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Assessment of the Antibacterial Activity of Secondary Metabolites of Endophytic Bacteria Isolated from *Senna occidentalis* against some Clinical Multidrug-Resistant Bacteria

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Abstract

Endophytic bacteria are endosymbiotic bacteria that colonize the inter- or intracellular regions of plants, providing them with protection through cross-communication and the release of metabolites via their biosynthetic gene clusters. The emergence of new diseases due to rapid multidrug resistance against existing drugs has created an indisputable need for biologically active secondary metabolites. This study aimed to assess the antibacterial activity of secondary metabolites from endophytic bacteria isolated from Senna occidentalis against multidrug-resistant clinical bacterial isolates. Endophytic bacteria were isolated from the leaves, stems, and roots of S. occidentalis (coffee senna) and identified based on their cultural, microscopic, and biochemical characteristics. The Agar well diffusion method was employed to evaluate the endophytes for antibacterial activity against clinical isolates of Staphylococcus aureus, Salmonella Typhi, and Pseudomonas aeruginosa. A total of twenty-three endophytes were isolated from Senna occidentalis, and molecular identification using the 16S rRNA gene sequencing method was carried out for the three most active endophytic bacteria, each from the leaf, stem, and root of the plant, respectively. Screening using agar well diffusion method yielded mean inhibitory zones ranging from 30.00±0.00 (mm) to 12.00±0.00 (mm) for the secondary metabolites. Molecular identification revealed 99-100% sequence identity to related type strains in two major classes: Gammaproteobacteria (Escherichia sp.) and Bacilli (Bacillus sp.). The findings of this study suggest that endophytic bacteria from S. occidentalis can serve as a promising source of novel antimicrobial compounds with broad therapeutic significance.

Key words: Antibacterial activity, endophytic bacteria, multidrug-resistant bacteria, Senna occidentalis

INTRODUCTION

Endophytes are an endosymbiotic group of microorganisms that includes bacteria and fungi capable of colonizing the inter- or intracellular regions of the plants. These microorganisms are found throughout or during some parts of the life cycle of plants without harming or causing any disease condition (Semenzato & Fani, 2024). The emergence of modern genomic and biotechnology tools has enabled the understanding of biological activities that occur during plant-endophyte interactions. Many metabolomic studies reported that endophytes can also serve as reservoirs of novel bioactive secondary metabolites, including alkaloids, tetralones, xanthones, terpenoids, flavonoids, phenolic compounds, benzopyrones, chinones, steroids, quinones, tannins, and many other subclasses (Narayanan & Glick, 2022). These groups of metabolites represent a promising resource for the isolation of novel natural products with significant biological activities, including anti-cancer, antioxidant, antiviral, antimicrobial, and immunosuppressive properties. Antimicrobial compounds may also be used as food preservatives, among other biotechnological applications. Furthermore, advances in chromatography and spectroscopy techniques have streamlined the process of rapidly identifying known and unknown secondary metabolites (Narayanan & Glick, 2022).

Senna occidentalis (L.) commonly known as coffee senna, stinking weed, negro-coffee, or septic weed is a medicinal plant belonging to the genus *Senna* and the family *Fabaceae* (*Leguminosae*). It is a wild flowering plant that is commonly found in many tropical countries. In Nigeria, it is commonly known as *rai dore, abo rere*, and akidi agbara in the Hausa, Yoruba, and Igbo languages, respectively (Isah *et al.*, 2018).

This plant is selected for this study due to it wide medicinal uses. Additionally, the medicinal properties of the plant have been utilized in traditional medicine as an excellent broad-spectrum antibacterial herb (Sadiq *et al.*, 2012). The leaves are used for the healing of wound infections (Taylor, 1996), while the seeds are dried, ground, and used as a coffee substitute; hence, the name coffee senna. The drink prepared from the plant has a reputation for treating malaria, typhoid fever, kidney disorders, dermatitis, and bladder troubles, as well as general aches and pains (Globinmed, 2014).

The emergence of new diseases due to the rapid resistance of existing drugs against harmful pathogenic microorganisms has created an indisputable need for biologically active secondary metabolites. The number of resistant bacterial isolates is increasing at an alarming rate (Ozma *et al.*, 2022), posing a significant threat to treatment options in modern medicine. Research has shown that by the year 2050, an estimated 390,000, 317,000, 392,000, 22,000, 4,730,000, and 4,150,000 people will die from the populations of Europe, North America, Latin America, Oceania, Asia, and Africa respectively, due to multidrug-resistant infections if new antibiotics are not found (O'Neil, 2024). Consequently, this ever-increasing development of resistance to antibiotics amongst the microbial pathogens lead the attention of scientists towards the immense need for the discovery of antibacterial compounds with resistance-breaking properties to effectively target the multidrug-resistant (MDR) pathogen bacteria that cause life-threatening infections (Bharadwaj *et al.*, 2022; Morens & Fauci, 2013). This intensive search for effective antimicrobial agents can be pursued by exploring new niches and habitats. Studies on plant-microbe interactions have encouraged scientists to foster valuable pharmaceutical resources, particularly through research on the plant-endophyte biome (Pal & Paul, 2020). In recent years, endophytes have been explored as a diverse group of microbes inhabiting unique and unusual environmental conditions, representing potential sources for new drugs in medicine and agriculture (Sarjono *et al.*, 2022).

Although many research studies have been conducted to explore plant endophytic fungi, the antibacterial activity of the endophytic bacteria from the medicinal plant S. occidentalis used in this research has not yet been sufficiently investigated. Hence, this research focused on isolating endophytic bacteria from the leaf, stem and root of *Senna occidentalis*, determining the antibacterial activity of secondary metabolites of endophytic bacteria isolates against the clinical isolates of *Staphylococcus aureus*, *Salmonella* Typhi and *Pseudomonas aeruginosa*, and identifying most active secondary metabolites producing endophytic bacteria each from the leaf, stem and root of the plant.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Healthy plant parts, i.e., leaf, stem, and root *Senna occidentalis* were collected from five individual plants of *S. occidentalis* within Kaduna metropolis using a sterile blade (Patle *et al.*, 2018) and transported to the Biology Laboratory of the Biological Science Department, Kaduna State University (KASU), Kaduna, in sterile polythene bags for authentication. The plants was identified by a curator as *Senna occidentalis*, given the voucher number: KASU/BSH/639, and kept for further analysis.

Collection of Clinical Isolates

The pure culture of the test pathogens *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa* was obtained from Barau Dikko Teaching Hospital, Kaduna, Kaduna State. The isolates were transferred to nutrient slants and maintained at a temperature of 4 °C until further analysis.

Surface Sterilization of Plant Material

The modified method of Patle *et al.* (2018) was used for surface sterilization of plant materials. The collected plant material was subjected to a surface sterilization procedure by brief washing in distilled water. Subsequently, surface sterilization was carried out sequentially by rinsing the plant material for 30 seconds in 70% ethanol, followed by 3.5% sodium hypochlorite (NaOCl) for 5 minutes, and then rinsing for 1-2 minutes in 70% ethanol. The plant material was then washed with three sets of sterile distilled water in respective conical flasks. The plant material was allowed to dry aseptically by placing it on a filter paper and keeping it in a fume hood.

Isolation of Endophytic Bacteria

After sterilization, the plant material was further cut aseptically using a sterile blade in order to expose its interior surfaces. For each plate, 6 to 8 segments were placed on a Tryptic Soy Agar (TSA) on petri dishes. Positive controls were prepared using the pour plate method, by pipetting 1 mL each of the last sterile water used to sterilize the plant part onto TSA, with sterile TSA used as the negative control. The dishes were sealed with parafilm to prevent contamination and then incubated at 37 °C for 48 hours (Pal & Paul, 2020).

Purification, Sectioning, and Preservation of Endophytic Bacteria

A sterilized wire loop was used to pick a colony from the master plate, which was then streaked onto nutrient agar to obtain pure cultures for further identification. The pure endophytic isolates were transferred to nutrient slant bottles and maintained at 4 °C until further analysis.

Cultural, Microscopic, and Biochemical Identification of Isolated Endophytic Bacteria

The appearance, size, and color of the colonies were observed from the petri plates containing the pure isolates. The clinical isolates were then differentiated into Gram-positive and Gram-negative bacteria using the Gram staining method (Cheesbrough, 2009). The Gram-negative bacteria appeared pinkish or reddish in color, while the Gram-positive bacteria appeared purple or blue-black in color. The endophytic bacteria obtained were subjected to further biochemical characterization using catalase, Simmons citrate, indole, methyl red, and oxidase tests, as described by Cheesbrough (2009).

Re-confirmation of pathogens

The obtained pathogens were reconfirmed using standard bacteriological methods and biochemical tests, as described by Cheesbrough (2009), by subculturing on Salmonella-Shigella agar for Salmonella Typhi, ceftriaxone agar for Pseudomonas aeruginosa, and mannitol salt agar for *Staphylococcus aureus*. The medium for each clinical isolate was prepared using the manufacturer's instruction as a guide. Microscopy and biochemical tests (catalase, methyl red, Simmons citrate, indole, oxidase, and urease tests) were also performed.

Antibacterial Activity of Secondary Metabolites of Endophytic Bacteria against the Clinical Pathogens

The Agar well diffusion method was used to assess the antibacterial activity of the crude secondary metabolites of the endophytes against pathogens. The endophytic bacteria were subcultured overnight and inoculated into a 500 mL conical flask containing 250 mL of nutrient broth, which was then fermented at 30 °C for 3 to 4 days at 160 rpm on a shaker incubator while monitoring the growth curve. The broth culture was transferred to a different centrifuge and centrifuged for 30 minutes at 5,000 rpm to separate the supernatant, which contains the secondary metabolites, from the microbial cells. Additionally, overnight cultures of the test organisms *S. aureus, S.* Typhi*, and P. aeruginosa were standardized to a 0.5 McFarland standard and then seeded with the help of a sterilized cotton swab on Mueller*-Hinton plates. Subsequently, using a sterile cork borer, wells of uniform diameter (6 mm) were created on the solidified agar. One hundred microliters of the supernatant was then dropped separately into each well using a micropipette and allowed to stand for 4-5 hours to facilitate the proper diffusion of the extract within the medium. Incubation (without inverting the plates) was carried out at 37ºC for 18-24 hours. The antibacterial drug ciprofloxacin served as the drug control, while seeded plates with no extract were used as the growth control. The zone of inhibition around the wells indicated bacterial growth inhibition by the supernatant, while the absence of zones indicated no inhibition. A centimeter rule was used to measure the zone of inhibition to the nearest millimeter. The experiment was performed in duplicates, and the most active extracts were recorded (Hayder & Mahmood, 2016).

Molecular Identification of Three Most Active Bacterial Endophytes

Extraction of Endophytic Bacterial DNA

The Zymo Research Fungal/Bacterial DNA Miniprep Kit was used for genomic DNA extraction from pure solid colonies, following the manufacturer's protocol (Zymo Research, USA). The extracted DNA was electrophoresed on 1% agarose gel. A Nanodrop Spectrophotometer was used to measure the concentration and purity of the DNA (Thermo Fisher Scientific, USA).

Amplification of Isolated Endophytic Bacterial DNA

Polymerase chain reaction (PCR) of the DNA of each bacterial isolate was conducted using modified 16S rRNA gene universal primers described by Makuwa and Serepa-Dlamini (2021). The amplification of the 16S rDNA was carried out in a reaction tube with a final volume of 20 µL containing 2 µL of each forward primer **5'-AGAGTTTGATCCTGGCTCAG-3'F** and reverse primer **5'-GGTTACCTTGTTACGACTT-3'R**, 5 µL of template DNA, 10 µL of PCR master mix with standard buffer (20 mM Tris-HCl, 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, and 25 units/mL One Taq® DNA polymerase) and the final volume was made up to with 20 µL nuclease-free water. Negative control (PCR mix without DNA) was included in all PCR experiments. The PCR reaction conditions were as follows: Initial denaturation at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 40 seconds and extension at 72°C for 1 minute, and a final extension of 72°C for 5 minutes using MyCycler™ -thermal Cycler (Bio-Rad, USA).

Gel Electrophoresis

ExoSAP-it™ (Thermo Fisher Scientific, USA) was used to purify the PCR products. The PCR product was then visualized in a 2% (wt/vol) agarose gel with ethidium bromide (0.5 µg/mL) staining using electrophoresis. It was sequenced at a commercial service provider, Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa (Makuwa & Serepa-Dlamini, 2021).

Sequence Identification

NCBI Basic Local Alignment Search Tool (BLAST) was used to analyse the 16S rRNA sequences against the rRNA sequence database to compare them with closely related bacterial species. Bacterial species with high similarity identity percentages and score values were selected for phylogenetic analysis. The nucleotide sequences of 16S rRNA genes were deposited to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and assigned accession numbers (Makuwa & Serepa-Dlamini, 2021).

Phylogenetic Analysis

MEGA 11 software was used to construct phylogenetic trees after alignment by multiple sequence comparison by log-expectation (MUSCLE) as described by Makuwa and Serepa-Dlamini (2021). DNA substitutions were performed according to the Tamura-Nei model. The maximum-likelihood method was used to cluster the phylogenetic relatedness of the endophytes with their close relatives. Bootstrap replications of 1000 were used to establish statistical confidence in the nodes of the phylogenetic trees and outgroups, which were considered to support differences among species (Makuwa & Serepa-Dlamini, 2021).

Statistical Analysis

The agar well diffusion method was conducted in duplicate. The values of the zone of inhibition were expressed as mean ± standard deviation (SD), and significant differences were analyzed using one-way analysis of variance (one-way ANOVA) computed with SPSS V 23.0 to compare the means of bioactive endophytic bacteria at $p \leq 0.05$.

RESULTS

Cultural and Biochemical Characteristics of Endophytic Bacteria Isolated from *Senna occidentalis*

A total of twenty three endophytic bacteria were isolated. Eight endophytic bacteria were isolated from the leaf, six from the stem and nine from the root of S. *occidentalis* as shown in Table 1. The colonial morphology observed in the isolates varied, ranging from green, milky, whitish, and yellow coloration to small and large colony sizes. Colonies were either mucoid, flat, or white, creamy, with smooth or rough margins. The Gram reaction showed that four of the endophytic isolates of S. *occidentalis* were Gram-negative, and the remaining were Gram-positive. The biochemical tests revealed that all the organisms are catalase- and citrate-positive, but negative for oxidase and methyl red. Meanwhile, four isolates were indole-positive.

Mean Zone of Inhibition of *Senna occidentalis* Bioactive Endophytic Bacteria Secondary Metabolites against the Bacterial Isolates

The mean zones of inhibition of bioactive secondary metabolites of endophytes of S. *occidentalis* are presented in Table 2. The mean zone of inhibition of the metabolites against *Staphylococcus aureus* ranged between 23.50±2.12 (mm) to 16.75±6.01 (mm). Similarly, the mean zone of inhibition of endophytes against *Salmonella* Typhi ranges from 21.50±4.95 (mm) to 14.50±0.71 (mm), and from 29.00±0.00 (mm) to 17.00±0.00 (mm) against *Pseudomonas aeruginosa*. There was a significant difference in zone of inhibition at $p \leq 0.05$. The growth curves of the most active endophytic bacteria, each isolated from the leaf, stem, and root of S. occidentalis, achieved during the fermentation process, are presented in Figures 1, 2, and 3.

Table 1: Cultural and Biochemical Characteristics of Endophytic Bacteria from *Senna occidentalis*.

Plant Part	Endophytic Isolates	Colony Morphology	Gram Reaction	Cell Morphology	CA	SC	IN	MR	OX
Leaf	RL1, RL6, RL8	Milkish, Small, Circular, Mucoid and Raised	-	Rod	+	+	-	-	-
	RL2	Greenish, Small, Circular, Mucoid and Raised	-	Rod	+	+	-	-	-
	RL3	Milky, Large, Regular, Creamy and Flat	+	Rod	+	+	-	-	-
	RL4	Milky, Small, Irregular, Creamy and Flat	+	Rod	+	+	-	-	-
	RL5, RL7	Yellow, Small, Irregular, Mucoid and Raised	+	Rod	+	+	+	-	-
Stem	RS1, RS3, RS4	Milky, Small, Regular, Mucoid and Raised.	+	Rod	+	+	-	-	-
	RS2	Yellow, Small, Regular, Mucoid and Raised	+	Rod	+	+	+	-	-
	RS5, RS6	Milky, Large, Regular, Creamy and Raised.	+	Rod	+	+	+	-	-
Root	RR1	Yellow, Small, Regular, Mucoid and Raised	+	Rod	+	+	+	-	-
	RR2	Yellow, Small, Regular, Mucoid and Raised	+	Rod	+	+	-	-	-
	RR3, RR4	Whitish Small, Irregular, Mucoid and Flat	+	Rod	+	+	-	-	-
	RR5, RR6, RR7, RR9	Milky, Large, Regular, Creamy and Flat	+	Rod	+	+	-	-	-
	RR8	Milky, Small, Regular, Mucoid and Flat	+	Rod	+	+	-	-	-

Key: + = Positive, - = Negative, CA = Catalase, SC = Simmons Citrate, IN = Indole, MR = Methyl Red, OX = Oxidase, RL = Endophytes from the Leaf of *S. occidentalis*, RS = Endophytes from the Stem of *S. occidentalis* and RR = Endophytes from Root of *S. occidentalis*

Table 2: Mean Zone of Inhibition of Secondary Metabolites of Endophytic Bacteria from *Senna occidentalis* against the Test Pathogens.

		Zone of Inhibition (mm)		
Plant Part	**Isolates**	***Staphylococcus aureus***	***Salmonella* Typhi**	***Pseudomonas aeruginosa***
Leaf	RL1	15.00 ± 1.41^{ak}	-	16.00 ± 0.00^{a}
	RL6	20.00 ± 5.66^{abeghi}	16.50 ± 7.07^{a}	-
	RL8	20.50 ± 4.95^{cj}	19.00 ± 4.24^{b}	-
Stem	RS2	17.00 ± 0.00^{bd}	16.00 ± 5.66^{c}	-
	RS5	12.00 ± 0.00^{cefk}	17.50 ± 0.71^{d}	-
	RS6	20.50 ± 6.36^{f}	18.50 ± 2.12^{e}	30.00 ± 0.00^{ab}
Root	RR5	16.50 ± 4.95^{bg}	-	24.00 ± 0.00^{ac}
	RR6	15.00 ± 0.00^{h}	17.50 ± 0.71^{f}	16.50 ± 2.12^{bg}
	RR7	16.00 ± 5.66^{i}	17.25 ± 1.06^{g}	17.00 ± 0.00^{bcg}
	RR9	12.00 ± 0.00^{fj}	27.50 ± 0.00^{abcdefg}	20.00 ± 0.00^{abe}
	Positive Control (Ciprofloxacin)	29.50 ± 0.71^{abcfhik}	28.00 ± 1.41^{abcefg}	29.50 ± 0.71^{aeg}
	Negative Control (Sterile Nutrient Broth)	-	-	-

Values are presented as Mean ± Standard deviation. Mean values with the same superscript in the same column have statistically significant differences using the Tukey post-hoc test at $p \leq 0.05$.
Key: RL = Endophytes from the Leaf of *S. occidentalis*, RS = Endophytes from the Stem of *S. occidentalis* and RR = Endophytes from Root of *S. occidentalis* and - = No Zone Observed

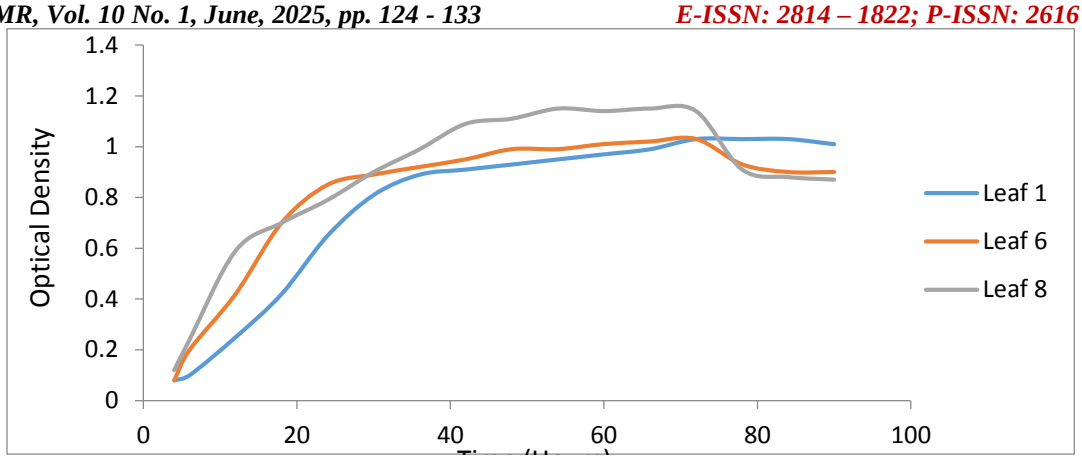

Figure 1: Growth curve of bioactive endophytic bacteria from leaf of *S. occidentalis*.

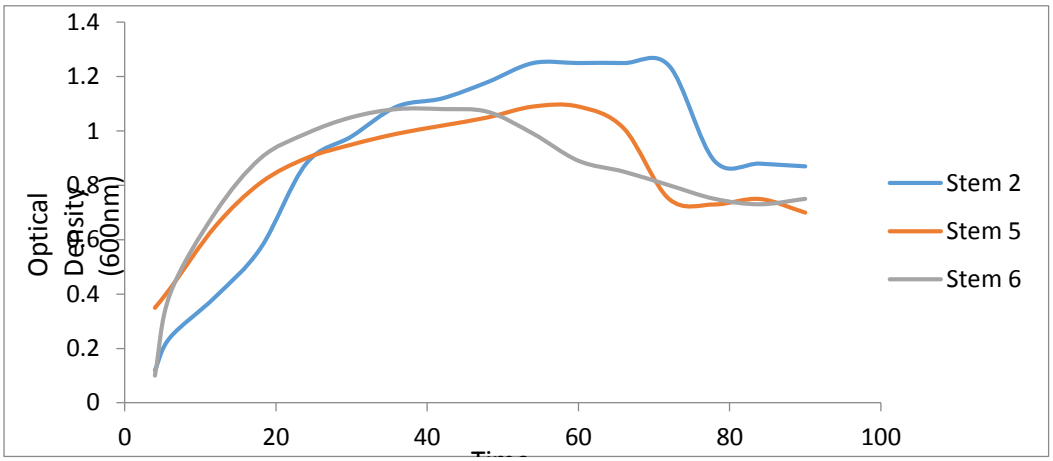

Figure 2: Growth curve of bioactive endophytic bacteria from stem of *S. occidentalis*.

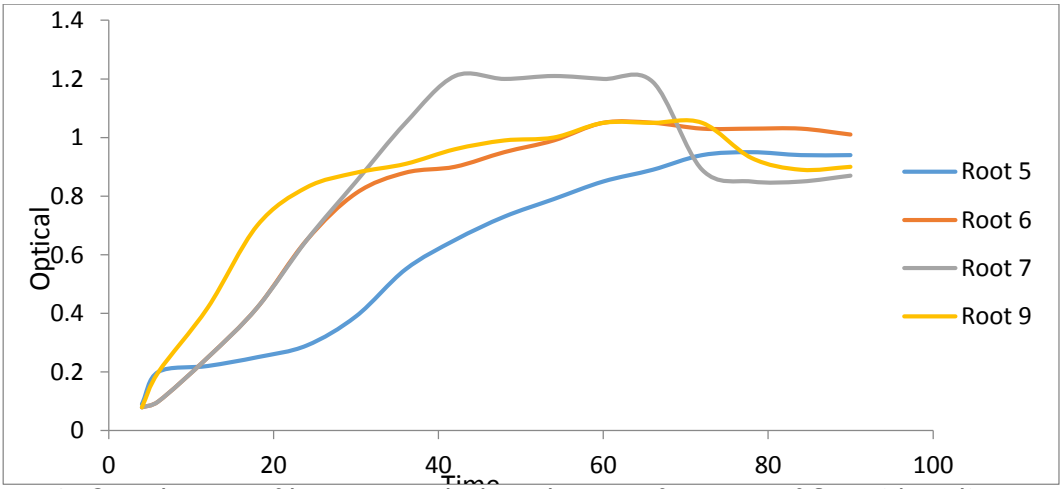

Figure 3: Growth curve of bioactive endophytic bacteria from root of *S. occidentalis*.

Molecular Identification of the Three Most Active Endophytic Bacteria

The 16S rRNA gene sequence analysis was used to identify the three most active endophytic bacteria, as presented in Table 3. Two of them, RS6 and RL8, belong to the class Gammaproteobacteria, and RR9 belongs to Bacilli, with sequence identities ranging from 99% to 100%. The phylogenetic relationship of the three identified endophytic organisms (RL8, RS6, and RR9) was inferred, as shown in Figure 5, with branching patterns remaining constant and a bootstrap value of 1000. Figure 4 shows the result of the gel electrophoresis of the three bioactive endophytic bacteria, displaying the bands of the three most endophytes using a universal 16S rRNA sequence as a primer.

Table 3: Taxonomic Identification of the Three Most Active Endophytes from *Senna occidentalis*.

LAB NO	Closely Related Type Strain	Accession Number	Percentage Identity (Accession Number)	Class
RL8	*Escherichia coli*	PQ724465	100 (LC4559552.1:13-840)	Gammaproteobacteria
RS6	*Escherichia fergusonii*	PQ724466	100 (MN817666.1:1-770)	Gammaproteobacteria
RR9	*Bacillus cereus*	PQ724467	99.25 (AJ577275.1:67-865)	Bacilli

Key: RL = Endophytes from the Leaf of *S. occidentalis*, RS = Endophytes from the Stem of *S. occidentalis* and RR = Endophytes from Root of *S. occidentalis*

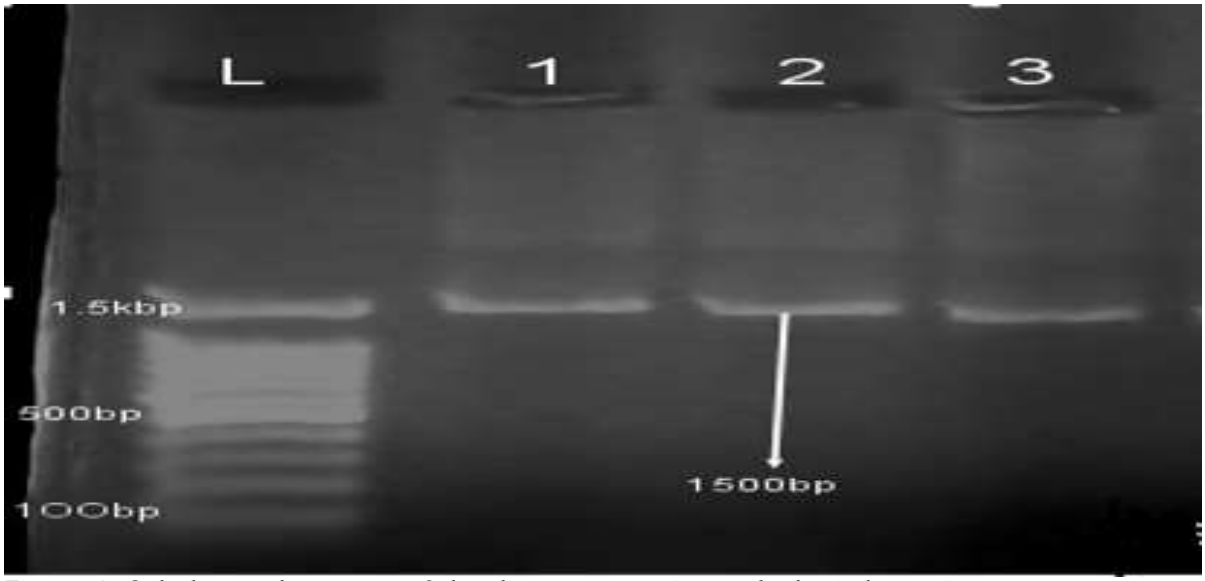

Figure 4: Gel electrophoregram of the three most active endophytic bacteria.
Key: L = Molecular Ladder, 1 = RL8, 2 = RS6, 3 = RR9, bp = basepairs and kbp = kilobasepairs

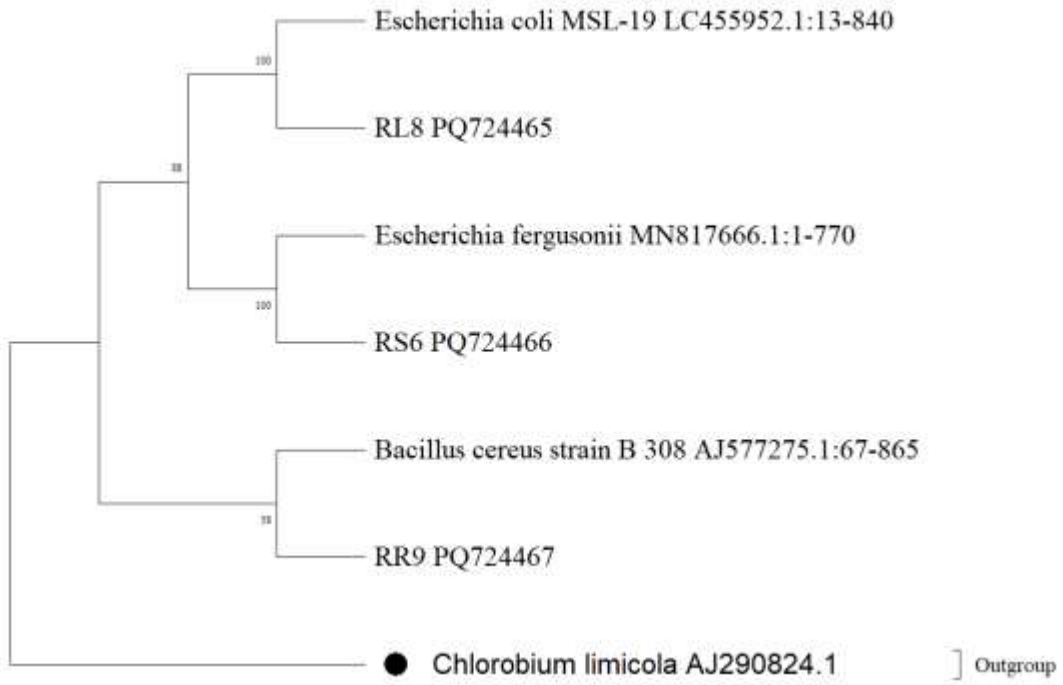

Figure 5: Phylogenetic distribution of the three most active endophytic bacteria isolated from the leaf, stem and root of *Senna occidentalis*. The strains used in this study were labeled as RL8, RS6, and RR9. Bootstrap values (1,000 replicates) are shown next to the branches with GenBank accession numbers beside the names of the bacteria.

DISCUSSION

Endophytic bacteria and host plants have a mutual relationship, which enables them to manage various environmental stresses by supporting each other. Hence, they are a unique treasure trove of underexplored natural products due to their biodiversity richness and biosynthetic versatility (Jasim *et al.*, 2016). The preference for their endophytic lifestyle may be a result of microbial production of diverse chemical scaffolds with broad phyto-interaction and protective properties. The identification of such promising endophytic isolates can have immense applications in various fields, ranging from industry and agriculture to medicine and biotechnology (Jasim *et al.*, 2016).

Senna occidentalis is selected in this current study as the source of endophytic bacteria due to its diverse medicinal applications. The remarkable ability of *S. occidentalis* to produce biomass within a short time, in addition to its medicinal properties, makes it reasonable to expect the endophytic microbiome of *S. occidentalis* to have great biosynthetic potential (Isah *et al.*, 2018). This informed the isolation of endophytic bacteria from *S. occidentalis* to unravel the chemical basis of its antibacterial activity.

The results of this study showed the presence of bacteria within the plant tissues, which could be a result of their role in providing protection to the plants against external forces, such as phytopathogens, and their ability to obtain nutrients from the plants. Eight endophytic bacteria were isolated from the leaf, six from the stem and nine from the root. This is in line with the work of Myo *et al.* (2020), who reported a total of thirty-one endophytic bacteria from four medicinal plants, indicating the presence of endophytic bacteria in plants. Maulani *et al.* (2019) stated that factors such as plant species, method of isolation, and other environmental factors, including weather and soil type, affect the population density of endophytic bacteria.

Most of the isolated endophytic bacteria exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. Based on the agar well diffusion method, all the endophytes were active against *S. aureus* and *S.* Typhi, with a few isolates also active against P. aeruginosa, exhibiting relatively higher zones of inhibition in the secondary screening. Many of these bacteria synthesize bioactive compounds, and some of these compounds have been proven useful for drug discovery (Kaari *et al.*, 2012). Plant growth, health, and beneficial effects are often mediated and characterized by the metabolic interactions of the bacterial endophytic microbiome (Brader *et al.*, 2014). This finding aligns with the work of Tavarideh et al. (2022), where endophytic bacteria isolated from the medicinal plant Scrophularia striata demonstrated antagonistic activity against test isolates of *Staphylococcus aureus*, *Bacillus cereus*, and *Escherichia coli*. In another study by Morare *et al.* (2018), bacterial endophytes belonging to genera *Staphylococcus*, *Bacillus*, and *Acinetobacter*, were isolated from the traditional medicinal plant *Crinum macowanii* Baker, which exhibited antibacterial activity against five bacterial pathogens, viz., *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *B. cereus*. The variation in the susceptibility of the test organisms to the endophytic organisms depends on the metabolic pathways and molecular structure of the test bacterial strains, as well as their antibacterial mechanism of action (Maulani *et al.*, 2019). The difference in the *in vitro* activity among the endophytes could be due to the production of either a broad-spectrum antimicrobial compound or several compounds with different activities. Additionally, differences in levels of antagonism may also depend on the concentration of the active compound. Higher zones of inhibition in the root of *B. pinnatum* and the stem of *S. occidentalis* could be due to the presence of more secondary metabolites in the bacteria found within those plant parts (Maulani *et al.*, 2019).

The selected endophytic bacteria with high activity in this study were identified as endophytic *Escherichia coli* (PQ724465), *Escherichia fergusonii* (PQ724466), and Bacillus cereus (PQ724467), isolated from the leaf stem and root of S. occidentalis, respectively. Chen *et al.* (2019) reported the isolation of endophytic *Bacillus* sp. from peanut root with inhibitory effects against *Aspergillus flavus* and *R. solanacearum*. Alaylar (2022) reported the isolation of endophytic *Bacillus* sp. from *Mentha longifolia, which has high potential for being utilized as a promising natural plant growth-promoter (PGP) for* sustainable crop production. Wu *et al.* (2018) reported an endophytic bacterium (*Pseudomonas aeruginosa* L10), from a reed (*Phragmites australis*) with biosurfactant-producing, hydrocarbon-degrading, and plant-growth-promoting properties, and Tharek *et al.* (2021) reported that endophytic *Escherichia coli* USML2 associated with rice seedlings has high beneficial plant-promoting potentials. Evidence showed that endophytic bacteria such as *Bacillus* sp. and *Pseudomonas* sp. could have antimicrobial effect on crops such as potato, tomato and cotton.).

The presence of these endophytic bacteria within the plant may be due to their ability to provide protection against phytopathogens and serve as growth promoters, biofertilizers, biostimulants, and bioprotectants in crop production (Alaylar, 2022).

While this study provides valuable insights into the effect of endophytic bacteria from this plant against multidrug-resistant clinical isolates, factors such as the limited number of bacterial species and the lack of a control plant species for comparison may limit the findings. Despite its limitations, this research makes a significant contribution to our understanding of the potential biotechnological applications of endophytic bacteria from *S. occidentalis* and underscores the need for further research in this area.

CONCLUSION

Endophytic bacteria were successfully isolated from *Senna occidentalis*. The endophytes exhibited significant antibacterial activities against the test isolates of *Staphylococcus aureus, Pseudomonas aeruginosa*, and Salmonella Typhi, with a significant difference in zone of inhibition ranging from 30.00±0.00 (mm) to 12.00±0.00 (mm) at $p \leq 0.05$. The molecular identification revealed that endophytic *Escherichia coli, Escherichia fergusonii, and Bacillus cereus were isolated from the leaf, stem, and root of Senna occidentalis*, respectively. Endophytes from the stem are more active than those from the root and the leaf.

Future studies should focus on utilizing plant control and other bacterial species to determine the versatility of the results. The study also recommends further research into the purification of these bioactive compounds on a larger scale, as well as genetic engineering of the bacterial strains to overproduce or modify the elaborated bioactive compounds.

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