Assisted in manuscript review and organization

ChidimmaRuthChukwunwejim assisted in laboratory investigations and manuscript organization.

OlusojiLekanAdeboye contributed to data interpretation, laboratory investigations literature review. All authors read and approved the final version of the manuscript

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References

Abuh, S. A., Adeboye, O. L., Iheanacho, C. O., Akpanke, J., Otakagu, E. C., & Akeem, A. A. (2025). Molecular identification and antibiogram studies of bacterial isolates from public sachet drinking water in Calabar metropolis. *Journal of Science and Practice of Pharmacy, 12*(1), 628–636.

Abuh, S. A., Akpanke, J., Lekan, A. O., & Ruth, C. (2026). Antibacterial potential of marine *Aspergillus austroafricanus*: Metabolite optimization, GC–MS characterization, in vitro activity and in silico mechanistic evaluation. *World Journal of Pharmaceutical Research, 15*(10), 1129–1154.

Agbo, B. E., Ikpoh, I. S., Ekpenyong, E. M., Abuh, S. A., Abdulraheem, A. B., Adie, F. U., & Gber, N. I. (2025). Fate of rhizosphere microorganisms and growth of leguminous plants in cassava mill effluent simulated soil. *Asian Journal of Microbiology and Biotechnology, 10*(2), 21–33. https://doi.org/10.56557/ajmab/2025/v10i29498

Ageevets, V., Sulian, O., Avdeeva, A., Chulkova, P., Ageevets, I., Gostev, V., Alieva, K., Golikova, M., & Sidorenko, S. (2024). Minimum inhibitory concentrations of aztreonam–avibactam, ceftazidime–avibactam and meropenem in clinical isolates of *Klebsiella pneumoniae* harboring carbapenemase genes. *The Journal of Antibiotics*, *77*(10), 706-710. https://doi.org/10.1038/s41429-024-00758-8

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. *Nature Reviews Drug Discovery*, *20*(3), 200-216. https://doi.org/10.1038/s41573-020-00114-z

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

Chen, T.-A., Chuang, Y.-T., & Lin, C.-H. (2024). A decade-long review of the virulence, resistance, and epidemiological risks of *Klebsiella pneumoniae* in ICUs. *Microorganisms, 12*(12), Article 2548. https://doi.org/10.3390/microorganisms12122548

CLSI (Clinical and Laboratory Standards Institute). (2023). Performance Standards for Antimicrobial Susceptibility Testing (33rd ed.). CLSI supplement M100

Conrado, R., Gomes, T. C., Calaço Roque, G. S., & De Souza, A. O. (2022). Overview of Bioactive Fungal Secondary Metabolites: Cytotoxic and Antimicrobial Compounds. *Antibiotics*, *11*(11), 1604. https://doi.org/10.3390/antibiotics11111604

Deshmukh, S. K., Dufossé, L., Chhipa, H., Saxena, S., Mahajan, G. B., & Gupta, M. K. (2022). Fungal endophytes: A potential source of antibacterial compounds. *Journal*

of Fungi, 8(2), Article 164. https://doi.org/10.3390/jof8020164

Effah CY, Sun T, Liu S, Wu Y. *Klebsiella pneumoniae*: an increasing threat to public health. Ann Clin Microbiol Antimicrob. 2020 Jan 9;19(1):1. doi: 10.1186/s12941-019-0343-8. PMID: 31918737; PMCID: PMC7050612.

Elias, R., Melo-Cristino, J., Lito, L., Pinto, M., Gonçalves, L., Campino, S., Clark, T. G., Duarte, A., & Perdigão, J. (2021). *Klebsiella pneumoniae* and colistin susceptibility testing: Performance evaluation for broth microdilution, agar dilution and MIC test strips. *Diagnostics, 11*(12), Article 2352. https://doi.org/10.3390/diagnostics11122352

Golikova, M. V., Strukova, E. N., Alieva, K. N., Ageevets, V. A., Avdeeva, A. A., Sulian, O. S., & Zinner, S. H. (2023). Meropenem MICs at standard and high inocula and mutant prevention concentration inter-relations: Comparative study with non-carbapenemase-producing and OXA-48-, KPC-, and NDM-producing *Klebsiella pneumoniae*. *Antibiotics, 12*(5), Article 872. https://doi.org/10.3390/antibiotics12050872

Adeosun, I. J., Baloyi, I. T., & Cosa, S. (2023). Extracts of selected South African medicinal plants mitigate virulence factors in multidrug-resistant strains of *Klebsiella pneumoniae*. *Evidence-Based Complementary and Alternative Medicine*, 2023, Article 3146588. https://doi.org/10.1155/2023/3146588

Huy, T. X. N. (2024). Overcoming *Klebsiella pneumoniae* antibiotic resistance: New insights into mechanisms and drug discovery. *Beni-Suef University Journal of Basic and Applied Sciences, 13*, Article 13. https://doi.org/10.1186/s43088-024-00470-4

Jacobs, M. R., Good, C. E., Hujer, A. M., Abdelhamed, A. M., Rhoads, D. D., Hujer, K. M., Rudin, S. D., et al. (2020). ARGONAUT II study of the*In vitro* activity of plazomicin against *Klebsiella pneumoniae*. *Antimicrobial Agents and Chemotherapy*. **64**(5), **e00012-20**. https://doi.org/10.1128/AAC.00012-20

Martin, R. M., & Bachman, M. A. (2018). Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. *Frontiers in Cellular and Infection Microbiology, 8*, Article 4. https://doi.org/10.3389/fcimb.2018.00004

Ntshonga, P., Gobe, I., Koto, G., Strysko, J., & Paganotti, G. M. (2024). Biocide resistance in Klebsiella pneumoniae: A narrative review. *Infection Prevention in Practice*, *6*(2), 100360. https://doi.org/10.1016/j.infpip.2024.100360

Rahmat Ullah, S., Jamal, M., Rahman, A., & Andleeb, S. (2024). Comprehensive insights into *Klebsiella* pneumoniae: unravelling clinical impact, epidemiological trends and antibiotic-resistance challenges. *The Journal of antimicrobial chemotherapy*, *79*(7), 1484–1492. https://doi.org/10.1093/jac/dkae184

RSC Advances. (2024). Antibacterial activity and MIC/MBC evaluation of nanocomposite materials. *RSC Advances*. https://doi.org/10.1039/D4RA03758H

Li, H., Fu, Y., & Song, F. (2023). Marine *Aspergillus*: A Treasure Trove of Antimicrobial Compounds. *Marine*

Drugs, *21*(5), 277. https://doi.org/10.3390/md21050277

Schön, T., Werngren, J., Machado, D., Borroni, E., et al. (2021). Multicentre testing of the EUCAST broth microdilution reference method for MIC determination. *Clinical Microbiology and Infection, 27*(2), 288–292. https://doi.org/10.1016/j.cmi.2020.10.019

Tan, R., Yu, A., Liu, Z., Liu, Z., Jiang, R., Wang, X., Liu, J., Gao, J., & Wang, X. (2021). Prediction of Minimal Inhibitory Concentration of Meropenem Against Klebsiella pneumoniae Using Metagenomic Data. *Frontiers in Microbiology*, *12*, 712886. https://doi.org/10.3389/fmicb.2021.712886

World Health Organization. (2024). *Antimicrobial resistance and hypervirulent Klebsiella pneumoniae: Global situation*. World Health Organization

Zhu, J., Song, L., Shen, S., Fu, W., Zhu, Y., & Liu, L. (2023). Bioactive Alkaloids as Secondary Metabolites from Plant Endophytic Aspergillus Genus. *Molecules*, *28*(23), 7789. https://doi.org/10.3390/molecules28237789