Content available at Bio-Research

Journal of Biological Research and Biotechnology
Journal Home page: https://bio-research.com.ng/index.php/home

ORIGINAL ARTICLE

Antibacterial Activity and Metabolite Profile of Endophytic Bacteria from *Azadirachta Indica* and *Khaya Senegalensis* against Poultry-Associated Antibiotic-Resistance *Escherichia Coli* and *Salmonella* Spp

[1*]Muhammad A. Ahmad✉, and [2]Abdullahi Bello.
[1]Department of Biochemistry, Kaduna state university, Kaduna state, Nigeria.
[2]Department of Biotechnology, Modibbo Adama University, Yola, Nigeria

ARTICLE INFO

Received: 13th May 2026
Revised: 24th August 2026
Accepted: 26th August 2026
Published online: 31st August 2026
DOI:https://dx.doi.org/10.4314/br.v24i2.30

Publisher's Note: *Bio-Research remains neutral with regard to jurisdictional claims in published maps and institutional affiliations. Bio-Research or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.*

e-ISSN: 2705-3822
print-ISSN: 1596-7409

Official. Faculty of Biological Sciences, University of Nigeria.

ABSTRACT

The escalating prevalence of multidrug-resistant bacteria in poultry production poses significant threats to both animal health and public safety through zoonotic transmission pathways. This study investigated the antimicrobial potential of endophytic bacteria isolated from two medicinal plants, *Azadirachta indica* (neem) and *Khaya senegalensis* (African mahogany), against multidrug-resistant Escherichia coli and Salmonella species obtained from poultry droppings in Kaduna State, Nigeria. Twenty endophytic bacteria were isolated from surface-sterilized roots and leaves of the medicinal plants using starch casein agar. Preliminary antimicrobial screening identified four isolates (ENDVI, ENDVII, ENDVIII, and ENDIX) exhibiting broad-spectrum activity against the multidrug-resistant poultry pathogens. These bioactive endophytes were subjected to fermentation, ethyl acetate extraction, and chromatographic purification. Secondary screening of purified fractions revealed substantial antimicrobial activity with inhibition zones ranging from 20.33±0.33 mm to 26.33±0.88 mm. Molecular identification using 16S rRNA gene sequencing identified the active endophytes as *Bacillus cereus* (ENDVI and ENDVIII, 92.96% and 98.72% identity respectively) and *Lysinibacillus species* (ENDVII as *L. fusiformis*, 98.85 % identity; ENDIX as *L. cavernae*, 88.00% identity). Liquid chromatography-mass spectrometry (LC-MS) analysis of active fractions revealed secondary metabolites such as Kaempferol, Vinclozolin, 2-Aminobenzimidazole, Tropicamide, and Docosapentaenoic acid in some of the fractions. Statistical analysis using one-way ANOVA confirmed significant antimicrobial activity ($p < 0.05$) of the partially purified fractions. The findings demonstrate that endophytic bacteria from *A. indica* and *K. senegalensis* are promising sources of antimicrobial compounds effective against multidrug-resistant poultry pathogens.

Keywords: *Azardirachta indica*; Antimicrobial activity; Endophytic Bacteria; *Khaya senegalensis*; Multidrug resistance; Secondary metabolite.

Cite this article: Ahmad A. M., & Abdullahi B. (2026). Antibacterial activity and metabolite profile of endophytic bacteria from *Azadirachta Indica* and *Khaya Senegalensis* against poultry-associated antibiotic-Resistance *Escherichia Coli* and *Salmonella* Sp. *Journal of Biological Research and Biotechnology*, 24(2), 732-741.
✉**Corresponding author:** Ahmad Aliyu Muhammad. Email Address: aaliyumuhammad6@gmail.com. ORCID: https://orcid.org/0009-0002-6646-4388
DOI: https://dx.doi.org/10.4314/br.v24i2.30

1.0 INTRODUCTION

The global health crisis precipitated by antimicrobial resistance (AMR) represents one of the most formidable challenges confronting modern medicine and food production systems in the twenty-first century. The overuse and misuse of antibiotics in human medicine, veterinary practice, and agricultural applications have accelerated the emergence and dissemination of multidrug-resistant bacterial pathogens, creating a scenario where previously treatable infections now pose life-threatening risks (Alara & Alara, 2024). The World Health Organization has identified AMR as a critical priority requiring urgent intervention, with projections suggesting that by 2050, antimicrobial-resistant infections could cause ten million deaths annually if current trends continue unabated (WHO, 2019). The poultry industry, in particular, has become a significant reservoir for antibiotic-resistant bacteria, with intensive farming practices necessitating the prophylactic and therapeutic use of antimicrobial agents, thereby exerting selective pressure that favors the survival and proliferation of resistant strains (Mak et al., 2022). This agricultural context has transformed poultry operations into hotspots for the development and transmission of resistant pathogens, particularly *Salmonella species* and *Escherichia coli*, which pose substantial risks to both animal health and human consumers through the food chain (Collignon et al., 2018).

Endophytic microorganisms residing within plant tissues without causing apparent disease, have emerged as prolific producers of bioactive secondary metabolites with remarkable antimicrobial properties (Anand et al., 2023). These symbiotic relationships between plants and endophytic organism have evolved over millions of years, resulting in sophisticated chemical defense systems that protect host plants from pathogens while simultaneously providing a rich source of novel compounds for drug discovery (Chen & Jiang, 2026). The exploration of endophytic bacteria from medicinal plants, particularly those with documented ethnobotanical uses such as *Azadirachta indica* (neem) and *Khaya senegalensis* (African mahogany), represents a rational bioprospecting strategy that leverages traditional knowledge and ecological relationships to identify promising antimicrobial candidates (Gakuubi et al., 2021). These medicinal trees have been utilized for centuries in traditional African and Asian medical systems for treating various infectious diseases, suggesting that their associated endophytic communities may harbor specialized metabolic capabilities for producing antimicrobial compounds (Guan et al., 2022). The intersection of endophyte biology, natural product chemistry, antimicrobial pharmacology, and agricultural microbiology creates a fertile ground for addressing the critical challenge of antibiotic resistance in poultry-associated pathogens. The investigation of endophytic bacteria from *A. indica* and *K. senegalensis* specifically targeting multidrug-resistant

Salmonella spp. and *E. coli* strains represents a strategic approach that combines several promising research directions (Musa et al., 2020). This research paradigm acknowledges the ecological wisdom encoded in plant-microbe associations while employing cutting-edge analytical technologies to unlock the therapeutic potential of these natural systems (Manganyi & Ateba, 2020). Furthermore, understanding the metabolomic profiles of bioactive endophytic bacteria can provide insights into biosynthetic pathways, regulatory mechanisms, and ecological functions that may inform both fundamental biology and applied biotechnology (Witte et al., 2021).

2.0 MATERIALS AND METHODS

2.1 Study area
The study area is Kaduna town, the capital of Kaduna State, located in the northwestern part of Nigeria. Kaduna State is located on latitude 10.52224N and longitude 7.43828N.

2.2 Collection of plant material
Roots and leaves of *A. indica* and *K. senegalensis* were collected within Kaduna metropolis in the month of May, 2025, in sterile polythene bags using a sterile blade and transported to the Biological Science Department, Kaduna State University for authentication by a curator. Voucher number of KASU/BSH/908 and KASU/BH/1702 were assigned to *A. indica* and *K. senegalensis* respectively.

2.3 Surface sterilization of plant material
The surface sterilization of plant samples was done according to procedure described by Mahdi et al. (2022), with some slight modifications. This was done by washing the plant material with running tap water to remove contaminations and physical debris. The samples were further cut into pieces using a sterile razor and tweezers and dried after washing three times with distilled water. Each sample was immersed in sterile 0.1% Tween 20 for five min. The samples were then treated with 70% ethanol for five min, and then further rinsed with freshly prepared 6% sodium hypochlorite (NaOCl) solution. Samples were then washed several times with distilled water to remove chemical residues and immersed in sterile 10% (w/v) sodium bicarbonate ($NaHCO_3$) for 10 min to delay the growth of endophytic fungi and then washed three times with sterile distilled water and dried in a laminar flow. In order to ensure the efficacy of the surface sterilization, 100 µL of the distilled water used for the last wash, was cultivated onto nutrient media and incubated at 28°C for 24 h. The absence of microbial growth on the culture media after plating the last washing water will demonstrate the effectiveness of surface sterilization (Mahdi et al., 2022).

2.4 Isolation of endophytic bacteria from plant material
Treated plant material were cut aseptically using sterile blades to expose the interior surface to the isolation media and then directly inoculated onto the media, starch casein agar, SCA. The plates were then incubated at 37°C for 5 days in an incubator (Ezeobiora et al., 2024). A sterilized wire loop was used to pick individual colonies from the plate and streak on nutrient agar to obtain pure culture. The isolates were transferred to nutrient slants and were maintained at 4°C until further use.

2.5 Poultry droppings' bacteria isolates
The test organisms used in this study comprised of multidrug-resistant bacterial isolates previously recovered from poultry droppings and characterized in our previous study (Muhammad et al., 2025). The isolates were preserved on nutrient agar slant at 4°C until required. Prior to use, each isolate was revived on nutrient media agar and re-verified using biochemical test. The biochemical test used included; Citrate utilization test, urease test, SIM (sulfide, indole, motility), methyl red and Voges-Proskauer tests.

2.6 Preliminary screening of endophytic bacteria for antimicrobial activity
The isolated endophytic bacteria were screened for antagonistic activity against the pathogens spot inoculation method as described by Panigrahi et al. (2018). Endophytic bacterial isolates were cultured in conical flasks containing Nutrient agar at 37˚C for 24 h. Overnight freshly grown culture of the test organisms were standardized according to 0.5 McFarland standards and seeded on NA plates with the help of a sterilized cotton swab separately. Isolated endophytes were spot inoculated on the test organism seeded plates with the help of an inoculating loop. Incubation was done for 24 h at 37˚C and observed for zone of inhibition around the colonies.

2.7 Fermentation of putative endophytic bacteria for secondary metabolite production
The fermentation of the endophytic actinomycetes was done using method described in a previous study by Sreenivasan et al. (2022). Antibiotic producing endophytes was inoculated into 250 mL conical flask containing 50 mL of Nutrient broth. The flask was incubated at 37 ºC for 2 days in an incubator-shaker. Fifty milliliters of the culture was transferred to 200 mL of the fermentation medium. The fermentation medium consisted of glucose 4 g/L, bacto peptone 15 g/L, yeast extract 3 g/L, Fe (III) citrate hydrate 0.3 g/L, and distilled water 200 mL. The pH value of the medium will be adjusted to 7.6 before sterilization. The fermentation was carried out at 37ºC for 5 days in an incubator-shaker.

2.8 Ethyl acetate extraction of secondary metabolites from fermentation broth
Ethyl acetate extraction was carried out as described by Sreenivasan et al. (2022). The culture broth was centrifuged at 10,000 x g for 15 min using an ambient benchtop centrifuge. The broth supernatants were divided and extracted using ethyl acetate as the solvent. The supernatant and the organic solvent were mixed thoroughly by shaking them in a 1:1 capacity separating funnel and then allowed to stand for 30 min. Two layers were separated, the aqueous layer and the organic layer, which contained the solvent and the antimicrobial agent. The organic layer was concentrated by evaporation under vacuum to the least possible volume. The aqueous layer was re-extracted, and the organic layer added to the above organic layer.

2.9 Column chromatography and thin-layer chromatography (TLC) of ethyl acetate extracts
Column chromatographic separation was done as descried by Sreenivasan et al. (2022) with slight modification. The dry extract of the supernatant was purified using silica gel column chromatography. The column used has a height of 300 mm and a diameter of 25 mm, the silica gel was loaded to 150 mm of the column. Dry extract was injected onto the column and then eluted stepwise with the chloroform-methanol solvent system as follows: First, the column was eluted with 100% chloroform (Fraction 1). Then the chloroform was reduced by 10% in each fraction while the methanol was increased by 10% in each fraction, until the methanol percentage was 100%. Thirty fractions were collected (20 mL each) and then concentrated. The fractions obtained was spotted on pre-coated thin layer chromatography plates. The plates were eluted with chloroform: methanol solvent system (4:1). The spotted TLC plates were kept in iodine chamber for the development of spots and Rf values where calculated. Samples with similar Rf values where combined and used for antimicrobial analysis (Reshma & Brindha-Priyadarisini, 2021).

2.10 Secondary antimicrobial screening of fractions against test organisms
The Fractions obtained from column chromatography and TLC were screened for antagonistic activity against the test organisms using agar well diffusion method as described by Ezeobiora et al. (2024) with slight modifications. Overnight freshly grown culture of the test organisms was standardized according to 0.5 McFarland standards and seeded on Mueller-Hinton agar plates with the help of a sterilized cotton swab separately. Wells of uniform diameter (6 mm) were made on the solidified agar using a sterile Cork borer. Exactly 100 µL of each fractions were dropped separately in each well using a micropipette. It was then allowed to stand for 3 h to allow proper diffusion of the extract within the medium. Incubation (without inverting the plates) was done for 24 h at 37˚C and observed for zone of inhibition around the wells and colonies as an indicative of antibacterial activities. All the experiments were carried out in triplicates.

2.11 Liquid chromatography- mass spectrometry (LC-MS) analysis of active fractions
Liquid chromatography-mass spectrometry (LC-MS) was carried out as described by Garba et al. (2023). The chemical constituents of the samples were analyzed using a liquid chromatography–mass spectrometry (LC–MS) system. The samples were reconstituted in methanol and filtered through a 0.45 µm membrane filter prior to injection. The analysis was

performed using a Waters e2695 LC system coupled with a photodiode array (PDA) detector and an electrospray ionization (ESI) mass spectrometer operating in both positive and negative ionization modes. Chromatographic separation was achieved on a SunFire C18 column (5.0 µm, 4.6 mm × 150 mm). The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The gradient elution program was as follows: A/B (95:5) for 1 min, then linearly changed to 5:95 over 13 min and maintained until 15 min, followed by re-equilibration to 95:5 at 17 min, maintained until 20 min. The flow rate was set at 1.0 mL min^{-1}, with both sample and column temperatures maintained at 25°C. Detection was performed using a PDA detector set between 210–400 nm, with a resolution of 1.2 nm and a sampling rate of 10 points s^{-1}. The MS data were acquired in full-scan mode (m/z 100–1250) under the following conditions: ESI source operated at a capillary voltage of 0.8 kV for both positive and negative modes, probe temperature at 600°C, nebulizer gas pressure of 45 psi, and desolvation gas flow rate of 10 mL min^{-1}. Fragmentation voltage was set to 125 V. Data acquisition and processing were carried out using Empower 3 software. The mass to charge ratio (m/z) and retention time of the fragments were used to search for possible matches on metabolomic workbench database.

2.12 Molecular identification of active endophytic bacteria

Endophytic bacteria were identified using the 16S rDNA. Deoxyribonucleic acid (DNA) was isolated using the Wizard© genomic DNA purification kit. Polymerase chain reaction (PCR) was performed to amplify the DNA using forward primer 27F (5^{I}-AGAGTTTGATCCTGGCTCAG -3^{I}) and reverse primer 1492R (5^{I}-TACGGTTACCTTGTTACGACTT -3^{I}), primers described by Mahdi et al. (2022). The amplification of the 16S rDNA was carried out in a reaction tube with a final volume of 20 µL containing 5 µL of template DNA, 2 µL of each forward primer and reverse primer, 10 µL of PCR Master mix with standard buffer (20 mM Tris-HCI, 1.8 mM MgCl2, 22 mM NH4Cl, 22 mM KCl, 0.2 mM dNTPs, 5% glycerol, 0.06% IGEPAL® CA-630, 0.05% Tween® 20, 25 units/mL One Taq® DNA polymerase) and final volume were filled up with 20 µL nuclease-free water. The PCR reaction conditions were as follows: Initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 40 s and extension at 72°C for 1 min, and a final extension of 72°C for 5 min. The PCR product was then visualized by electrophoresis in 2% (wt/vol) agarose gel with ethidium bromide (0.5 µg/mL) staining. The PCR products were taken for sequencing at a commercial service provider Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa.

2.13 Phylogenetic analysis of 16S rDNA sequences of endophytic bacteria obtained after sequencing

Phylogenetic analysis of the 16S rRNA gene sequences was conducted to determine the taxonomic placement of two endophytic bacterial isolates obtained from the leaves and roots of *Azadirachta indica*. The sequences of isolates ENDVI and ENDVII were aligned using MAFFT v7.520 together with closely related reference sequences retrieved from GenBank based on BLAST similarity (Katoh & Standley, 2013). A maximum-likelihood (ML) phylogenetic tree was reconstructed using IQ-TREE with automatic model selection, and branch support was evaluated using 1,000 ultrafast bootstrap and SH-aLRT replicates (Minh et al., 2020). The bacterial endophytes isolated from the leaves and roots of *Khaya senegalensis* were analyzed using the same approach described above.

2.14 Statistical analysis of data

The significant differences were analyzed using SPSS V 23.0. One way analysis of variance (One-Way ANOVA) was carried out, followed by Tukey's Honestly Significant Difference (HSD) post-hoc test to compare means of bioactive endophytic bacteria at $p < 0.05$.

3.0 RESULTS

3.1 Isolated endophytic bacteria

A total of twenty endophytes, ten from *A indica* and ten from *K senegalensis*, were isolated. Five endophytes were isolated from the leaves of *A indica* and five from the roots of this plant. Similarly, five endophytes where each isolated from the roots and leaves of *K senegalensis*. All the isolated endophytes were observed to be Creamy, white and flat on starch casein agar.

3.2 Preliminary antimicrobial screening of endophytic bacteria against poultry isolates

Amongst the 20 endophytes, nine (ENDI, ENDIV, ENDVI, ENDVII, ENDVIII, ENDIX, ENDX, ENDXI) where antagonistic against our test poultry organisms. Five isolates from *A indica* showed activity against the test organisms, while four only four where active from *K senegalensis*. Isolates ENDVI, ENDVII, ENDVIII, and ENDIX showed the strongest antagonistic activity against all the test organism. These four Endophytes were chosen for subsequent analysis.

3.3 Column chromatography and thin-layer chromatography of microbial extract

The dried ethyl acetate extracts of ENDVI, ENDVII, ENDVIII, and ENDIX was subjected to column chromatography and thirty fractions were collected from each sample. After running TLC, fractions with similar Rf values were pooled together for each sample. For ENDVI, we got three fractions (fraction 1-5, 6-9, 10-30; labelled A, B, C, respectively), for ENDVII we also got three fractions (fraction 1-6, 7-9, and 10-30; labelled D, E, F, respectively), for ENDVIII, we got 5 fractions (fraction 1-4, 5-12, 13-16, 17-18, and 19-30; labelled G, H, I, J, K, respectively), and ENDIX gave two fractions (fraction 1-10 and 11-30; labelled L and M respectively).

3.4 Secondary antimicrobial screening of chromatographic fractions

Secondary screening using disc diffusion method shown in Figure 1, showed that fraction H has the highest mean zone of inhibition against POLC1 with a value of 26.33, additionally Fraction B showed the mean zone of inhibition against POLE2 with a value of 23.00. Mean zones of inhibition of all the fractions against poultry droppings' isolates are tabulated as shown in Table 1.

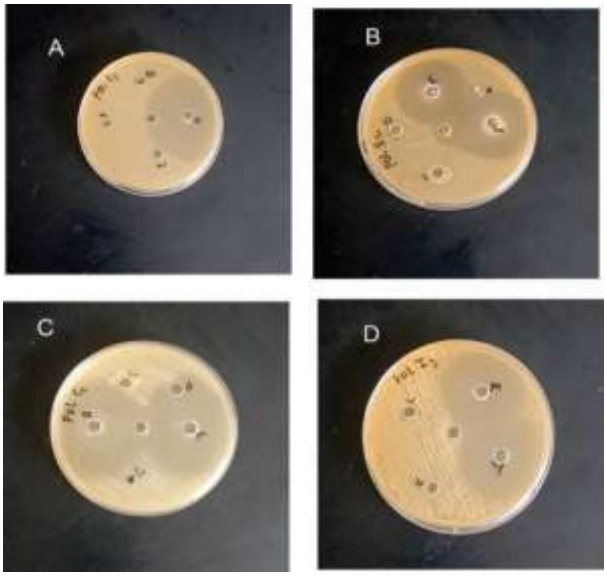


Figure **1.** Antibacterial screening of fractions against poultry-associated multidrug-resistant isolates

3.5 Isolates identified using 16S rDNA

The sequences of isolates were aligned with those sequences of bacteria available in the public database and determined their similarity values. The presumptive relationships of these sequences were obtained from database comparison. The phylogenetic relationship of isolates based on 16S rDNA gene sequences is shown in Figure 2 and 3. The final alignment of *A. indica* bacterial endophytes comprised 30 sequences and 760 nucleotide positions, of which 649 sites were conserved and 111 were variable. Among the variable sites, 51 were parsimony-informative and 60 were singleton sites, yielding 69 distinct alignment patterns that reflect several single nucleotide polymorphisms (SNPs) among the analyzed sequences. The phylogenetic tree resolved two distinct lineages within the phylum Firmicutes. Isolate ENDVI clustered within the genus *Bacillus*, showing close affiliation with members of the *Bacillus cereus* group, including *Bacillus thuringiensis*. On the other hand, isolate ENDVII grouped within the genus *Lysinibacillus* and showed the closest relationship with *Lysinibacillus fusiformis*, indicating that the two isolates belong to different genera within the phylum Firmicutes. For *K. senegalensis* bacterial endophytes, the final multiple sequence alignment consisted of 30 sequences and 774 nucleotide positions, of which 405 were conserved and 369 were variable. Among the variable sites, 56 were parsimony-informative and 313 were singleton sites, producing 135 distinct alignment patterns that indicate several SNPs across the dataset. The phylogenetic tree resolved the sequences into two major lineages within the phylum Firmicutes. Isolate ENDVIII clustered within the genus *Bacillus* and showed a close relationship with members of the *Bacillus cereus* group, including *Bacillus cereus, Bacillus tropicus*, and *Bacillus paramycoides*. In contrast, isolates ENDVII and ENDIX grouped within the genus *Lysinibacillus* and were most closely related to *Lysinibacillus fusiformis*, suggesting a very close evolutionary relationship with previously reported strains of this species. The similarity, and accession numbers on National center for biotechnology information (NCBI) of the endophytic bacterial isolates with their closely related strains are summarized in Table 2. All the 16S rDNA gene sequences of our isolates were submitted to GenBank and were assigned GenBank accession numbers: PX482399.1**,** PX446468.1, PX446467.1, and PX482400.1.

Table **1.** Mean zone of inhibition of four active chromatographic fractions from endophytes against multidrug-resistant poultry isolates

Endophyte Isolate	Fraction	R_f Value	Zone of inhibition against poultry droppings isolate (mm)			
			POLC1	POLC3	POLE2	POLI3
ENDVI	6-9 (B)	0.91	22.67± 2.31^{c}	25.67± 0.58^{b}	23.00± 0.00^{b}	25.67± 1.53^{b}
ENDVII	7-9 (E)	0.82	23.00± 0.00^{c}	21.67± 0.58^{c}	25.67± 0.58^{a}	24.67± 0.58^{b}
ENDVIII	5-12 (H)	0.75	26.33± 1.53^{b}	25.67± 0.58^{b}	20.33± 0.58^{c}	22.33± 0.58^{c}
ENDIX	11-30 (M)	0.78	21.00± 1.73^{c}	20.67± 2.31^{c}	21.00± 0.00^{c}	25.33± 1.15^{b}
	DMSO (Negative control)	NA	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}
	CIP (Positive control)	NA	33.67±1.53^{a}	30.67±1.15^{a}	25.33±1.53^{a}	33.67±1.53^{a}

Values are mean ± SD of three replicates. Within the same column (isolate), mean values bearing the same superscript letter are not significantly different at p < 0.05. POLC1= Poultry farm C1, POLC3= Poultry farm C3, POLE2= Poultry farm E2, POLI3= Poultry farm I3, CIP= Ciprofloxacin, DMSO= Dimethyl Sulfoxide, NA= Not applicable

Table **2.** Endophytic bacteria identified based on 16s rDNA similarity search on NCBI database and EZbiocloud database

Isolates	Length of Sequence (bp)	Most similar hit on EZbiocloud	% Identity	Most similar hit on NCBI	% Identity	Accession No.
END VI	770	*Bacillus cereus* (AE016877)	89.75	*Bacillus cereus* (MF445133.1)	92.96	PX482400.1
END VII	708	*Lysinibacillus fusiformis* (AB271743)	98.02	*Lysinibacillus fusiformis* (OP811537.1)	98.85	PX446467.1
END VIII	789	*Bacillus cereus* (AE016877)	98.97	*Bacillus cereus* (DQ648272.1)	98.72	PX446468.1
END IX	667	*Lysinibacillus cavernae* (MN611148)	84.66	*Lysinibacillus cavernae* (MN611148.1)	88.00	PX482399.1

Key: bp=base pairs

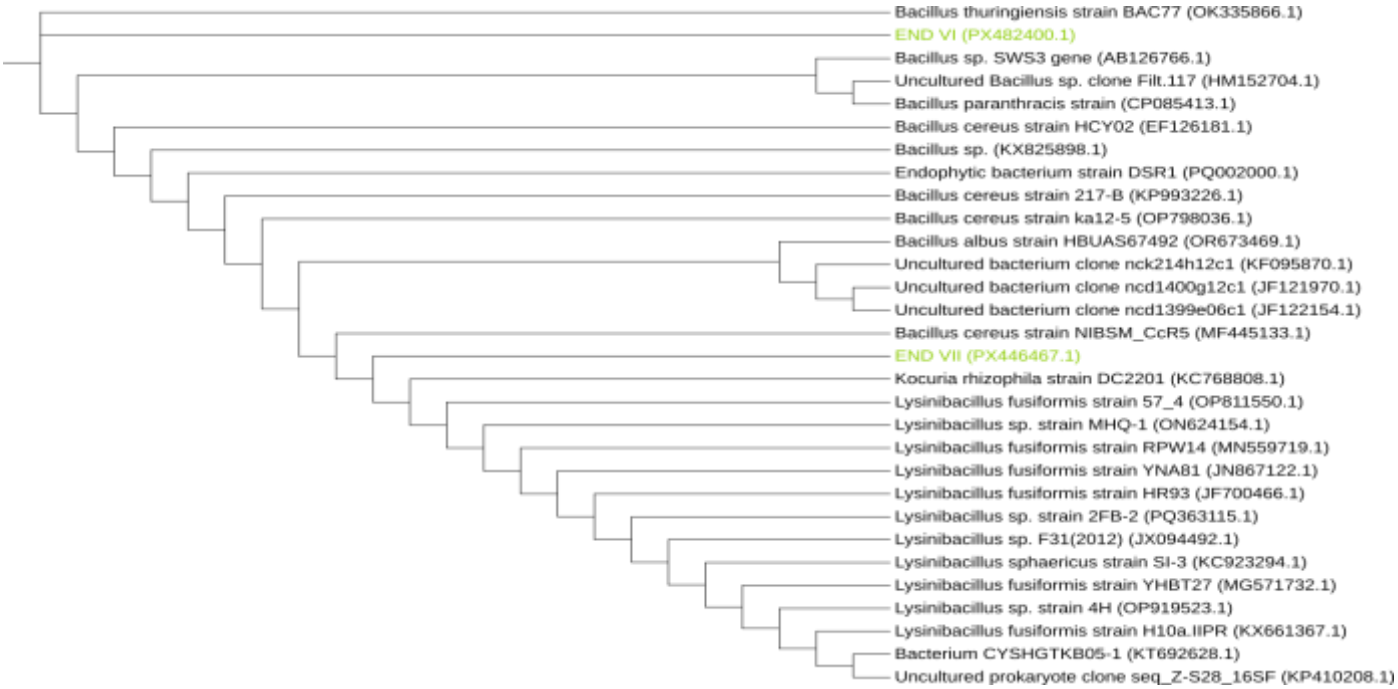


Figure **2**. Maximum-likelihood phylogenetic tree of endophytic bacteria (ENDVI (PX482400.1), and ENDVII (PX446467.1)) Based on 16s rDNA sequence using Iqtree software.

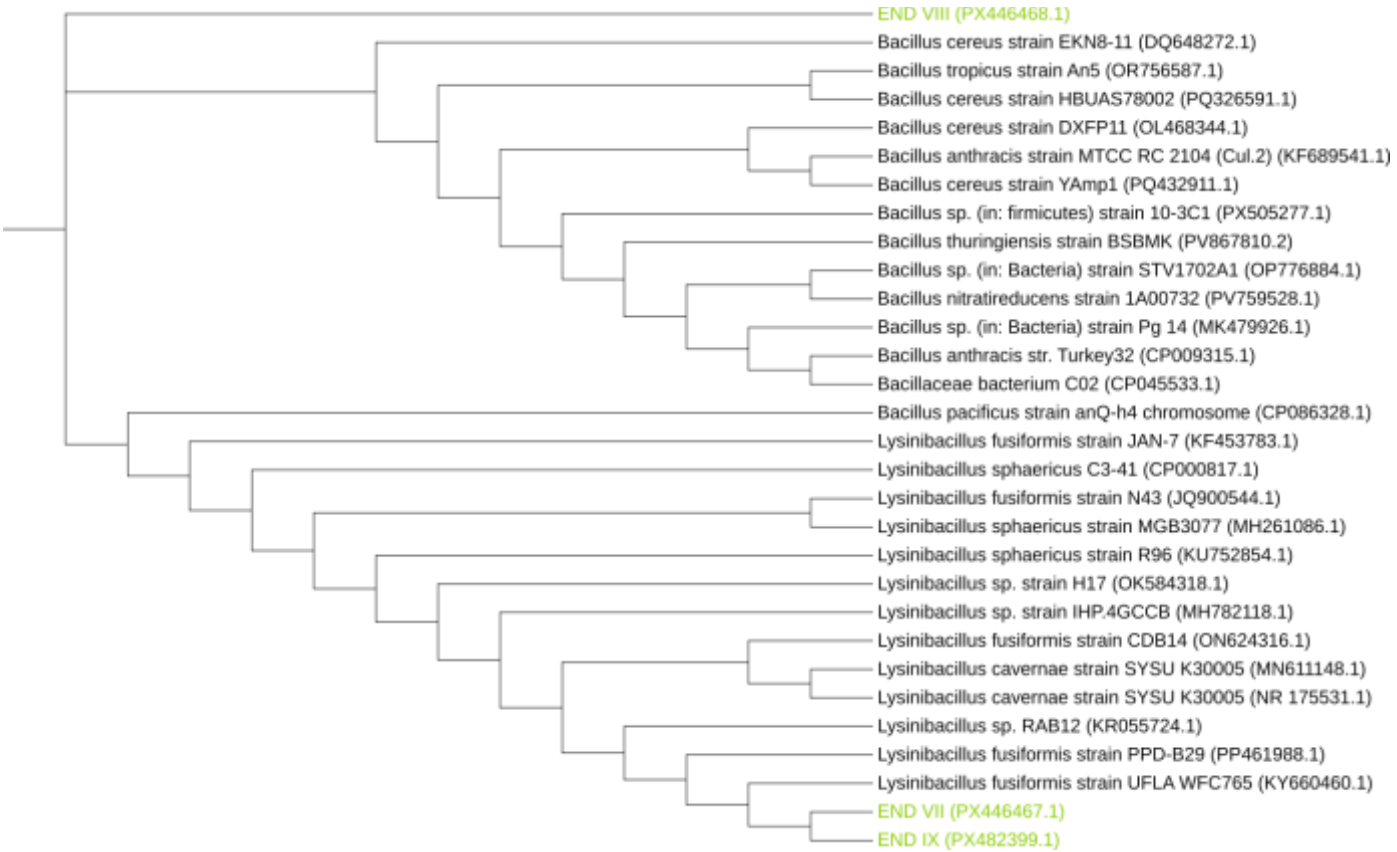


Figure **3.** Maximum-likelihood phylogenetic tree of endophytic bacteria (ENDVIII (PX446468.1), and ENDIX (PX482399.1)) Based on 16s rDNA Sequence using Iqtree software.

3.6 LC-MS metabolite profiling of secondary metabolites produced by bioactive endophytes

LC-MS analysis of extracts from END VI, VII, VIII, and IX lead to the prediction of a number of secondary metabolites which is presented in tables 3-10. A total of 21 different metabolites were predicted in both positive and negative ion mode for ENDVI. 12 different metabolites were discovered from the positive ion mode while 9 different metabolites were predicted from the negative ion mode. A total of 7 different metabolites were predicted in both positive and negative ion mode for ENDVII. 4 different metabolites were discovered from the positive ion mode while 3 different metabolites were predicted from the negative ion mode. A total of 11 different metabolites were predicted in both positive and negative ion mode for ENDVIII. 9 different metabolites were discovered from the positive ion mode while 2 different metabolites were predicted from the negative ion mode. A total of 7 different metabolites were predicted in both positive and negative ion mode for ENDIX. 5 different metabolites were discovered from the positive ion mode while 2 different metabolites were predicted from the negative ion mode.

Table **3.** Secondary metabolites in positive ion mode for END VI fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_T (min)	Predicted Compound	Main Class	Molecular Formula
1	285.135	2.312	Cyclo(-his-phe)	Amino acids and peptides	$C_{15}H_{16}N_4O_2$
2	241.934	2.626	Quinclorac	Quinolines	$C_{10}H_5Cl_2NO_2$
3	215.036	5.23	Allylphenol sulfate	Phenylpropanoids	$C_9H_{10}O_4S$
4	132.234	6.163	bis(3-aminopropyl) amine	Ornithine alkaloids	$C_6H_{17}N_3$
5	176.094	6.29	N-Carboxyethyl-gamma-aminobutyric acid	Fatty acids	$C_7H_{13}NO_4$
6	285.401	8.128	16-Oxoheptadecanoic acid	Fatty acids	$C_{17}H_{32}O_3$
7	215.271	8.359	12-Aminododecanoic acid	Fatty amides	$C_{12}H_{26}N_2O$
8	285.086	9.253	Xanthosine	Purines	$C_{10}H_{12}N_4O_6$
9	284.583	9.69	Glycitein	Flavonoids	$C_{16}H_{12}O_5$
10	215.386	9.786	Harmaline	Tryptophan alkaloids	$C_{13}H_{14}N_2O$
11	241.534	12.448	Palmitaldehyde	Fatty aldehydes	$C_{16}H_{32}O$
12	285.536	17.45	1,1,3,3-Tetrabutylurea	Organonitrogen compounds	$C_{17}H_{36}N_2O$

Table **4.** Secondary metabolites in negative ion mode for ENDVI fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t (min)	Predicted Compound	main class	molecular formula
1	285.185	6.163	2-Hydroxyestrone	Steroids	$C_{18}H_{22}O_3$
2	286.485	6.29	Procyclidine	Amines	$C_{19}H_{29}NO$
3	285.985	6.519	Vinclozolin	Halobenzenes	$C_{12}H_9Cl_2NO_3$
4	285.14	8.517	Kaempferol	Flavonoids	$C_{15}H_{10}O_6$
5	285.105	9.253	6-Chloropurine riboside	Purine Nucleosides	$C_{10}H_{11}ClN_4O_4$
6	269.198	9.544	4-Octylbenzenesulfonic acid	Benzenoids	$C_{14}H_{22}O_3S$

7	299.096	9.69	1,3-dihydroxy-6-(hydroxymethyl)-8-methoxyanthracene-9,10-dione	Benzenoids	$C_{16}H_{12}O_6$
8	299.108	9.786	1-[4-Hydroxy-5-(hydroxymethyl)oxolan-2-yl]-5-trimethylsilylpyrimidine-2,4-dione	Pyrimidine nucleosides	$C_{12}H_{20}N_2O_5Si$
9	269.188	17.45	Margaric acid	Fatty acids	$C_{17}H_{34}O_2$

Table **5.** Secondary metabolites in positive ion mode for ENDVII fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	284.291	12.794	Hexadecyl-trimethylammonium	Organonitrogen compounds	$C_{19}H_{42}N^+$
2	285.296	14.37	Docosapentaenoic acid	Fatty acids	$C_{22}H_{34}O_2$
3	285.285	17.482	17alpha-Hydroxypregn-4-ene-3,20-dione	Steroids and steroid derivatives	$C_{21}H_{30}O_3$
4	285.435	17.631	10-Methyl-heptadecanoic acid	Fatty acids	$C_{18}H_{36}O_2$

Table **6.** Secondary metabolites in negative ion mode for ENDVII fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	284.235	1.693	Alpha-hydroxy-metoprolol	Benzenoids	C15H25NO4
2	285.208	9.063	Tropicamide	Pyridine alkaloids	$C_{17}H_{20}N_2O_2$
3	284.035	5.7	4-[(4-cyanophenyl)-(1,2,4-triazol-1-yl)methyl]benzonitrile	Benzenoids	$C_{17}H_{11}N_5$

Table **7.** Secondary metabolites in positive ion mode for ENDVIII fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	285.181	1.678	5,7-Dimethoxyflavanone	Phenylpropanoids and polyketides	$C_{17}H_{16}O_4$
2	241.346	1.967	Anserine	Amino acids and peptides	$C_{10}H_{16}N_4O_3$
3	132.15	2.351	2-Aminobenzimidazole	Benzimidazoles	$C_7H_7N_3$
4	101.779	6.255	2-aminobutanoic acid	Amino acids and peptides	$C_4H_9NO_2$
5	176.166	7.743	1-Methoxy-1H-indole-3-carboxaldehyde	Tryptophan alkaloids	$C_{10}H_9NO_2$
6	101.869	9.76	Methionine sulfoximine	Amino acids and peptides	$C_5H_{12}N_2O_3S$
7	79.895	12.769	Cyclamate	Cyclohexylamines	$C_6H_{13}NO_3S$
8	284.406	13.868	Hexadecyltrimethylammonium	Organic nitrogen compounds	$C_{19}H_{42}N^+$
9	79.79	17.648	2-Aminoethanesulfonic acid	Amino acids and peptides	$C_2H_7NO_3S$

Table **8.** Secondary metabolites in negative ion mode for ENDVIII fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	285.115	9.041	6-Chloropurine riboside	Nucleosides, nucleotides, and analogues	$C_{10}H_{11}ClN_4O_4$
2	285.935	6.88	Vinclozolin	Halobenzenes	$C_{12}H_9Cl_2NO_3$

Table **9.** Secondary metabolites in positive ion mode for ENDIX fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	285.197	2.486	5,7-Dimethoxyflavanone	Phenylpropanoids and polyketides	$C_{17}H_{16}O_4$
2	285.309	6.939	N-[3-(Dimethylamino)propyl]dodecanamide	Fatty amides	$C_{17}H_{36}N_2O$
3	285.314	17.658	1,4-Androstadiene-3,17-dione	Lipids and lipid-like molecules	C19H24O2
4	284.235	7.246	Hexadecyl-trimethylammonium	Organic nitrogen compounds	$C_{19}H_{42}N^+$
5	285.314	17.658	3-O-Methylgalangin	Flavonoids	$C_{16}H_{12}O_5$

Table **10:** Secondary metabolites in negative ion mode for END IX fraction on metabolomics workbench

S/No	Observed M/Z	Retention Time R_t	Predicted Compound	Main Class	Molecular Formula
1	285.111	9.065	Kaempferol	Flavonoids	$C_{15}H_{10}O_6$
2	285.181	17.467	17alpha-Hydroxypregn-4-ene-3,20-dione	Steroids and steroid derivatives	$C_{21}H_{30}O_3$

4.0 DISCUSSION

The choice of *Azadirachta indica* and *Khaya senegalensis* as source plants for endophyte isolation reflects strategic bioprospecting based on ethnobotanical knowledge (Wylie & Merrell, 2022). Neem has extensive traditional medicinal applications across Africa and Asia, with documented antimicrobial, anti-inflammatory, and antiparasitic properties attributed to compounds such as azadirachtin, nimbin, and various limonoids (Wylie & Merrell, 2022). African mahogany similarly possesses a rich history of traditional therapeutic uses, particularly for treating infections and inflammatory conditions (Adamu et al., 2022). The bioactive chemistry of

these plants likely creates selective environments within their tissues that favor colonization by endophytes with complementary metabolic capabilities (Chen & Jiang, 2026). The preliminary antimicrobial screening conducted against four randomly selected poultry droppings' isolates which were already established as MDR isolates in our previous study (Muhammad et al., 2025), revealed differential antagonistic activities among the twenty endophytes tested. Four endophytes—ENDVI and ENDVII from *A. indica*, and ENDVIII and ENDIX from *K. senegalensis*—demonstrated consistent inhibitory activity against all four test organisms. This universal antagonistic capability suggests these isolates produce broad-spectrum antimicrobial compounds effective against diverse enterobacterial strains. The selection of these four endophytes for detailed characterization proved strategic, as subsequent analyses confirmed their capacity for bioactive secondary metabolite production. The variable antimicrobial activity observed across the endophyte collection reflects the heterogeneous nature of endophytic communities and their diverse metabolic capabilities (Singh et al., 2021). Several isolates showed selective activity against specific test organisms, while others demonstrated no detectable antimicrobial effects under the screening conditions employed. Such variability is typical in natural product discovery programs, where only a subset of microbial isolates produces compounds with desired biological activities at detectable levels (Hashem et al., 2023). The factors influencing antimicrobial production by endophytes include genetic capacity for biosynthetic gene cluster expression, regulatory mechanisms controlling secondary metabolism, nutritional conditions, and environmental signals perceived by the organisms (De Simeis & Serra, 2021). The extraction of bioactive compounds using ethyl acetate represents a strategic choice based on this solvent's physicochemical properties. This differential extraction provides initial purification by separating bioactive organic compounds from bulk fermentation components. The liquid-liquid extraction procedure employed, utilizing a separating funnel with repeated extractions, maximizes compound recovery while the subsequent vacuum evaporation concentrates the extract to manageable volumes for further purification (Abdelmohsen et al., 2022). The stepwise increase in methanol polarity from 0% to 100% in 10% increments ensures comprehensive elution of compounds across the polarity spectrum. This approach capitalizes on differential retention of compounds based on their interactions with the silica gel surface and relative solubility in the mobile phase (Sreenivasan et al., 2022). The collection of thirty fractions from each extract provided fine resolution of compound mixtures, critical for isolating individual bioactive entities from complex natural product matrices (Sreenivasan et al., 2022). Thin-layer chromatography served dual purposes in this investigation: confirming successful separations achieved through column chromatography and enabling combination of fractions with similar compound profiles (Reshma & Brindha-Priyadarisini, 2021). The Rf values calculated for active fractions—0.91, 0.82, 0.75, and 0.78 for ENDVI, ENDVII, ENDVIII, and ENDIX respectively—indicate the bioactive compounds possess relatively high polarity and mobility in the chloroform-methanol (4:1) system employed. These Rf values suggest moderately polar compounds, consistent with many known antimicrobial secondary metabolites including various alkaloids, modified peptides, and oxygenated terpenes (Ujam et al., 2021). The magnitude of inhibition zones observed in secondary screening of the fractions (generally 20-26 mm) compares favorably with those produced by conventional antibiotics against susceptible strains and exceeds activity levels typically reported for crude natural product extracts (Gajic et al., 2022). These substantial zones of inhibition suggest the purified fractions contain bioactive compounds at significant concentrations and that these compounds possess genuine antimicrobial potency rather than marginal effects. Research by Gajic et al. (2022) established that inhibition zones exceeding 15 mm typically indicate promising antimicrobial activity worthy of further investigation, with zones above 20 mm suggesting compounds with therapeutic potential. The antibacterial activity recorded in this study is significant considering that the poultry droppings' bacterial isolates used as test organisms were previously confirmed to show multidrug-resistant to several conventional antibiotics (Muhammad *et al.*, 2025). This suggest that endophytes from *A. indica* and *K. senegalensis* may represent a promising source of antimicrobial metabolites that could serve as alternative or complements to existing antibiotics in controlling MDR bacteria associated with poultry environments.

The findings of this investigation align with and extend previous research on endophytic bacteria from medicinal plants. A study by Tshikhudo et al. (2023) isolated endophytic actinomycetes from medicinal plants in Egypt and demonstrated their antimicrobial activities against multidrug-resistant clinical isolates. Their work identified *Streptomyces* species as predominant bioactive endophytes, contrasting with the *Bacillus* and *Lysinibacillus* species suspected in this study. Research conducted by Raihan et al. (2021) in Bangladesh isolated endophytic bacteria from neem (*Azadirachta indica*) and screened them for antimicrobial activities against both Gram-positive and Gram-negative pathogens. They identified several *Bacillus* species with significant bioactivities, supporting findings from this investigation. However, their study did not extend to detailed metabolite profiling or testing against multidrug-resistant agricultural isolates, representing areas where the current work provides additional insights (Newman & Cragg, 2020). Additionally, a comprehensive investigation by Ek-Ramos et al. (2019) examined endophytic bacteria from African medicinal plants, including species closely related to *Khaya senegalensis*. They documented diverse antimicrobial activities and identified novel bioactive compounds including modified peptides and polyketides. Their emphasis on African medicinal plants aligns with the rationale for selecting study plants in this investigation, recognizing the untapped potential of African biodiversity for antimicrobial discovery (Newman & Cragg, 2020). The 16S rDNA gene sequencing and phylogenetic analysis provided taxonomic identification of the four bioactive endophytes, closely related to members of the *Bacillus* and *Lysinibacillus* genera. ENDVI and ENDVIII were identified as *Bacillus cereus* based on 92.96% and 98.72% sequence identity respectively with NCBI database sequences. ENDVII showed 98.85% identity with *Lysinibacillus fusiformis*, while ENDIX demonstrated 88.00% identity with *Lysinibacillus cavernae*. The genus *Bacillus* represents one of the most prolific sources of antimicrobial compounds in nature, with species producing a vast array of bioactive metabolites including lipopeptides (surfactins, iturins, fengycins), polyketides, bacteriocins, and various modified peptides (Caulier et al., 2019; Patience et al., 2026). *Bacillus cereus*, while often discussed in food safety contexts due to pathogenic strains, also includes environmental isolates with significant biocontrol and antimicrobial production capabilities. Research by Caulier et al. (2019) comprehensively reviewed the antimicrobial arsenal of the *Bacillus cereus* group, identifying numerous biosynthetic gene clusters responsible for producing bioactive compounds. The isolation of *B. cereus* strains from medicinal plants as endophytes with antimicrobial activity has been reported by Mohamad et al. (2018), who documented diverse bioactive compounds from *B. cereus* endophytes including novel antimicrobial peptides. *Lysinibacillus fusiformis* and *L. cavernae* belong to a genus historically included within Bacillus but reclassified based on phylogenetic and chemotaxonomic criteria (Kan et al., 2020). Despite this taxonomic separation, *Lysinibacillus* species retain metabolic capabilities characteristic of *Bacillus*, including secondary metabolite production (Harirchi et al., 2022). *L. fusiformis* has been investigated for antimicrobial compound production by several research groups, with identified bioactive metabolites including cyclic lipopeptides and modified peptides (Jamal & Ahmad, 2022). Studies by Passera et al. (2020) demonstrated that *L. fusiformis* isolates from diverse environments produce antimicrobial compounds, supporting the findings of this investigation.

The ENDVI extract contained diverse compound classes including amino acid derivatives, fatty acids, alkaloids, and flavonoids. Cyclo(-his-phe), identified at m/z 285.135, represents a cyclic dipeptide formed from histidine and phenylalanine. Cyclic dipeptides, also termed diketopiperazines, constitute a major class of bacterial secondary metabolites with documented antimicrobial, antifungal, and cytotoxic activities (Song et al., 2021). Research by Song et al. (2021) comprehensively reviewed diketopiperazine bioactivities, noting their production by numerous Bacillus species and their potential as antimicrobial lead compounds. The presence of cyclo(-his-phe) in the active fraction suggests potential contribution to observed antibacterial effects, either independently or synergistically with other components. Several long-chain fatty acid derivatives were identified, including 16-oxoheptadecanoic acid (m/z 285.401) and 12-aminododecanoic acid (m/z 215.271). Modified fatty acids have received increasing attention as antimicrobial agents, with mechanisms including membrane disruption, interference with fatty acid biosynthesis, and modulation of bacterial signaling pathways (Casillas-Vargas et al., 2021). Studies by Casillas-Vargas et al. (2021) demonstrated that bacterial fatty acids, particularly those with unusual structural features, exhibit significant antimicrobial activities against both Gram-positive and Gram-negative bacteria. The identification of xanthosine (m/z 285.086), a purine nucleoside, suggests potential involvement in nucleotide metabolism interference or purine analog-based antimicrobial mechanisms (Iacob et al., 2025; Usman et al., 2026). Glycitein (m/z 284.583), an isoflavonoid, represents a flavonoid

class compound traditionally associated with plant metabolism but increasingly recognized as products of bacterial biotransformation or biosynthesis (Ahmadi & Ebrahimzadeh, 2020). Isoflavonoids possess various biological activities including antimicrobial effects, potentially contributing to the observed bioactivity of ENDVI extracts. In negative ion mode, kaempferol (m/z 285.14) was identified as a flavonoid with well-documented antimicrobial properties. Multiple studies have established kaempferol's effectiveness against bacterial pathogens through mechanisms including membrane disruption, inhibition of bacterial enzymes, and interference with quorum sensing (Shamsudin et al., 2022). Research by Shamsudin et al. (2022) comprehensively reviewed antimicrobial mechanisms of flavonoids, noting kaempferol's activity against various *E. coli* and *Salmonella* strains, directly relevant to this study's test organisms. The metabolite profile of ENDVII showed a predominance of lipid-derived compounds including docosapentaenoic acid (m/z 285.296), a polyunsaturated fatty acid with 22 carbons and five double bonds. Polyunsaturated fatty acids have demonstrated antimicrobial activities, with mechanisms proposed to involve membrane perturbation and alteration of membrane fluidity affecting bacterial physiology (Casillas-Vargas et al., 2021). The identification of 17alpha-hydroxypregn-4-ene-3,20-dione (m/z 285.285 and 285.181), a steroid derivative, represents an unusual finding in bacterial extracts, as steroid biosynthesis is traditionally associated with eukaryotic organisms (Donova, 2022). However, bacterial steroid production has been increasingly documented, particularly in organisms inhabiting eukaryotic hosts or environments rich in steroid precursors (Fernández-Cañón & Chamizo-Ampudia, 2026). The presence of hexadecyl-trimethylammonium (m/z 284.291), a quaternary ammonium compound, suggests potential surfactant-type antimicrobial activity. Quaternary ammonium compounds act as cationic surfactants that disrupt bacterial membranes through electrostatic interactions with negatively charged membrane components, leading to membrane depolarization and cell lysis (Vereshchagin et al., 2021). Tropicamide (m/z 285.208), identified in negative ion mode, typically represents a synthetic pharmaceutical compound used as a mydriatic agent. Its identification in bacterial extracts may reflect structural similarity with produced metabolites rather than actual tropicamide presence, highlighting limitations of database matching for novel natural products (Rutz & Wolfender, 2023). ENDVIII exhibited a diverse metabolite profile including 5,7-dimethoxyflavanone (m/z 285.181), a flavonoid with documented antimicrobial properties. Methoxylated flavonoids often demonstrate enhanced bioactivities compared to unmethoxylated counterparts due to improved membrane permeability and altered receptor interactions (Tan et al., 2022). The presence of anserine (m/z 241.346), a dipeptide consisting of beta-alanine and 3-methyl-histidine, represents another amino acid derivative potentially contributing to antimicrobial activity (Tan et al., 2022). Several indole derivatives were identified, including 1-methoxy-1H-indole-3-carboxaldehyde (m/z 176.166). Indole compounds serve as important signaling molecules in bacterial communities and possess various biological activities including antimicrobial effects (Razzaq et al., 2025). The tryptophan alkaloid category encompassing these compounds has been extensively studied for bioactive properties. Research by Razzaq et al. (2025) documented diverse biological activities of bacterial indole derivatives, including antimicrobial, antifungal, and anti-biofilm effects. The identification of 2-aminobenzimidazole (m/z 132.15) represents a nitrogen-containing heterocycle with known antimicrobial properties. Benzimidazole derivatives have been investigated as antimicrobial agents due to their ability to interfere with microbial DNA synthesis and enzyme function (Brishty et al., 2021).

The metabolite profile of ENDIX included 5,7-dimethoxyflavanone (m/z 285.197), also identified in ENDVIII extracts, suggesting convergent metabolic capabilities across different endophyte species. This flavonoid's recurrent identification in bioactive fractions strengthens evidence for its potential contribution to observed antimicrobial effects (Shamsudin et al., 2022). The fatty amide N-[3-(Dimethylamino)propyl]dodecanamide (m/z 285.309) represents another potentially bioactive component, as fatty amides with amino substituents often exhibit enhanced antimicrobial properties compared to simple fatty acids (Casillas-Vargas et al., 2021). Kaempferol (m/z 285.111) was identified in ENDIX extracts, similar to its detection in ENDVI, indicating this flavonoid may be a common metabolite among bioactive endophytes from these medicinal plants (Shamsudin et al., 2022). The identification of 1,4-androstadiene-3,17-dione (m/z 285.314), a steroid compound, parallels findings from ENDVII and suggests steroid metabolism may be more common in bacterial endophytes than previously recognized (Donova, 2022). Steroid compounds can possess antimicrobial activities through membrane interactions and interference with cellular processes dependent on membrane integrity (Fernández-Cañón & Chamizo-Ampudia, 2026). The compound 3-O-methylgalangin (m/z 285.314) represents a methylated flavonol with structural similarity to other identified flavonoids. The methylation pattern may influence antimicrobial potency through effects on lipophilicity, membrane penetration, and molecular target interactions (Tan et al., 2022). Studies by Tan et al. (2022) demonstrated that methylation patterns in flavonoids significantly influence their antimicrobial activities, with specific substitution patterns conferring enhanced effectiveness against particular bacterial species.

Limitations

All biological test were performed at one concentration, which prevents the calculation of MIC. The liquid chromatography–mass spectrometry (LC-MS) analysis used for metabolite profiling of the active endophytic fractions was conducted using a single analytical platform and relied on database matching for compound identification. No confirmatory structural elucidation techniques — such as nuclear magnetic resonance (NMR) spectroscopy, high-resolution mass spectrometry (HRMS), or tandem mass spectrometry (MS/MS) fragmentation analysis — were employed to validate the identity of the putative compounds identified. These constraints mean that compound identities should be regarded as tentative rather than confirmed, and the specific contributions of individual metabolites to the observed antimicrobial activity cannot be conclusively established without further validation.

Conclusion

This investigation successfully demonstrated that endophytic bacteria isolated from *Azadirachta indica* and *Khaya senegalensis* produce diverse secondary metabolites with potent antimicrobial activities against multidrug-resistant *Escherichia coli* and *Salmonella* species from poultry sources. The identification of *Bacillus cereus* and *Lysinibacillus* species as bioactive endophytes expands recognition of antimicrobial production capabilities within these genera. The metabolite profiles revealed through LC-MS analysis indicate production of multiple compound classes including flavonoids, cyclic dipeptides, modified fatty acids, and alkaloids, suggesting diverse biosynthetic capabilities with potential for further exploration. The promising antimicrobial activities demonstrated by endophyte-derived secondary metabolite against resistant pathogens suggest genuine therapeutic potential worthy of continued development. The substantial inhibition zones observed, combined with chemical diversity of identified metabolites, position these findings as valuable starting points for antimicrobial development programs targeting agricultural and potentially human applications. The integration of traditional ethnobotanical knowledge with modern analytical and molecular techniques exemplified in this study provides a productive model for natural product discovery in regions with rich biodiversity and traditional medicine practices.

Acknowledgements

The authors extend their sincere thanks to professor Timothy Bulus, Dr. Maimuna Zubairu for their guidance, suggestions and assistance throughout this work. Special thanks to Mallam Mahmud Abdulaziz of the Department of Veterinary and Public Health, Ahmadu Bello University for his Laboratory assistance and recommendations during this work

Funding

The authors declare that no funds, grants, or other support were received for this work

Conflict of interest

The authors report no conflict of interest and are responsible for the content and writing of the manuscript.

Data availability

The 16S rDNA sequences generated and analyzed in this study have been deposited in the GenBank database under accession numbers PX446467.1, PX446468.1, PX482400.1 and PX482399.1. The datasets generated and analyzed during current study, including raw AMR screening measurements and LC-MS data, are available from the corresponding author upon reasonable request.

Authors contribution

AAM conceptualized the study; AAM performed the methodology; AAM wrote the original draft; AB performed writing review, editing, validation and proofreading; AAM did Project administration. All authors read and agreed to the final version of the manuscript.

REFERENCES

Abdelmohsen, U.R., Sayed, A.M., & Elmaidomy, A.H. (2022). Natural products' extraction and isolation–between conventional and modern techniques. *Frontiers in Natural Products*, 1, 873808.

Adamu, I., Majiyebo, A. J., Alozieuwa, U. B., Lawal, A. A., Alawode, B. O. O. R. A., & Berinyuy, E. B. (2022). Ethnopharmacology, chemical constituents, and biological activities of Khaya senegalensis (Desr.) A. Juss a forest plant with multi-purpose applications. *AROC. Natural Products Research*, *2*(2), 10-15.

Ahmadi, R., & Ebrahimzadeh, M.A. (2020). Resveratrol – a comprehensive review of recent advances in anticancer drug design and development. *European Journal of Medicinal Chemistry*, 200, 112356.

Alara, J. A., & Alara, O. R. (2024). An overview of the global alarming increase of multiple drug resistant: a major challenge in clinical diagnosis. *Infectious Disorders-Drug Targets Disorders)*, *24*(3), 26-42.

Anand, U., Pal, T., Yadav, N., Singh, V.K., Tripathi, V., Choudhary, K.K., Shukla, A.K., Sunita, K., Kumar, A., Bontempi, E., Ma, Y., Kolton, M., & Singh, A.K. (2023). Current scenario and future prospects of endophytic microbes: promising candidates for abiotic and biotic stress management for agricultural and environmental sustainability. *Microbial Ecology*, 86(3), 1455-1486.

Brishty, S.R., Hossain, M.J., Khandaker, M.U., Faruque, M.R.I., Osman, H., & Rahman, S.M.A. (2021). A comprehensive account on recent progress in pharmacological activities of benzimidazole derivatives. *Frontiers in Pharmacology*, 12, 762807.

Casillas-Vargas, G., Ocasio-Malavé, C., Medina, S., Morales-Guzmán, C., García Del Valle, R., Carballeira, N.M., & Sanabria-Ríos, D.J. (2021). Antibacterial fatty acids: an update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. *Progress in Lipid Research*, 82, 101093.

Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., & Mahillon, J. (2019). Overview of the antimicrobial compounds produced by members of the *Bacillus subtilis* group. *Frontiers in Microbiology*, 10: 302.

Chen, Z., & Jiang, S. (2026). Chemical Signaling and Metabolomic Crosstalk in Endophytic Fungi–Medicinal Plant Symbioses for Natural Product Discovery and Sustainable Bioproduction. *Metabolites*, *16*(3), 164

Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S., & Laxminarayan, R. (2018). Anthropological and socioeconomic factors contributing to global antimicrobial resistance. *The Lancet Planetary Health*, 2(9), e398-e405.

De Simeis, D., & Serra, S. (2021). *Actinomycetes*: a never-ending source of bioactive compounds—an overview on antibiotics production. *Antibiotics*, 10(5), 483.

Donova, M.V. (2022). Microbial steroid production technologies: current trends and prospects. *Microorganisms*, 10(1), 53.

Ek-Ramos, M.J., Gomez-Flores, R., Orozco-Flores, A.A., Rodríguez-Padilla, C., González-Ochoa, G., & Tamez-Guerra, P. (2019). Bioactive products from plant-endophytic gram-positive bacteria. *Frontiers in Microbiology*, 10: 463.

Ezeobiora, C.E., Igbokwe, N.H., Stephen, C.M., Mendie, U.E., & Amin, D.H. (2024). Diversity, molecular phylogenetics, and antibiotic biosynthetic potential of endophytic Actinobacteria isolated from medicinal plants in Nigeria. *Brazilian Journal of Microbiology.* 55, 179-190.

Fernández-Cañón, J.M., & Chamizo-Ampudia, A. (2026). Toward sustainable testosterone manufacturing: green chemistry and microbial biotransformation approaches. *International Journal of Molecular Sciences*, 27(5), 2444.

Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L., & Opavski, N. (2022). Antimicrobial susceptibility testing: a comprehensive review of currently used methods. *Antibiotics*, 11(4), 427

Gakuubi, M.M., Munusamy, M., Liang, Z.-X., & Ng, S.B. (2021). Fungal endophytes: a promising frontier for discovery of novel bioactive compounds. *Journal of Fungi*, 7(10), 786.

Garba, Y., Bello, N. U., Bashir, M., & Mohammed, S. Y. (2023). *In Vitro* and *in Vivo* Analysis of the Antioxidant Potentials of Methanolic Extract of *Tamarindus indica* Leaves. *Nigerian Journal of Biochemistry and Molecular Biology*, *38*(3), 104-111.

Guan, S., Zhong, L., Yu, H., Wang, L., Jin, Y., Liu, J., Xiang, H., Yu, H., Wang, L., & Wang, D. (2022). Molecular docking and proteomics reveals the synergistic antibacterial mechanism of theaflavin with β-lactam antibiotics against MRSA. *Frontiers in Microbiology*, 13, 993430.

Harirchi, S., Sar, T., Ramezani, M., Aliyu, H., Etemadifar, Z., Nojoumi, S.A., Yazdian, F., Awasthi, M.K., & Taherzadeh, M.J. (2022). Bacillales: from taxonomy to biotechnological and industrial perspectives. *Microorganisms*, 10(12), 2355.

Hashem, A.H., Attia, M.S., Kandil, E.K., Fawzi, M.M., Abdelrahman, A.S., Khader, M.S., Khodaira, M.A., Emam, A.E., Goma, M.A., & Abdelaziz, A.M. (2023). Bioactive compounds and biomedical applications of endophytic fungi: a recent review. *Microbial Cell Factories*, 22, 107.

Iacob, S., Stefan, C.-S., Nechita, A., Matei, M.-N., Lisa, E.-L., Tutunaru, D., Fulga, I., Fulga, A., Cristea (Hohota), A.-G., & Dragostin, O.-M. (2025). Hybrid molecules with purine and pyrimidine derivatives for antitumor therapy: news, perspectives, and future directions. *Molecules*, 30(13), 2707.

Jamal, Q.M.S., & Ahmad, V. (2022). Lysinibacilli: A Biological Factories Intended for Bio- Insecticidal, Bio-Control, and Bioremediation Activities. *Journal of Fungi,* 8(2), 1288

Kan, Y., Niu, X.K., Rao, M.P.N., Dong, Z.Y., Xie, Y.G., Kang, Y.Q., & Li, W.J. (2020). *Lysinibacillus cavernae* sp. nov., isolated from cave soil. *Archives of Microbiology*, 202(6), 1529-1534.

Kato, K., & Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, 30, 772-780.

Mahdi, R.A., Bahrami, Y., & Kakaei, E. (2022). Identification and antibacterial evaluation of endophytic actinobacteria from Luffa cylindrica. *Scientific Reports,* 12, 18236.

Mak, P.H.W., Rehman, M.A., Kiarie, E.G., Topp, E., & Diarra, M.S. (2022). Production systems and important antimicrobial resistant-pathogenic bacteria in poultry: a review. *Journal of Animal Science and Biotechnology*, 13, 148.

Manganyi, M.C., & Ateba, C.N. (2020). Untapped potentials of endophytic fungi: a review of novel bioactive compounds with biological applications. *Microorganisms*, 8(12), 1934.

Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, 37(5), 1530-1534.

Mohamad, O.A.A., Li, L., Ma, J.B., Hatab, S., Xu, L., Guo, J.W., Rasulov, B.A., Liu, Y.H., Hedlund, B.P., & Li, W.J. (2018). Evaluation of the antimicrobial activity of endophytic bacterial populations from Chinese traditional medicinal plant licorice and characterization of the bioactive secondary metabolites produced by *Bacillus* atrophaeus against *Verticillium dahliae*. *Frontiers in Microbiology*, 9: 924.

Muhammad, A.A., Bulus, T., & Zubairu, M. (2025). Antibiotic susceptibility profiles and multiple antibiotic resistance index of *Salmonella* spp and *Escherichia coli* from poultry droppings from selected farms in Samaru, Zaria. *Nigerian Journal of Scientific Research*, 24(1), 140-148.

Musa, Z., Ma, J., Egamberdieva, D., Abdelshafy Mohamad, O., Abaydulla, G., Liu, Y., Li, W.-J., & Li, L. (2020). Diversity and antimicrobial potential of cultivable endophytic actinobacteria associated with the medicinal plant Thymus roseus. Frontiers in Microbiology, 11, 191.

Newman, D.J., & Cragg, G.M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3): 770-803.

Panigrahi, S., Dash, D., & Rath, C. C. (2018). Characterization Of Endophytic Bacteria with Plant Growth Promoting Activities Isolated from Six Medicinal Plants. *Journal of ExperimentalBiology and Agricultural Sciences*, 6(5), 782 – 791.

Passera, A., Rossato, M., Oliver, J.S., Battelli, G., Shahzad, G.-I.-R.., Cosentino, E., Sage, J.M., Toffolatti, S.L., Lopatriello, G., & Davis, J.R. (2020). Characterization of Lysinibacillus fusiformis strain S4C11: In vitro, in planta, and in silico analyses reveal a plant-beneficial microbe. *Microbiological Research,* 244, 126665,

Patience, A. C., Christiana, O. C., & Ukamaka, O. E. (2026). Biodegradation of cypermethrin and its metabolic products by *Bacillus flexus* strain PC5 isolated from cypermethrin-treated agricultural soil. *Bio-Research*, *24*(1), 360–373.

Raihan, T., Azad, A.K., Ahmed, J., Shepon, M.R., Dey, P., Chowdhury, N., Aunkor, T.H., Ali, H., & Suhani, S. (2021). Extracellular metabolites of endophytic fungi from *Azadirachta indica* inhibit multidrug-resistant bacteria and phytopathogens. *Future Microbiology*, 16, 557-576.

Razzaq Meo, S., Van de Wiele, T., & Defoirdt, T. (2025). Indole signaling in *Escherichia coli*: a target for antivirulence therapy? *Gut Microbes*, 17(1), 2499573.

Reshma, A., & Brindha-Priyadarisini, V. (2021). Eco friendly antimicrobial finishing of textiles using bioactive metabolite from endophytic *Streptomyces fradiae* CQLW against bio deterioration. *Journal of the Textile Institute*, 112(6), 902-913.

Rutz, A., & Wolfender, J.-L. (2023). Automated composition assessment of natural extracts: untargeted mass spectrometry-based metabolite profiling integrating semiquantitative detection. *Journal of Agricultural and Food Chemistry*, 71(46), 18010-18023.

Shamsudin, N.F., Ahmed, Q.U., Mahmood, S., Ali Shah, S.A., Khatib, A., Mukhtar, S., Alsharif, M.A., Parveen, H., & Zakaria, Z.A. (2022). Antibacterial effects of flavonoids and their structure-activity relationship study: a comparative interpretation. *Molecules*, 27(4), 1149.

Singh, A., Singh, D.K., Kharwar, R.N., White, J.F., & Gond, S.K. (2021). Fungal endophytes as efficient sources of plant-derived bioactive compounds and their prospective applications in natural product drug discovery: insights, avenues, and challenges. *Microorganisms*, 9(1), 197.

Sreenivasan, A., Manikkam, R., & Manigundan, K. (2022). Isolation, characterization, and antimicrobial activity of endophytic actinobacterial from medicinal plant. *Indian Journal of Pharmaceutical Science,* 84(5), 1150-1160.

Song, Z., Hou, Y., Yang, Q., Li, X., & Wu, S. (2021). Structures and biological activities of diketopiperazines from marine organisms: a review. *Marine Drugs*, 19(8), 403.

Tan, Z., Deng, J., Ye, Q., & Zhang, Z. (2022). The antibacterial activity of natural-derived flavonoids. *Current Topics in Medicinal Chemistry*, 22(12), 1009-1019.

Tshikhudo, P.P., Ntushelo, K., & Mudau, F.N. (2023). Sustainable applications of endophytic bacteria and their physiological/biochemical roles on medicinal and herbal plants: review. *Microorganisms*, 11(2), 453.

Ujam, N.T., Ajaghaku, D.L., Okoye, F.B.C., & Esimone, C.O. (2021). Antioxidant and immunosuppressive activities of extracts of endophytic fungi isolated from *Psidium guajava* and *Newbouldia laevis*. *Phytomedicine Plus*, 1(2), 100028.

Usman, J., Gusau, M. A., Abubakar, I., & Hussaini, S. (2026). Mutation in the quinolone resistance-determining region (QRDR) of the *gyrA* gene in ciprofloxacin-resistant *Salmonella typhi*. *Bio-Research*, *24*(1), 490–496

Vereshchagin, A.N., Frolov, N.A., Egorova, K.S., Seitkalieva, M.M., & Ananikov, V.P. (2021). Quaternary ammonium compounds (QACs) and ionic liquids (ILs) as biocides: from simple antiseptics to tunable antimicrobials. *International Journal of Molecular Sciences*, 22(13), 6793.

Witte, T.E., Villeneuve, N., Boddy, C.N., & Overy, D.P. (2021). Accessory Chromosome-Acquired Secondary Metabolism in Plant Pathogenic Fungi: The Evolution of Biotrophs Into Host-Specific Pathogens. *Frontiers in Microbiology*, 12, 664276.

WHO (World Health Organization). (2019). *Global antimicrobial resistance surveillance system (GLASS) report: Early implementation 2016-2017*. Geneva: World Health Organization.

Wylie, M.R., & Merrell, D.S. (2022). The antimicrobial potential of the neem tree *Azadirachta indica*. *Frontiers in Pharmacology*, 13, 891535.