ZIBELINE INTERNATIONAL™ PUBLISHING

Acta Scientifica Malaysia (ASM)

DOI: http://doi.org/10.26480/asm.02.2025.82.88

ISSN: 2521-5051 (Print)
ISSN: 2521-506X (Online)
CODEN: ASMCCQ

RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF METABOLITES OF BACTERIAL ENDOPHYTES FROM HIBISCUS ROSA-SINENSIS

Lucky Evbuomwan[a]*, Latifat Lukman[b], Emeka Patrick Chukwuka[c], Fortune Itoje Ebiala[d], Andrew E. Ojo[e] , Phebean Ozolua[a] , John Odidi Ekharemounu[f]

[a] Department of Microbiology, Edo State University, Uzairue, Auchi, Edo State, Nigeria
[b]Department of Science Lab Technology, Auchi Polytechnic
[c]Laboratory Department, Maternity and Children Hospital, Al Hasa 31928, Saudi Arabia
[d]Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria
[e]Nigerian Institute for Oil Palm Research (NIFOR)
[f]Department of pedratrics Edo State University Teaching Hospital
**Corresponding Author Email: evbuomwan.lucky@edouniversity.edu.ng*

ARTICLE DETAILS

Article History:

Received 10 June 2025
Revised 15 July 2025
Accepted 19 August 2025
Available online 23 September 2025

ABSTRACT

Bacterial endophytes, dwelling in the internal tissues of plants, have been reported to secrete many biologically active secondary metabolites which have found useful medical applications. This study aimed at evaluating antibacterial and antioxidant activity of endophytic bacteria from Hibiscus rosa-sinensis flower. Fresh flowers were collected and identified. Endophytic bacteria were isolated by imprint method after proper sterilization. Cultural and polymerase chain reaction were used to confirm the identity of the bacterial endophyte. Four clinical bacterial isolates including Salmonella enterica, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus, were collected from Cottage hospital and identified culturally. Isolated endophytic bacteria were screened for antimicrobial potential using agar well diffusion method. Crude metabolite was prepared by centrifugation of broth culture of the bacterial isolates. Antibacterial activity of the crude metabolites was carried out by agar well diffusion while its antioxidant activity was done using DPPH and FRAP assays. Different compounds and their functional group present in the crude metabolites were detected using GC-MS/FTIR analyses. Antibiotic resistance of the clinical isolates was carried out by disk diffusion. Isolated bacterial endophytes included Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis, Bacillus amyloliquefaciens and Bacillus siamensis. Screening test revealed Bacillus cereus to have the highest antibacterial potential of 50%. At 100% concentration of Bacillus cereus metabolite, zones of inhibition ranged from 6.67±0.80mm in Staphylococcus aureus to 17.33±1.20mm in Salmonella enterica. Minimum inhibitory concentration of the metabolite ranged from 25% in Salmonella enterica to 100% in Klebsiella pneumoniae while minimum bactericidal concentration was only observed against Salmonella enterica at 100%. The four clinical isolates were multidrug resistance with resistance index ranging from 0.5 to 1. Percentage reduction of DPPH increased from 9.2% at 10% concentration of crude metabolite to 53% at 100% concentration, although lower than that of the ascorbic acid. However, the crude metabolites reduced FRAP better than ascorbic acid. Compounds present in the crude metabolite of Bacillus cereus included butylated hydroxytoluene, undec-10-ynoic acid, squalene and oleic acid. This study has shown that endophytic Bacillus cereus from Hibiscus rosa-sinensis flower is a rich source of biologically active metabolites.

KEYWORDS

Endophyte, Hibiscus, Antibacterial, Resistance, Bacillus

1. INTRODUCTION

Nature has been a source of medicine for thousands of years and a striking number of modern drugs have been isolated from a natural source, many bases on their use in traditional medicine or phytomedicines. Over the years, researchers have advocated traditional medicine as a safe medicine for ailments of microbial and non-microbial origins (Etminani and Harighi, 2018; Feng et al., 2022). Over 50% of all clinical modern drugs are of natural product origin and natural products play a vital role in drug development programs in the pharmaceutical industry (Atanasov et al., 2021).

Microbial pathogens have repeatedly developed resistance to most antibiotics, making microbial infections difficult to treat. One way of solving this problem is to source for new antimicrobials (Peterson and Kaur, 2018). Endophytic bacteria are considered a subclass of rhizospheric bacteria, commonly called plant growth-promoting rhizobacteria (PGPR) (Etminani and Harighi, 2018). These are a specialized group of rhizobacteria that have acquired the ability to invade their plant host. They share all the important traits consistent with the host plant growth promotion found in rhizobacteria, and they seem to be ubiquitous in most plant species and have been isolated from roots, leaves, and stems, and a few from flowers, fruits, and seeds (Santoyo et al., 2016). The plant Hibiscus rosa-sinensis (H. rosa-sinensis) belongs to the family Malvaceae. Traditionally, the flower can be used as an anti-asthmatic agent (Bhakta and Das, 2017). Many chemical constituents such as cyaniding, quercetin, hentriacontane, calcium oxalate, thiamine, riboflavin, niacin, and ascorbic acids have been isolated from this plant (Gomare and Mishra,

Quick Response Code	Access this article online	
	Website: www.actascientificamalaysia.com	**DOI:** 10.26480/asm.02.2025.8288

Cite The Article: Lucky Evbuomwan, Latifat Lukman, Emeka Patrick Chukwuka, Fortune Itoje Ebiala, Andrew E. Ojo , Phebean Ozolua, John Odidi Ekharemounu (2025). In Vitro Antibacterial And Antioxidant Activities Of Metabolites Of Bacterial Endophytes From Hibiscus Rosa-Sinensis. *Acta Scientifica Malaysia, 9(2): 82-88*.

2018). Bacterial endophytes in herbal plants are important sources of secondary metabolites of divers biological activities such as antimicrobial and antioxidant activities (Nongkhlaw and Joshi, 2015). There is increasing research in endophytic bacteria because of their ability to produce secondary metabolites of various biological impacts (Pandey et al., 2017). Secondary metabolites from bacterial endophytes of plants have wide usefulness in various fields including agriculture, pharmaceutical and biotechnological industries. For example, campothecin, a secondary metabolite from endophytic bacteria isolated from the climbing shrub Miquelia dentate Bedd. is a potent anticancer agent (Khan et al., 2020). The analysis reported Pseudomonas entomophila and Bacillus tequilensis endophytes of Aloe barbadensis to have broad spectrum of antimicrobial activities (Akinsanya et al., 2015). In another study, pumilacidin, a secondary metabolite from endophytic Bacillus pumilus obtained from Manikhot esculenta Crantz, was found to have antifungal activity against some fungal pathogens of plant, including Sclerotium rolfsii and Rhizoctonia solani (Indrawati et al., 2018).

Studies on bacterial endophytes from Hibiscus rosa sinensis flower are scanty and as such, the current study was aimed at investigating the antibacterial and antioxidant potentials of crude metabolites of bacterial endophytes isolated from the flower of Hibiscus rosa sinensis.

2. Materials And Methods

2.1 Sample Collection

Hibiscus rosa-sinensis flower sample was collected from the Botanic Garden of Federal Polytechnic Auchi, School of Applied Science and Technology, Department of Science Laboratory Technology (SLT). Sample was tagged and placed in separate polythene bags and processed within 24 hours of collection.

2.2 Sample Pretreatment and Isolation of Endophytic Bacteria

For the pre-treatment of samples and isolation of endophytic bacteria, H. rosa-sinensis flower was excised and subjected to a surface sterilization procedure described by (Indrawati et al., 2018). Briefly, the plant material was washed in running tap water, then washed with 70% ethanol, followed by washing with 2% sodium hypochlorite and finally, it was washed with sterile distil water five times to remove sterilizing agent. The efficiency of surface sterilization was checked by the imprint method. Isolation of endophytic bacteria was carried out by imprint method using tryptic soy agar medium. The plates were incubated for 24 hr at 37ºC. Developed colonies were selected based on specific cultural features.

2.3 Identification of Endophytic Bacteria

Preliminary identification was carried out based on the cultural, morphological and biochemical activities of the respective bacteria isolates. Bacterial identification was confirmed using polymerase chain reaction method. Bacteria DNA were extracted and amplified using PCR. PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each 27F 5' AGA GTT TGA TCM TGG CTC AG3' and 1525R, 5'AAGGAGGTGATCCAGCC3' primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a Pcr profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 50°C for 60s and 72°C for 1 minute 30 seconds ; and a final termination at 72°C for 10 mins. And chill at 4oC. The amplified DNA was separated using agarose gel electrophoresis. Each band was sequenced to get the bacteria DNA sequences. The sequences were then sent for Blasting in order to identify the specific bacterium. (Tian and LiY, 2017).

2.4 Screening Antibacterial Activity of Endophytic Bacterial Metabolites by Agar Well Diffusion

The anti-bacterial activity was determined using agar well diffusion method. Isolated endophytic bacterial strains were grown in 100ml nutrient broth (peptone: 0.5g; yeast extract: 0.5g and NaCl: 0.5g in 100ml distilled water) and was incubated for five days in rotary shaker at 120rpm. The culture media was centrifuged at 10,000rpm for 15 minutes. The supernatant was monitored for antibacterial activity against human pathogenic bacteria inoculated on MHA (Muella Hinton agar) by agar well dilution. Four clinical bacteria including Staphylococcus aureus, Salmonella entterica, Klebsiella pneumoniae, Escherichia coli were used for the antibacterial studies. They were inoculated in 10ml nutrient broth and incubated for 18hrs at 37°C. A total of 20µl of cellfree supernatant was applied on 6mm diameter well on Muella Hinton agar plate. The diameter of the inhibition clear zone was measured after 24hrs of incubation at 37°C (Nongkhlaw and Joshi, 2015). The Bacterium with the highest Antibacterial activity was selected for further analyses.

2.5 Antibacterial Activity

Antimicrobial activity of the bacterial endophyte with the highest activity from the screening experiment was examined according to Vijayalakshmi et al. (2016). 20µl of crude metabolites of bacterial endophytes was pipetted into 6mm wells punched in MH agar plates already inoculated with fresh culture of test bacterial isolates. The plates were inoculated at 37oC for 24 hrs. clear zones of inhibition were measured and recorded. The experiment was performed in triplicate Antioxidant Procedure

2.6 Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1–diphenyl–2–picrylhydrazyl (DPPH) radical was determined by a slightly modified method of (Indrawati et al., 2018). The assay was based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour from deep violet to light yellow was measured at 517nm using a spectrophotometer. One (1) ml of 0.1mM DPPH solution in methanol was added to 1ml of various concentrations (10 - 100 µg/ml) of the extracts. The reaction tubes were shaken and incubated for 30 min at room temperature in the dark; absorbance read at 517 nm. All tests were performed in triplicate. Ascorbic acid was used as standard control, with similar concentrations as the test samples prepared. A blank containing 1ml of 0.1 mM DPPH and 1mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula: DPPH radical scavenging activity (%) = [(A0−A1)/ (A0)] × 100, where A0 was the absorbance of DPPH radical + methanol; A1 was the absorbance of DPPH radical + sample extract or standard.

The 50% inhibitory concentration value (IC50) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

2.7 Ferric Ion Reducing Antioxidant Power (Frap) Assay

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay which depended on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593nm. 1.5ml of freshly prepared FRAP solution (25ml of 300mM acetate buffer ppH 3.6, 2.5ml of 10mmM 2,4,6-tripyridyltriazine (TPTZ) in 40mmM HCl, and 2.5ml of 20mM ferric chloride (FeCl3.6H2O) solution) was mixed with 1ml of the extracts at concentration of 1.0 mg\ml. The reaction mixtures were incubated at 37°C for 30min and increase in absorbance 593nm measured. FeSO4was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)\g of the extract) for the extracts were then extrapolated from the standard curve.

2.8 Determination of Bioactive Metabolite in Endophytic Bacteria

The crude metabolites were analyzed using gas chromatography–mass spectrometry in order to identify the bioactive compounds while FTIR analysis was used to detect their functional groups (Leo et al., 2014).

2.9 Data Analysis

The results were analyzed using SPSS version 24. All experiments were performed in triplicates. Descriptive statistics was used to present data in mean±standard error. Comparisms between two variables was done using independent T- test while comparison among three or more variables was done using one way analysis of variance. P- value was set at 0.05.

3. Results

3.1 Results of Endophytic Bacteria Isolates from Hibiscus rosa-sinensis Flower

Result of the identity of endophytic bacteria isolated are shown in table 1a. The isolated endophytic bacteria were identified based on their cultural, morphological and biochemical features and included three genera with seven different species. The identified genera were Proteus, Bacillus and Enterobacter. Molecular characterization revealed seven different species including Proteus mirabilis, Bacillus thuringiensis, Bacillus cereus, Enterobacter cloacae, Bacillus subtilis, Bacillus amyloliquefaciens and Bacillus siamensis. All isolates had percentage identity of above 95% (Table 1c). Phylogenetic tree showing the evolutionary relationship of the isolates is shown in figure 1.

The clinical isolates were screened following cultural, morphological and

biochemical characteristics. Their identity was confirmed to be Salmonella enterica, Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus as initially classified by the hospital where they were obtained (Table 1b).

3.2 Screening of Endophytic Bacterial Metabolite for Antibacterial Potential

Result of the screening test to determine which endophytic bacteria had antibacterial potential is presented in Table 2. Proteus mirabilis was only active against Staphylococcus aureus with inhibition zone of 6.33±0.66mm. Bacillus amyloliquefaciens and Bacillus siamensis metabolites were only active against Klebsiella pneumoniae with zones of inhibition of 5.67±0.33mm and 7.66±1.44mm respectively. Bacillus subtilis metabolite was active only against Escherichia coli with zone of inhibition of 7.33±1.44mm. Endophytic bacterium with the highest antibacterial potential was Bacillus cereus which was active against two clinical isolates including Salmonella sp and Escherichia coli with inhibition zones of 15.00±2.88mm and 7.33±1.44mm. Bacillus cereus had percentage antibacterial potential of 50% compared to other bacterial endophytes which had 25% each.

3.3 Antibacterial Activity of Bacillus cereus metabolites Against Clinical Isolates

Finding of antibacterial activity of crude metabolites of Bacillus cereus is shown in Table 3. At 100% concentration of Bacillus cereus metabolite, zones of inhibition ranged from 6.67±0.80mm in Staphylococcus aureus to 17.33±1.20mm in Salmonella enterica, at 75% concentration, zone of inhibition ranged from 4.70±0.88mm in Staphylococcus aureus to 8.33±0.88mm in Escherichia coli. At 50%, the metabolite was only active against Salmonella enterica and Escherichia coli with zones of inhibition 5.30±0.66mm and 4.66±0.88mm respectively. Bacillus cereus metabolite was solely active against Salmonella enterica at 25% with inhibition zone of 3.12±0.88mm. Minimum inhibitory concentration of the metabolite ranged from 25% in Salmonella enterica to 100% in Klebsiella pneumoniae while minimum bactericidal concentration was only observed against Salmonella enterica at 100%. The most sensitive or least resistant clinical bacteria isolate to Bacillus cereus metabolite was Salmonella enterica while the least sensitive or the most resistant was Klebsiella pneumoniae as only 100% concentration produced minimal inhibition zone of 4.66±0.88mm against the pathogen.

3.4 Antibiotic Resistance Pattern of Clinical Bacterial Isolates

Antibiotics result of clinical bacterial isolates before curing is shown in Table 4. High antibiotic resistance was observed in all clinical isolates as all were resistance to at least five commercially available antibiotics including gentamicin, streptomycin, septrin, pefloxacin and ciprofloxacin while sparfloxacin, chloramphenicol and zinnacef were able to inhibit some of the clinical isolates. The most resistant clinical bacterial isolates were Klebsiella pneumoniae and Escherichia coli with multidrug resistance index of 1.0 while the least resistant was Staphylococcus aureus with multidrug resistance index of 0.5.

3.5 Antioxidant Properties of Crude Metabolites of Bacillus cereus

Findings of the antioxidant potential of crude metabolite of Bacillus cereus using Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity as presented in Figure 2 revealed increasing antioxidant potential as the concentration of metabolites increased. Percentage inhibition of DPPH increased from 9.2% at 10% concentration of crude metabolite to 53% at 100% concentration of crude metabolite. Antioxidant activity of the metabolite followed the same pattern as the standard Vit C antioxidant which produced increasing percentage of 79.14% at 10% Vit C concentration to 95.07% percentage inhibition at 100% concentration of Vit C. the standard antioxidant (Vit C) proved to be a better antioxidant as a result of its ability to greatly reduce DPPH compared to crude metabolite of Bacillus cereus.

Antioxidant potential of crude metabolites of Bacillus cereus using Ferric Ion Reducing Antioxidant Power (Frap) Assay is presented in Table 5. Findings showed that crude metabolite of Bacillus cereus had more potential in reducing FRAP compared to the standard Vit C antioxidant used. Percentage reduction of FRAP by crude metabolite increase from 0.094±0.002 at 10% concentration to 0.180±0.001 at 100% concentration while for Vit C, percentage reduction of FRAP was 0.010±0.00 and 0.042±0.001 at 10% and 100% concentration of Vit C respectively.

3.6 GC-MS/ FTIR Analysis Result

Gas chromatographic analysis of the crude metabolite of Bacillus cereus as shown in Figure 3, reveled different peaks at different retention time in the chromatogram. Twelve different compounds were found to be present in the crude metabolite as presented in Table 4a. They included Butylated Hydroxytoluene, Undec-10-ynoic acid, undecyl ester, Butyl 9-tetradecenoate, Cyclohexane, 1,2,3-trimethyl-, Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-, Hexadecanoic acid, ethyl ester, Oleic Acid, alpha.-Aminooxy-propionic acid, ethyl ester, Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-, Cyclopentadecanone, 2-hydroxy-, 9,12-Octadecadienoic acid, methyl ester, (E,E)- and Squalene. These twelve different compounds were found to belong to either of three functional group as revealed by FTIR analysis in figure 4.4. The functional groups included alcohol, alkynes and amines (Table 6b)

Table 1a: Cultural, morphological and biochemical characteristics of endophytic bacterial isolates

Table 1a: Cultural, morphological and biochemical characteristics of endophytic bacterial isolates

PARAMETERS	1	2	3	4	5	6	7
CULTURAL							
Shape	Circular	Irregular	Irregular	Circular	Irregular	Irregular	Irregular
Colour	Cream	Cream	Cream	Cream	Cream	Cream	Cream
Size	Medium	Large	Large	Large	Large	Large	Large
Elevation	Flat	Flat	Flat	Raised	Flat	Flat	Flat
Transparency	Opaque	Opaque	Opaque	Translucent	Opaque	Opaque	Opaque
MORPHOLOGICAL							
Gram stain	-	+	+	-	+	+	+
Cell type	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Singly	Single	Single	Single	Single	Single	Single
BIOCHEMICAL							
Indole	-	-	-	-	-	-	-
Citrate	-	-	-	+	-	+	+
Catalase	+	-	-	+	-	-	-
Coagulase	+	-	-	-	-	-	-
Oxidase	-	+	-	-	+	+	+
H_2S	-	-	-	-	-	-	-
OXIDATIVE FERMENTATION TEST							
Glucose	AG	+	+	AG	+	+	+
Sucrose	A	+	+	A	+	+	+

Lactose	A	-	-	A	-	-	-
Isolates	*Proteus* sp.	*Bacillus* sp.	*Bacillus cereus*	*Enterobacter* sp.	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.

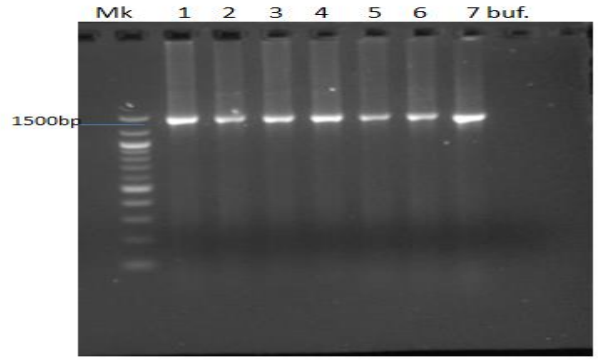

Plate 1: PCR product of 16SrRNA on 1% Agarose Gel.

Lane M = molecular size marker, 1= Proteus mirabilis , 2= Bacillus thuringiensis, 3= Bacillus cereus, 4= Enterobacter cloacae, 5=Bacillus subtilis, 6= Bacillus amyloliquefaciens, 7=Bacillus siamensis

Table 1c: Molecular identification of endophytic bacteria from Hibiscus flower

Sample ID	Scientific Name	Per. Ident	Accession
L.BE(1)	*Proteus mirabilis*	99.90%	OR467446
L.B.G(2)	*Bacillus thuringiensis*	100.00%	OR467447
L.B.G(3)	*Bacillus cereus*	99.90%	OR467448
L.B.G(4)	*Enterobacter cloacae*	100.00%	OR467449
L.B.G(5)	*Bacillus subtilis*	99.70%	OR467450
L.B.G(6)	*Bacillus amyloliquefaciens*	100.00%	OR467451
L.B.G(7)	*Bacillus siamensis*	99.70%	OR467452

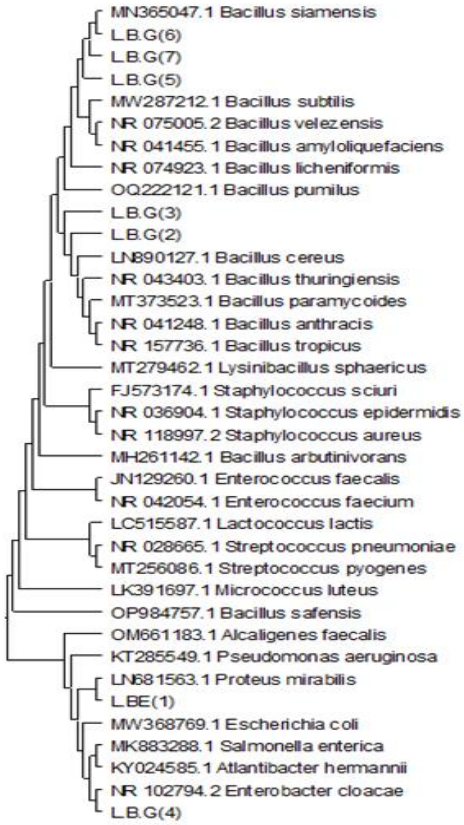

Figure 1: Phylogenetic tree of bacterial isolates

Table 2: Screening for antibacterial activity of endophytic bacterial metabolite at 100% concentration against clinical isolates

Clinical isolates			Conc. (%)			MIC (%)	MBC (%)
	100	75	50	25	12.5		
Salmonella enteric	17.33 ±1.20	7.53± 1.45	5.30± 0.66	3.12± 0.88	0.00± 0.00	25	100
Klebsiella pneumonia	4.66± 0.88	0.00± 0.00	0.00± 0.00	0.00± 0.00	0.00± 0.00	100	-
Escherichia coli	12.00 ±2.00	8.33± 0.88	4.66± 0.88	0.00± 0.00	0.00± 0.00	50	-
Staphylococcus aureus	6.67± 0.80	4.70± 0.88	0.00± 0.00	0.00± 0.00	0.00± 0.00	75	-

Table 3: Antibacterial activity (zone of inhibition, mm) Bacillus cereus metabolite against clinical isolates

Clinical isolates			Conc. (%)			MIC (%)	MBC (%)
	100	75	50	25	12.5		
Salmonella enteric	17.33 ±1.20	7.53± 1.45	5.30± 0.66	3.12±0 .88	0.00± 0.00	25	100
Klebsiella pneumonia	4.66± 0.88	0.00± 0.00	0.00± 0.00	0.00±0 .00	0.00± 0.00	100	-
Escherichia coli	12.00 ±2.00	8.33± 0.88	4.66± 0.88	0.00±0 .00	0.00± 0.00	50	-
Staphylococcus aureus	6.67± 0.80	4.70± 0.88	0.00± 0.00	0.00±0 .00	0.00± 0.00	75	-

Table 4: Antibiotic susceptibility pattern of bacterial isolates

G-ve	CPX	AM	AU	CN	PEF	OFX	S	SXT	CH	SP	MRI
Salmonella enteric	R	R	R	R	R	R	S	R	S	S	0.7
Klebsiella pneumoniae	R	R	R	R	R	R	R	R	R	R	1.0
Escherichia coli	R	R	R	R	R	R	R	R	R	R	1.0
G+ve	SXT	E	PEF	CN	APX	Z	AM	R	CPX	S	
Staphylococcus aureus	S	S	S	R	R	S	R	R	S	R	0.5

Note:

SXT = septrin, SP = sparfloxacin, CPX = ciprofloxacin, AM = amoxicillin, AU = augmentin, 1PEF = pefloxacin, OFX = ofloxacin, St = streptomycin, CN = gentamicin, Ro = rocephin, Z = zinnacef, E = erythromycin, APX = ampicillin

I= Intermediate R= Resistant S= Sensitive, MRI= multidrug resistance index

Resistance (R) = ≤10 mm. Intermediate (I) = 11-17 mm. Sensitivity (S) ≥ 18mm

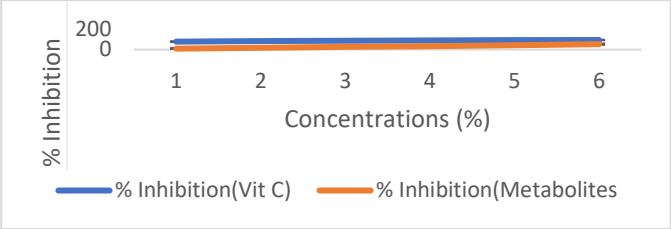

Figure 2: in vitro antioxidant (DPPH) potential of metabolites of Bacillus subtilis

Key: 1 =10%, 2=20%, 3= 40%, 4= 60%, 5=80%, 6=100%

Table 5: Antioxidant of B. cereus metabolite using FRAP

Conc. (%)	Vit C	Metabolite	p-value
10	0.010±0.00	0.094±0.002	0.477
20	0.013±0.001	0.120±0.002	0.163
40	0.019±0.00	0.140±0.003	0.097
60	0.025±0.001	0.150±0.001	0.089
80	0.031±0.001	0.170±0.001	1.000
100	0.042±0.001	0.180±0.001	0.442

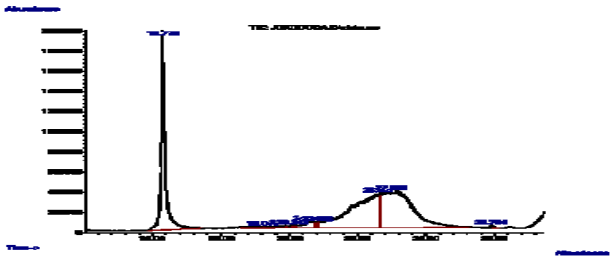

Figure 3: Gas chromatogram forcrude metabolites of Bacillus cereus.

Table 6a: Metabolites of Endophytic Bacillus subtilis

PK	RT	Library/ID
1	10.7776	Butylated Hydroxytoluene
2	18.0413	Undec-10-ynoic acid, undecyl ester
3	19.7223	Butyl 9-tetradecenoate
4	20.1175	Cyclohexane, 1,2,3-trimethyl-
5	20.2427	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-
6	21.4806	Hexadecanoic acid, ethyl ester
7	21.8529	Oleic Acid
8	21.9456	.alpha.-Aminooxy-propionic acid, ethyl ester
9	22.0382	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-
10	26.6209	Cyclopentadecanone, 2-hydroxy-
11	27.5053	9,12-Octadecadienoic acid, methyl ester, (E,E)-
12	34.7936	Squalene

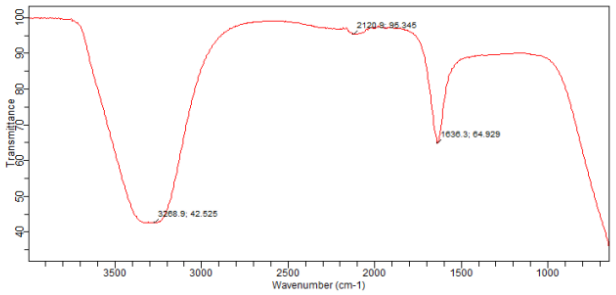

Figure 4: Fourier Transform Infrared Spectrum analysis for Bacillus subtilis metabolite

Table 6b: Different functional groups of metabolites of Bacillus subtilis

S/N	Wavelength	Functional group	Compound class
1	3268.9	O-H	Alcohol
2	2120.9	C≡C	Alkyne
3	1636.3	N-H	Amines

4. DISCUSSION

Endophytes living within a healthy plant are a good source of antimicrobial agents, enzymes and secondary metabolites (Narkhede, 2021). The presence of seven different species of endophytic bacteria in Hibiscus rosa-sinensis reveals the plant to be a host for many nutrients that support the growth of microorganism.

Bacillus species have been reported as endophytes from various plants, with varieties of secondary metabolites that have various applications in the medical, food and pharmaceutical industries (Feng et al., 2022). In a study by Aljuraifani et al. (2019), endophytic bacteria isolated were in the genera of Bacillus and Proteus, with Bacillus having over 80% of the total bacteria species. Their findings agree with this current study as many of the identified bacteria species were in the genus Bacillus

Many of the endophytic bacteria were not active against tested clinical isolates because the isolates may have possessed many drug resistance factors. Microorganisms especially bacteria pathogens have developed various mechanisms of resisting antimicrobial agents including the use of efflux pump or actively inactivating the agent. In this study, crude metabolite of Bacillus cereus was found to be the most active against clinical bacterial isolates of all the seven endophytic bacteria isolated. This may be due to the fact that many Bacillus species are notable antibiotic producers and the sporing nature of Bacillus genus enable them to also withstand plants' phytochemicals within the plant as well as functioning as biocontrol agent against phytopathogens for the plant.

Crude metabolite of Bacillus cereus was found to be active against multidrug resistant bacterial pathogens. This could be due to the secretion of bioactive secondary metabolites by Bacillus cereus. Salmonella enterica was found to be highly sensitive to the crude metabolite compared to other clinical bacterial pathogens. The reason for this may be that the metabolite active component easily penetrated the cell membrane of the pathogen and caused intracellular damage. Also, it was noted in this study that Klebsiella pneumoniae was the most resistant to the crude metabolite. This may be due to the ability of the pathogen to produce capsule, a layer external to the cell wall, which enable pathogenic bacteria to become resistant to many antimicrobial agents. Capsules usually prevent the penetration of antimicrobial agents into microbial cells, thereby rendering them inactive. It was also observed that at higher concentrations of the crude metabolites, there were higher zones of inhibition. This could be explained by the fact that higher concentrations contained more of the specific bioactive metabolites compared to lower.

Findings in this study agree with earlier study who reported high antibacterial activity of metabolites of endophytic Bacillus against clinical pathogens by (Aljuraifani et al., 2019). As pathogenic organisms are developing resistance to the majority of antibiotics available, it is important to find new antibiotics to tackle this problem. Findings also agree with early work carried out who reported that endophytic bacteria exhibit antimicrobial activity against human pathogenic microbes by (Seo et al., 2010).

Findings of antibiotics resistance of pathogen using commercially available antibiotics revealed high level of bacterial resistance of bacterial

isolates to the antibiotics. This high resistance suggests that they were highly pathogenic. This also implies that there is need for alternative antimicrobial agents that can help resist pathogens' resistance to commonly used antibiotics. Example of this alternative search is secondary metabolites produced by endophytic bacteria. This analysis reported that most pathogenic bacteria have developed antibiotics resistance using different mechanisms, including effluxing, target degradation of the antibiotics, target modification of the antibiotics and even antibiotic target by pass (Peterson and Kaur, 2018)

Crude metabolites of Bacillus cereus showed moderate to high level of antioxidant activity. With DPPH, ascorbic acid proved to be a better electron donor compared to crude metabolites. The antioxidant capacity of both standard and crude metabolite increased at increased concentrations suggesting that at higher concentrations, there would be higher antioxidant potential of the crude metabolite. With FRAP however, the crude metabolite served as a better electron donor to reduce the already oxidized agent. This is of great medical advantage as many diseases and weakness in the body arise from oxidative stress, an imbalance between prooxidants and antioxidant. With more antioxidants in the system, many free and toxic radicals would be easily reduced into innocuous state. This implies that the crude metabolites can be well purified and used to formulate drugs that will function as antioxidant agents. It was demonstrated that the crude metabolite showed a mechanism of action as electron donors and they terminated the oxidation chain reaction by reducing the oxidized intermediates into more stable forms. The observed antioxidant potential of the crude metabolites of Bacillus cereus is ascribed to the bioactive compounds secreted by the organism. This finding is in line with earlier study who reported high antioxidant activity of crude metabolites of Bacillus cereus isolated from marine soil (Ts et al., 2024). In another study, reported that Bacillus cereus isolated from seaweed has high in antioxidant potency (Indraningrat et al., 2023).

GC-MS/FTIR results revealed that twelve different bioactive compounds were present in the crude metabolite of Bacillus cereus. These compounds were responsible for the various activities of the crude metabolites observed in this study. these compounds were found to be in one of three functional groups of alcohol, alkynes and amines. Alcohol has been reported to possess strong antimicrobial activity (Koushik, 2013). The research also reported that alcohol can alter biofilm formation, which is a virulence factor for most pathogens (Koushik, 2013). The researcher reported that many essential oils are of alcohol group and possess high level of antibacterial and antioxidant potentials (Zengin et al., 2014). The study reported high level of antimicrobial and antioxidant activities of amines and amine derived compounds (Fayeulle et al., 2021). Therefore, the presence of amines derived compounds in the crude metabolite may have aided the antibacterial and antioxidant activities observed. An important metabolite secreted by Bacillus cereus in this study was squalene. Studies have revealed that squalene is an antioxidant compound that prevents development of free-radical induced oxidative injury particularly in the skin. In addition, it is known to be an important step in cholesterol synthesis. It shows effective physiological and biochemical activity in cancer treatment. Squalene has been reported that it is able to suppress development of tumor cells, prevents chemical-originated cancers, reduces tumor growth rate, stimulates reticuloendothelial system, and increases white blood cell count (Guneş, 2013). Another medically important metabolite revealed was oleic acid. The study Oleic acid, the principal component of olive oil, has properties that help to prevent cancer and Alzheimer's disease and to lower cholesterol (Santa-Maria, 2023). This analysis reported that butylated hydroxytoluene, also identified as one of the metabolites of Bacillus cereus has strong antioxidant activity (VKM, 2019).

5. CONCLUSION

This study has shown that the internal tissues of Hibiscus rosa-sinensis flower is a repository for many bacteria species whose metabolite are of great medical and pharmaceutical importance. The study showed that Bacillus cereus possessed the highest antibacterial potential of the seven endophytic bacteria isolated. Crude metabolite of the Bacillus cereus was highly active against Salmonella enterica clinical isolates and moderately active against E. coli, K. pneumoniae and S. aureus. Many bioactive compounds with notable antibacterial and antioxidant potential were reported to be present in the crude metabolite of Bacillus cereus. This is of great medical importance as drugs can be formulated from these metabolites to serve as better alternative to commonly used antibiotics to which most pathogens have developed resistance, as demonstrated in this study.

REFERENCES

Akinsanya, M.A., Goh, J.K., Lim, S.P. and Ting, A.S.Y., 2015. Diversity, antimicrobial and Antioxidant activities of culturable bacterial endophyte communities in Aloe vera.Microbiology Letters362: Pp. 1–8.

Aljuraifani, A., Sahar, A. and Ibtisam, A., 2019. In vitro Antimicrobial Activity of Endophytes, Isolated from Moringa peregrina Growing in Eastern Region of Saudi Arabia. Natl. Acad. Sci. Lett. 42(1): Pp. 75–80

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M. et al. Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov 20, Pp. 200–216 (2021).

Bhakta, S., Das, SK., 2017; A review on Hibiscus rosa-sinensis: a rural traditional medicine for contraception. International Journal of Engineering and Information Systems. 1(9): Pp. 77–79.

Etminani, F., and Harighi B., 2018. Isolation and identification of endophytic bacteria with plant growth promoting activity and biocontrol potential from wild pistachio trees. The Plant Pathology Journal 34:208.

Fayeulle, A., Elodie, T., Audrey, D., Alexandra, J., Nesrine, B.H. and Claire, C., 2022. Antimicrobial and antioxidant activities of amines derived from vanillin as potential preservatives: Impact of the substituent chain length and polarity, Sustainable Chemistry and Pharmacy 22: 9pp

Feng, B., Chen, D. and Jin, R., 2022. Bioactivities evaluation of an endophytic bacterial strain Bacillus velezensis JRX-YG39 inhabiting wild grape. BMC Microbiology 22: Pp. 170-176.

Feng, B., Chen, D. and Jin, R., 2022.Bioactivities evaluation of an endophytic bacterial strain Bacillus velezensis JRX-YG39 inhabiting wild grape. BMC Microbiology 22: Pp. 170-176.

Feng, Y., Zhang, Y., Shah, O.U., Luo, K., Chen, Y., 2023. Isolation and Identification of Endophytic Bacteria Bacillus sp. ME9 That Exhibits Biocontrol Activity against Xanthomonas phaseoli pv. manihotis. Biology 12, 1231

Gomare, K.S., Mishra, D.N., 2018. FTIR spectroscopic analysis of phytochemical extracts from Hibiscus rosa–sinensis L. used for hair disorder. International Journal of Recent Trends in Science and Technology. Pp. 70–75.

Guneş, F.E., 2013. Medical Use of Squalene as a Natural Antioxidant. MUSBED 3(4): Pp. 220-228

Indraningrat, A.A.G., Purnami, P.P.C., Aryastuti, A.A.S., Wijaya, M.D. and Horng, J.T., 2023. Antibacterial, antifungal and antioxidant activities of Bacillus cereus SMPRL-2 isolated from seaweeds Eucheuma cottonii. Earth Environ. Sci. 1271 012066.

IndrawatiI, Rossiana, N. and Diresna, D., 2018a. Bioprospecting of bacterial endophytes from Curcuma aeruginosa, Curcuma xanthorrhiza and Curcuma zedoaria as antibacterial against pathogenic bacteria. IOP Conference Series: Earth and Environmental Science 197: 012009

IndrawatiI, Rossiana, N. and Hidayat, T.., 2018b. Antibacterial activity of bacterial endophytes fromkupa plant (Syzygium Polycephalum Miq. (Merr and Perry) against pathogenic bacteria. IOP Conference Series: Earth and Environmental Science 166:012013

Khan, S.S., Vijeshwar, V. and Shafaq, R., 2020. Diversity and the role of endophytic bacteria: a review. Botanica Serbica 44 (2): Pp. 103-120

Koushik, M. (2013). Antibacterial activity of long-chain fatty alcohols against mycobacteria, FEMS Microbiology Letters338(2): Pp. 177–183,

Leo, V.V., Passari, A.K., Joshi, J.B., Mishra, V.K., Uthandi, S., Ramesh, N., Gupta, V.K., Saikia, R., Sonawane, V.C. and Singh, B.P., 2014.A novel triculture system (CC3) for simultaneous enzyme production and hydrolysis of common grasses through submerged fermentation. Frontiers in Microbiology 7:447.

Li, Q., Chang, W., Zhang, H., Hu, D. and Wang, X., 2019. The Role of Plasmids in the Multiple Antibiotic Resistance Transfer in ESBLs-Producing Escherichia coli Isolated From Wastewater Treatment Plants. Front. Microbiol. 10: Pp. 633-639

Narkhede, M.B., 2012. Investigation of in vitro α-amylase and α-glucosidase inhibitory activity of polyherbal extract. Int. J. Pharm.

Res. Dev. 3: Pp. 97–103.

Nongkhlaw, F.M. and Joshi, S.R., 2015. Investigation on the bioactivity of culturable endophytic andepiphytic bacteria associated with ethno medicinal plants. The Journal of Infection in Developing Countries 9: Pp. 954–961.

Pandey PK, Singh S, Singh MC, Singh AK, Pandey P, Pandey AK, Pathak M, Kumar M, Shaky warRC &PatidarRK., 2017. Inside the plants: bacterial endophytes and their natural products. International Journal of Current Microbiology and Applied Sciences 6: Pp. 33–41.

Peterson, E. and Kaur, P., 2018. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Front. Microbiol. 9:2928

Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda, M.C., Glick, B.R., 2016. Plant growth-promoting bacterial endophytes. Microbiol Res. 183: Pp. 92-9.

Seo, W.T., Lim, W.J., Kim, E.J., Yun, H.D., Lee, Y.H. and Cho, K.M., 2010. Endophytic bacterial diversity in the young radish and their antimicrobial activity against pathogens. Journal of the Korean Society for Applied Biological Chemistry 53: Pp. 493–503.

Temesgen, A.A. and Alebe, N.B., 2022. Assessment of antibacterial and antioxidant activity of aqueous crude flower, leaf, and bark extracts of Ethiopian Hibiscus rosa-sinensis Linn: geographical effects and Co2Res2 /Glassy carbon electrode. International Journal of Food Properties 25(1): Pp. 1875–1889

Tian, Y., and LiY, H., 2017. Comparative analysis of bacteria associated with different mosses by 16S rRNA And 16SrDNA sequencing. Journal of Basic Microbiology 57: Pp. 57–67.

Ts, S.G., Ragothaman, P. and Velu, P S., 2024. Secondary metabolite profiling using HR-LCMS, antioxidant and anticancer activity of Bacillus cereus PSMS6 methanolic extract: In silico and in vitro study. Biotechnol Rep (Amst) 2;42:e00842

Tshikhudo, P.P.,Ntushelo, K. and Mudau, F.N., 2023. Sustainable Applications of Endophytic Bacteria and Their Physiological/Biochemical Roles on Medicinal and Herbal Plants: Review. Microorganisms 11: Pp. 453.

Vijayalakshmi, R., Kairunnisa, K, Narender, S., Soumya, S.D. and Natarajan, S. , 2016. Enzyme Production and Antimicrobial Activity of Endophytic Bacteria Isolated from Medicinal Plants Indian Journal of Science and Technology 9(14),

Vijayalakshmi, R., Kairunnisa, K., Sivvaswamy, S.N., Dharan, S.S. and Natarajan, S., 2016. Enzyme production and antimicrobial activity of endophytic bacteria isolated from medicinal plants. Indian Journal of Science and Technology 9: Pp. 1–8.

VKM, 2019. Risk assessment of butylated hydroxytoluene (BHT).Opinion of the Panel on Food Additives, Flavourings, Processing Aids, Materials in Contact with Food, and Cosmetics of the Norwegian Scientific Committee for Food and Environment. VKM report. Norwegian Scientific Committee for Food and Environment (VKM), Oslo, Norway. 28pp.

Zachow, C., Jahanshah, G., deBruijn, I., Song, C.,m Ianni, F., Pataj, Z., Gerhardt, H., Pianet, I., Lämmerhofer, M. and Berg, G., 2015. Then ovellipo peptide poaeamide of the endophyte Pseudomonas poae RE* 1-1-14 is involved in pathogen suppression and root colonization. Molecular Plant-Microbe Interactions 28: Pp. 800–810.

Zengin, H. and Baysal, A.H., 2014. Antibacterial and Antioxidant Activity of Essential Oil Terpenes against Pathogenic and Spoilage-Forming Bacteria and Cell Structure-Activity Relationships Evaluated by SEM Microscopy. Molecules 19: Pp. 17773-17798.