Journal of Applied Biology & Biotechnology Vol. 13(1), pp. 192-201, Jan-Feb, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.182980

Evaluation and characterization of endophytic bacteria from *Capparis decidua* (Forssk.) Edgew. for their antifungal and antioxidant activities

Sudesh Kumari[1], Prity Gulia[1], Pooja Choudhary[1], Ritu Pasrija[2], Mehak Dangi[3], Anil Kumar Chhillar[1]*

[1]Centre for Biotechnology, Maharshi Dayanand University, Rohtak, Haryana, India.
[2]Department of Biochemistry, Maharshi Dayanand University, Rohtak, Haryana, India.
[3]Centre for Bioinformatics, Maharshi Dayanand University, Rohtak, Haryana, India.

ARTICLE INFO

Article history:
Received on: May 28, 2024
Accepted on: October 02, 2024
Available online: November 15, 2024

Key words:
Endophytes,
Antifungal,
Antioxidant,
Staphylococcus,
Bioactive Metabolites,
Candida.

ABSTRACT

This comprehensive research endeavor was undertaken to delve into the isolation and identification of endophytic bacteria from *Capparis decidua*, specifically focusing on their potential contributions to antifungal and antioxidant activities. The meticulously examined endophytes, identified as *Staphylococcus pasteuri*, *S. warneri*, and *Staphylococcus* sp., underwent a rigorous assessment. Their crude extracts demonstrated considerable potency, manifesting significant antifungal efficacy against various *Candida* species, as indicated by well-defined inhibition zones and minimum inhibitory concentrations spanning 6.51 ± 166 µg/mL. Furthermore, these extracts exhibited noteworthy antioxidant potential in DPPH assays, showcasing IC_{50} values ranging between 94.01 ± 1.40 and 186.73 ± 1.30 µg/mL. The qualitative analysis substantiated the presence of bioactive compounds, encompassing flavonoids, alkaloids, and phenolic compounds. Through meticulous metabolite identification using Fourier transform infrared and gas chromatography/mass spectrometry techniques, specific compounds previously associated with antifungal and antioxidant properties were unveiled. These compelling findings underscore the therapeutic promise encapsulated within endophytic bacteria derived from *Capparis decidua*. The harmonious alignment of robust antifungal and antioxidant activities with the identified bioactive metabolites offers valuable insights for the exploration of natural sources, potentially paving the way for impactful applications in pharmaceutical research.

1. INTRODUCTION

Due to global warming, more and more fungal pathogens can survive and multiply at human body temperature [1]. This is the reason that outbreaks of fungal infections are likely to occur in the coming years if not controlled [2]. Resistance to currently available antifungal drugs is increasing at an alarming rate, worsening the situation [3]. Therefore, the development of new antifungal drugs with broad-spectrum activity, low toxicity, and resistance is an important goal for both academia and the pharmaceutical industry [4]. The most common fungal infections in humans are caused by *Candida sp.* [5]. In the past two decades, the incidence of *Candida infections has increased due to the irregular use of* broad-spectrum antibiotics and the limited number of antifungal drugs to control fungal infections [6]. Therefore, there is an urgent need for new compounds with antifungal activity. One possible approach could be to screen endophytic bacterial isolates for the presence of competent antifungal chemotherapeutic metabolites.Endophytic microorganisms live symbiotically in various plant tissues. They contribute either directly or indirectly to increasing plant tolerance to abiotic (pressure, temperature) and biotic (microbial) stresses. To cope with environmental stresses, plants synthesize various bioactive metabolites, both individually and in enhanced production by endophytic strains [7,8]. However, this requires isolating endophytes and studying their metabolites, which not only saves time but also does not affect plant diversity.

Ethnomedicinal uses of *Capparis decidua*: *Capparis decidua* (Forssk) Edgew belongs to the class Magnoliopsida in the family Capparaceae. This small, branched shrub is native to the arid regions of the Asian continent. It has ethnobotanical importance in folk medicine and is also used to treat various ailments such as rheumatism, respiratory problems, diabetes, microbial infections, hypertension, free radical scavenger, and various stomach problems [9]. Since *C. decidua exhibits activity against microbes* [10], there is a possibility that its endophytes could be involved either directly or indirectly in various pharmaceutical activities.

**Corresponding Author:*
Anil Kumar Chhillar,
Centre for Biotechnology, Maharshi Dayanand University,
Rohtak, Haryana, India.
E-mail: anil.chhillar@gmail.com

© 2025 Sudesh Kumari, *et al*. This is an open access article distributed under the terms of the Creative Commons Attribution License -NonCommercial-ShareAlike Unported License (http://creativecommons.org/licenses/by-nc-sa/3.0/).

Table 1: Endophytic bacteria and their plant sources.

Sample No.	Endophytic Bacteria	Plant source with local name	Plant part
1	Sample (A/21)	*Capparis deciduas* (Forssk.) Edgew & Kher	Seed
2	Sample (B/22)	*Capparis deciduas* (Forssk.) Edgew& Kher	Seed
3	Sample (C/23)	*Capparis deciduas* (Forssk.) Edgew& Kher	Seed

2. MATERIALS AND METHODS

2.1. Collection of the Plant Sample

The healthy plant *Capparis decidua* (Forssk.) Edgew. (confirmed at https://www.gbil.org) was collected from Satnali village in Mahendragarh district (28º23'0" N, 75º58'0" E), Haryana. Plant leaves, seeds, and stems were cut with sterile scissors, collected in zip-lock bags, and carefully transported to the laboratory for further experimental work.

2.2. Isolation and Purification of Bacterial Endophytes

The origin of the endophytic bacterial strains is summarized in Table 1. These strains were isolated as described by Duhan et al., 2020, with some modifications. Plant leaves, stems, and seeds were washed under running tap water for 5 min, then rinsed with a mild detergent and washed twice more with distilled water. Then, the plant leaf and seed were immersed in 70% ethanol for 5 min, followed by soaking in a sodium hypochlorite solution (0.9%) for 15 min, after that they were washed three times with sterile distilled water. Plant leaves, stems, and seeds were drained or immersed in sodium bicarbonate solution (10%) for 15 min to inhibit any endophytic fungal growth [11]. Plant leaves, stems, and seeds were dried, aseptically cut into small pieces, placed on Petri dishes containing LB (Luria Bertani) agar supplements, and incubated at 37°C for 72 h. A control Petri dish containing only LB media and without endophytic extract was also included to check purity. The absence of growth in the control indicated a lack of contamination. Bacterial isolates were sub-cultured on fresh plates until monocultures were obtained, which were used to characterize bacterial strains by morphological, biochemical, and genotypic methods.

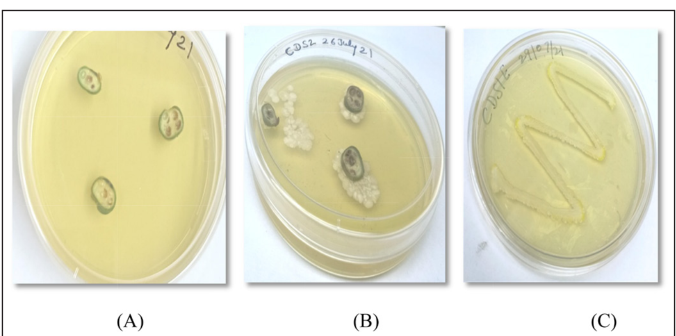

Figure 1: Endophytic bacterial growth from the inoculated plant seed and bacterial sub cultivation at 37 °C for 72 h. **(A)** Plant part inoculated on Nutrient agar **(B)** endophytes growth **(C)** Sub culturing of bacterial isolates.

Table 2: Qualitative screening of metabolites in ethyl acetate extracts of endophytic bacteria.

Sr. no.	Bioactive compound analysis	Method	Observations for positive analysis
1	Alkaloids	Addition of 2-3 drops of Meyer's reagent to endophyte extract	Precipitation
2	Flavonoids	Addition of 2 drops of 1% NaOH, few drops of dilute HCl to 1 mL endophyte extract	Yellow coloration
3	Saponins	Addition of 2 drops of olive oil to 1 mL endophyte extract	Formation of foam
4	Steroids	Addition of 1 mL acetate, followed by adding 2-3 drops of concentrated H2SO4 to 1mL of endophyte extract	Brown-yellow formation
5	Tannins	Addition of 2-3 drops of FeCl2 to 1 mL endophyte extract	Black-green color

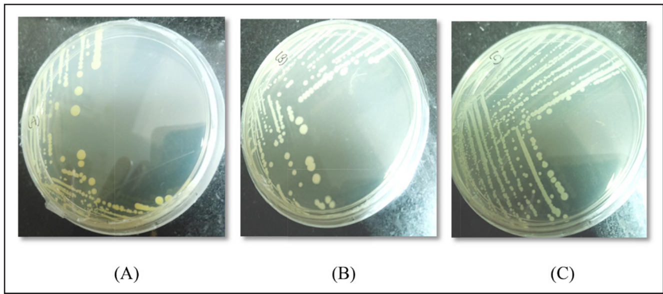

Figure 2: Purified endophytic bacterial strains after sub-culturing **(A)** Extract A **(B)** Extract B **(C)** Extract C.

2.3. Bacterial Identification

2.3.1. Morphological and biochemical screening

The isolated bacteria were sub-cultured and examined for the color, shape, and size of the colonies. Further Gram staining was performed using a commercially available Gram staining kit (HiMedia, K001-1KT). Various biochemical characterization tests, such as carbohydrate fermentation, buffered glucose broth test (MR-VP), catalase test, citrate test, and protease test, were performed, and the bacterial samples were identified using Bergey's Handbook of Systematic Bacteriology [11].

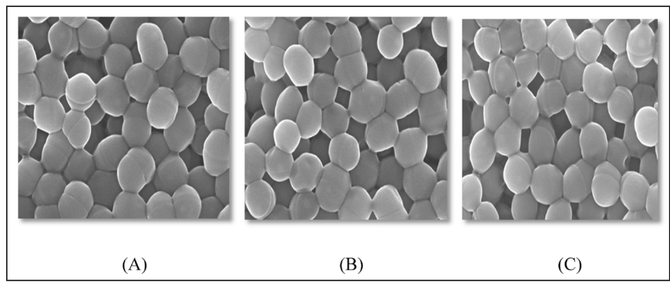

Figure 3: Field emission scanning electron microscopy showing the spherical morphology of *Staphylococcus* sp. endophytic bacteria: **(A)** Extract A/21 **(B)** Extract B/22 **(C)** Extract C/23 at 20,000 X magnification.

Table 3: Morphological and biochemical characteristics of the endophytic bacterial isolates of *Capparis decidua*.

Characteristic	Sample- A	Sample-B	Sample- C
Color of bacterial isolates	Yellow-golden	Whitish	Yellow-golden
Shape of the colony	Spherical	Spherical	Spherical
Size of colony	Large	Large	Large
Catalase Test	Mild	Mild	Mild
Phenol Red Broth(Carbohydrate fermentation)	+	+	+
Protease	–	–	–
Citrate	–	–	–
Buffered Glucose Broth (MR-VP)	+	+	+

Notes: + shows positive results and – shows negative results.

2.3.2. Molecular characterization

Molecular characterization was done based on 16S rRNA sequence phylogeny. The conserved 16SrRNA gene sequence was amplified from extracted bacterial genomic DNA using the universal forward primer 27F with sequence 5'-AGAGTTGATCCTGGCTCAG-3' and the reverse primer 1492R with sequence 5'-CGGTTACCTTGTTACGACTT-3'. Amplification was performed with 2 µL template DNA, 1 µL primer, and 7 µL master mix, and the PCR cycle was performed. The quality of the amplified product was checked on 2% agarose gel electrophoresis with ethidium bromide staining, and endophytic extract purification was performed using the QIAquick PCR Purification Kit from QIAGEN (Cat. No. 28104). The PCR reaction for sequencing was performed in the Applied Biosystems™ MiniAmp™ Plus Thermal Cycler using the Big Dye™ Terminator V3.1 kit. The software DNA STAR was used to generate contigs, and results were verified by comparing the contigs to the NCBI Gen Bank database using the nBLAST tool.**2.4. Field Emission Scanning Electron Microscopy (FESEM)**

FESEM was performed as described by Photolo et al. [12]. Briefly, endophytic bacterial isolates were grown in Luria broth at 32ºC with shaking at 170 rpm for 24 h and centrifuged at 10,000 rpm for ten min. The pellet was collected, and the cells were washed with distilled water. The pellet was then fixed with a mixture of 1% formaldehyde and 2% glutaraldehyde (1:1 v/v) at 25°C for 1 h. Fixation was followed by centrifugation at 10000 rpm for 10 min. The supernatant was then discarded, the pellet rinsed with distilled water, and dehydrated by treatment with ethanol at increasing concentrations (30%, 70%, 90%, 95%, and 100%). The endophytic extract was kept open at 4ºC for 12 h and mounted on SEM stubs. The endophytic extract was then coated with gold for 2 min using a JEOL Smart Coater and assayed using a FESEM (7610F Plus/JEOL).

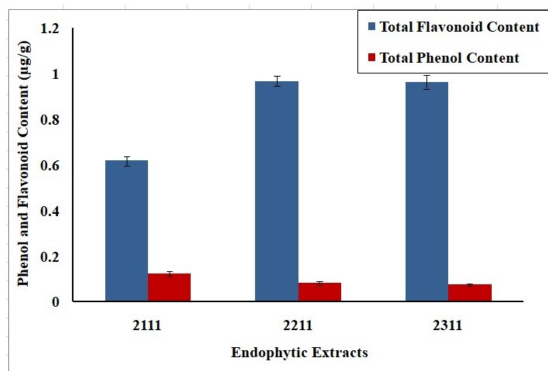

Figure 4: Comparison of total phenolic content (TPC) and total flavonoid content (TFC) in endophytic extracts, presented as mean ± standard deviation of triplicate specimens. TPC values are expressed as Gallic acid equivalent (GAE) g-1 dry weight, while TFC values are expressed as quercetin equivalent (QE) g-1 dry weight.

2.5. Extraction of Bioactive Metabolites From Bacterial Endophytes

Selected bacterial strains were cultivated in Luria Broth and cultured at 37°C for 72 h and at 120 rpm in a shaking incubator. The culture was then transferred to a 50 mL Falcon tube and centrifuged at 10,000 rpm for 10 min to recover the cell pellet. The pellets were suspended in ethyl acetate solvent and incubated for one day at 30°C. The following day, the endophytic extract was lysed by sonication for 30 min at 5 min intervals. The tubes were then centrifuged (10,000 rpm) for 10 min. In Falcon tube (A), the organic supernatant was collected, and the extraction solvent was added to the remaining pellet and centrifuged at 10,000 rpm for 10 min. A centrifuge tube was used to collect the supernatant (B). Both solvents (A) and (B) were combined, and the pellet was discarded. A crude extract of the bioactive compounds was obtained by evaporating the solvent at room temperature. Dimethyl sulfoxide (DMSO) was used to dissolve the bacterial extract [13] and stored at 4ºC for further experiments.

2.6. Quantitative and Qualitative Screening

2.6.1. Qualitative analysis

Qualitative screening for alkaloids, flavonoids, saponins, steroids, and tannins is summarized in Table 2 for further experimental work.

2.6.2. Quantitative analysis

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) in endophytic extract. The absorbance was measured with a spectrophotometer at 765 nm. The TPC was expressed as mg gallic acid equivalents (GAE) per gram of dry weight [14].The colorimetric method with aluminum chloride ($AlCl_3$) was used to determine the total flavonoid content of the extract. For each sample, 15 µL of bacterial extract (5 mg/mL dry weight) was mixed with 30 µL of absolute methanol, 3 µL of 10% $AlCl_3$, 3 µL of potassium acetate, 20 µL of 1% sodium hydroxide, and 30 µL of distilled water

Table 4: Endophytic bacterial isolates, identified to their genus and species by 16S rRNA sequencing. The deposit numbers and their matches are indicated for clarity.

Endophyte Isolates	Primer pair	Organism	Blast output Accession number	Identity (%)	Accession number of the sequence submitted to NCBI
Seq-1(A/21)	27F/1492R	*Staphylococcus pasteuri*	MH174447.1	97	OP572265
Seq-2(B/22)	27F/1492R	*Staphylococcus warneri*	OM604759.1	99.78	OP572266
Seq-3(C/23)	27F/1492R	*Staphylococcus* sp.	MH707151.1	99.86	OP572267

Table 5: Qualitative analysis of secondary metabolites in endophytic bacterial extracts.

Bioactive compound test	2111	2211	2311
Alkaloids	+	+	-
Flavonoids	+	+	+
Saponins	–	+	–
Steroids	+	–	–
Tannin	–	–	–

Notes: The (–) and (+) represents the absence and presence of respected bioactive compound.

*2111- Extract A/21 prepared in ethyl acetate solvent, 2211- Extract B/22 prepared in ethyl acetate solvent, 2311- Extract C/23 prepared in ethyl acetate solvent.

and incubated for 30 min at room temperature. The absorbance was measured at 417 nm. TFC (total flavonoid content) was expressed as mg of quercetin equivalents (QE) per gram of dry weight [15].

2.7. Antifungal Potential

Antifungal activities were confirmed by two different methods: the disk diffusion method and the minimum inhibitory concentration (MIC) of all bacterial extracts as described by Elooff, 1998, with some modifications [16]. Briefly, sterile 96 microtiter plates were filled with 50 µL of sterile Sabouraud dextrose broth (SDB). 50 µL of the endophytic bacterial extracts (100 µg/mL) were added to the first column of the microtiter plate. 50 µL of 100 µg/mL of amphotericin B (positive) and sterile distilled water (negative) were added as controls. Similarly, the culture control SDB for each microorganism was taken as the reference point for the growth indicator. The tested fungal strains were diluted with saline (0.85%) to achieve an approximate

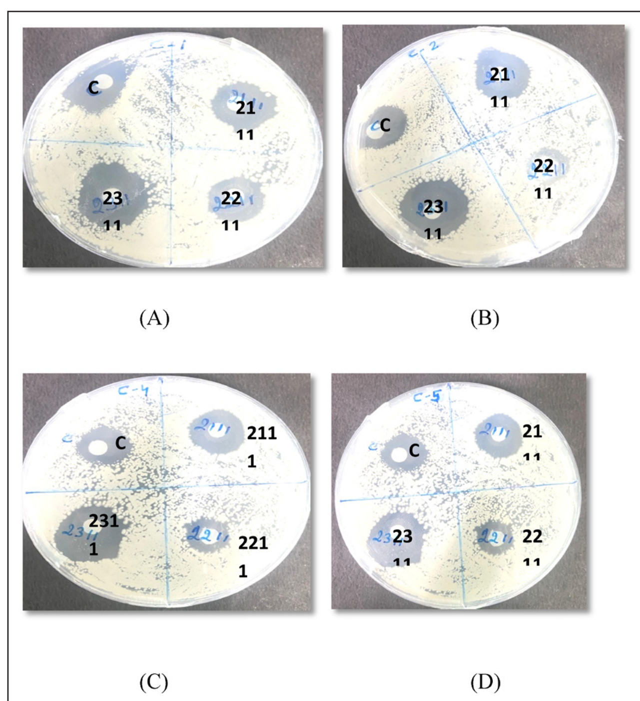

Figure 5: Antimicrobial activity of endophytic extracts (2111, 2211, 2311) and control (C) against *Candida strains:* **(A)** C-1 *(Candida albicans),* **(B)** C-2 *(Candida parapsilosis)*, **(C)** C-4 *(Candida glabrata)*, **(D)** C-5 *(Candida krusei)*.

Table 6: Minimum inhibitory concentration (MIC) of endophytic extracts and positive control (amphotericin b) against fungal strain reported as mean ± standard deviation (µg/mL).

Tested fungal strain	Endophytic crude extracts (µg/mL)			Antibiotic Control (µg/mL)
	2111	2211	2311	
Candida albicans	20.84 ± 9.1	13.02 ± 4.5	6.51 ± 2.25	20.84 ± 9.1
Candida parapsilosis	20.84 ± 9.1	13.02 ± 4.5	6.51 ± 2.25	20.84 ± 9.1
Candida glabrata	166 ± 72.17	20.84 ± 9.1	20.84 ± 9.1	20.84 ± 9.1
Candida krusei	20.84 ± 9.1	20.84 ± 9.1	83.33 ± 36.08	83.33 ± 36.08

concentration of 1×105 CFU/mL, and then 10 µL was added to all wells. The fungal strains used to test the efficacy of the endophytic extracts were *C. albicans* (ITCC 4718), *C. parapsilosis, C. glabrata* and *C. krusei.* The microtiter plates were incubated at 37ºC for one day. After incubation, 10 µL (1 mg/mL) of resazurin solution (HiMedia) was added to each well to determine cell viability and incubated for 2 h. The lowest concentration showing no microbial growth (pinkish-purple color) is considered as the MIC point for the extract.

For the disk diffusion assay, the method of Chhillar et al., 2009, was used with modifications. Briefly, 20 µL of fungal suspension (1×10^5 CFU/mL) was added to a petri dish containing potato dextrose agar (PDA). Then, the disks were impregnated with 20 µL of endophytic isolates (0.1 mg/mL) and amphotericin B solution (HiMedia) as a positive control. The test disks were placed on the plates and incubated at 32ºC for 24 h [17]. Antifungal activity was determined by measuring the clear zone of growth inhibition.

2.8. Free Radical Scavenging Activity

The antioxidant activity of the bacterial extracts in different solvents was measured by their free radical scavenging ability using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Takao et al., 2015 with some modifications [18]. In summary, different concentrations (100, 80, 60, 40, 20, 0 µg/mL) were added to each endophytic extract and solvent to obtain a final volume of 100 µL for each of the above concentrations, and 200 µL of 0.3 mM DPPH was added and incubated for 30 min in the dark, and further absorbance was measured at 517 nm using UV-VIS spectrophotometer. Radical scavenging activity was determined as a percentage of DPPH using the following equation:

Radical scavenging activity (%) = (A of control- A of endophytic extract)/ A of control× 100

Here, A = absorbance

The mean value of the radical scavenging concentration (%) of each extract concentration was calculated from three independent endophytic extracts. The IC_{50} (inhibitory concentration that scavenges 50% of DPPH radical) was determined using a linear equation, and the result was expressed in µg/mL of bacterial extract. Ascorbic acid was used as a control for radical scavenging

2.9. Bioactive Metabolites Profiling

Bioactive metabolites were identified by Fourier Transform Infrared (FTIR) and Gas chromatography-mass spectroscopy (GCMS) studies. The endophytic extracts were analyzed using a Nicolet iS50 FTIR

Table 7: Endophytic bioactive metabolites with antifungal and antioxidant potential identified through GC-MS analysis.

Sample	RT	Bioactive compounds	SI	Area%	MF	MW	Mol. Structure	Bioactivity
Endophytic extract (A), 2111	8.086	1-Dodecanol	83	0.21	$C_{12}H_{26}O_3$	186		Anticandidal activity[29]
	11.377	Ageratochromene	90	1.06	$C_{13}H_{16}O_3$	220		Antifungal [30]
	8.634	β-cis- Caryophyllene	75	0.16	$C_{15}H_{24}$	204		Antiphytopathogenic activity and Antioxidant [31,32]
Endophytic extract (B), 2211	4.940	Butyric Acid	81	0.09	$C_4H_8O_2$	88		Antifungal [33]
	5.894	NonanoicAcid	74	0.65	$C_9H_{18}O_2$	158		Antifungal [34]
	19.639	1,2-BenzenedicarboxylicAcid	89	0.62	$C_{24}H_{38}O_4$	390		Antibacterial, Antifungal [35]
	21.488	13-Docosenamide, (Z)	84	1.88	$C_{22}H_{43}NO$	337		Antibacterial, Antifungal [36]
	10.460	Diethyl Phthalate	80	1.06	$C_{12}H_{14}O_4$	222		Antibacterial and cytotoxic activities, Antioxidant and antifungal [37]
	12.482	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	92	2.74	$C_7H_{10}N_2O_2$	154		Antibacterial and antioxidant [38,39]
	9.565	2,4-Di-Tert-Butylphenol	85	0.79	$C_{14}H_{22}O$	206		Antioxidant [40]
Endophytic extract (B), 2211	7.804	DL-Pyroglutamic acid	74	0.05	$C_5H_7NO_3$	129		Antibacterial, Antifungal [41]
	10.126	4-Aminobenzoic Acid	78	1.47	$C_7H_7NO_2$	137		Antibacterial, Antifungal [42]
	15.200	NonanoicAcid	75	0.12	$C_9H_{18}O_2$	158		Antibacterial and antifungal [43]
	15.813	n-Nonadecanol-1	94	1.46	$C_{19}H_{40}O$	240		Antibacterial and antifungal [35]
	13.148	3-Isobutylhexahydropyrrolo [1,2-a]pyrazine-1,4-dione	83	8.57	$C_{11}H_{18}N_2O_2$	210		Antifungal, Antibacterial and cytotoxic activity [44]

(Continued)

Table: 7 (Continued)

Sample	RT	Bioactive compounds	SI	Area%	MF	MW	Mol. Structure	Bioactivity
	14.940	n-Tetracosanol-1	91	0.36	$C_{24}H_{50}O$	354		Antioxidant [45]
	16.553	2,5-Piperazinedione,3,6-bis (2-methylpropyl)-	84	1.00	$C_{12}H_{22}N_2O_2$	226		Antioxidant [46]
	12.511	Pyrrolo[1,2-a] pyrazine-1,4-dione,hexahydro-	95	6.44	$C_7H_{10}N_2O_2$	154		Antibacterial and antioxidant [38,39]
	21.490	9-Octadecenamide	92	3.11	$C_{18}H_{35}NO$	281		Antioxidant [47]
Common Among all three sample	18.485	Pyrrolo[1,2-a] pyrazine-1, 4-dione, hexahydro-3-(phenylmethyl)-	90	14.40	$C_{14}H_{16}N_2O_2$	244		Antifungal [48]
	11.990	Uric acid	77	3.04	$C_5H_4N_4O_3$	168		Antioxidant [49]

spectrometer, and OMINC 8 spectrum software was used for analysis to determine the composition of bio-metabolites. A small amount of the extract was placed in the attenuated total reflectance (ATR) head of the FTIR spectrometer, and spectra were recorded over a wave-number range of 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}.GC- MS analysis was performed using a Shimadzu GC-MS-QP plus with a thermal desorption system TD 20 under the following conditions. Helium gas at a flow rate of 1.21 mL/min was used to separate the metabolites, and an injection volume of 1 µL was injected in split mode. The injector temperature was maintained at 260°C, and the column temperature was programmed at 100°C for 2 min and then ramped at 300°C for 18 min. Mass detector settings included an ion source temperature of 220°C, an interface temperature of 270°C, an ionization energy of 70 eV, and a scan time of 0.2 seconds. The obtained spectra of the metabolites were identified by comparison with the mass spectra of the integrated libraries, such as GC-MS NIST.14 and WILEY8.

Statistical tool: We collected statistical data from all inoculation studies, which were performed in triplicate for each treatment. All results were expressed as mean ± standard deviation using Origin and Graph Pad Prism 9 software.

3. RESULTS

3.1. Isolation and Identification of Bacterial Endophytes

In this study, a total of 15 endophytic bacterial isolates were isolated and purified from different parts of the shrub, including the leaves, stems, and seeds of *Capparis decidua* (Forssk.) Edgew, as depicted in Figures 1 and 2. However, among all the purified isolates, only three showed considerable bioactivity. Therefore, these three plant seeds were further characterized by morphological, biochemical, and genomic sequencing using 16S rRNA sequencing.Morphological and biochemical identification was performed, and the differences in texture, color, type, shape, and size of colonies were observed and summarized in Table 3. The endophytic bacterial isolates were spherical, Gram-negative, and formed grape-like clusters as observed by FESEM, as shown in Figure 3. The information obtained from the morphological identification of the bacterial endophytes confirmed the identification at the molecular level by 16S rRNA sequencing, which revealed species-specific sequences. Therefore, it was concluded that the bacterial identification was correct [11].Sequencing of the genome of the endophytic bacteria was performed using Oxford nanopore technology (ONT), and 16S rRNA sequencing was conducted using the Sanger method. The identified bacterial isolates are listed in Table 4 along with their BLAST homology searches and Genbank accession numbers. The sequences of the isolated endophytic strains - *Staphylococcus pasteuri, Staphylococcus warneri*, and *Staphylococcus* sp. were deposited with NCBI under accession numbers OP572265, OP572266, and OP572267, respectively.

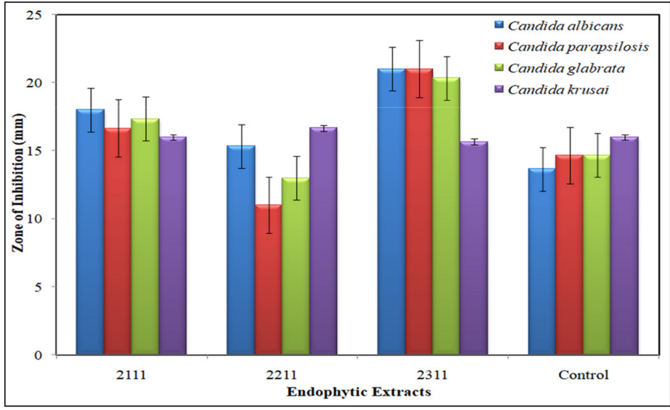

Figure 6: Zone of inhibition in mm (mean ± SD) of control and endophytic extracts against phytopathogenic fungi

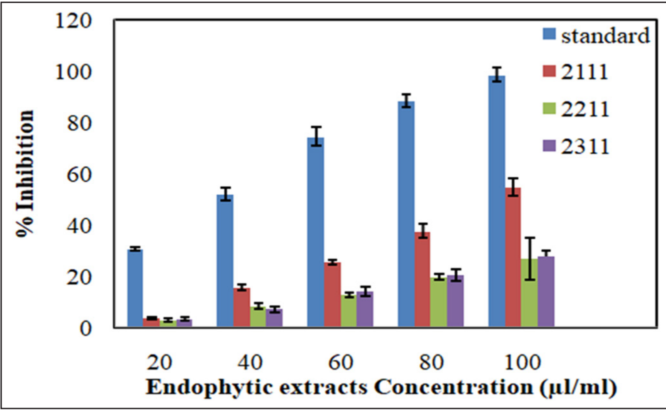

Figure 7: DPPH radical scavenging activity (%) of ascorbic acid and ethyl acetate solvent extracts of *Staphylococcus pasteuri* (2111), *S. warneri* (2211), and *Staphylococcus* sp. (2311).

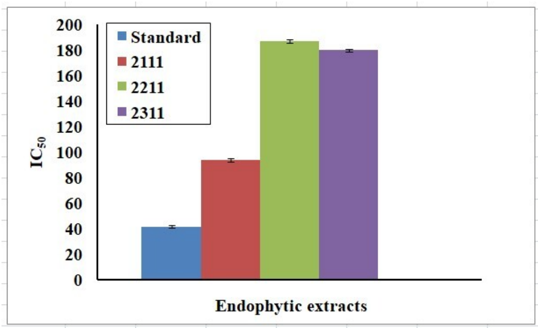

Figure 8: IC_{50} values (µg/mL) of ascorbic acid and different solvent extracts of *Staphylococcus pasteuri* (2111), *S. warneri* (2211), and *Staphylococcus* sp. (2311). Statistical significance was determined using ANOVA, with differences considered significant at $p < 0.05$.

3.2. Quantitative and Qualitative Screening

We thoroughly analyzed the endophytic extracts using both quantitative and qualitative methods. The qualitative analysis for bioactive compounds is shown in Table 5. The total content of flavonoids and phenols in the endophytic bacterial extracts is summarized in Figure 4. Medicinal plants contain a group of polyphenolic flavonoids that provide various health benefits, including combating pathogenic fungi and bacteria, preventing cancer and inflammation, and acting as antioxidants and antiviral agents. They have also been shown to be effective against allergies. Additionally, alkaloids possess veterinary, pharmacological, and medicinal properties [12].

Endophytes have evolved to produce secondary metabolites that help them thrive in host tissues and ensure their survival. These metabolites can also help the host plant adapt to and withstand stressful conditions. The presence of secondary metabolites in bacterial extracts indicates their therapeutic potential for their hosts, making them valuable resources for medical research.

3.3. Evaluation of Antifungal Activity

We investigated the efficacy of three endophytic bacterial strains in four solvent extracts against *Candida albicans* (SC5314), *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* through a disk diffusion assay to determine their antifungal properties. Endophytic isolates extracted with ethyl acetate showed the greatest antifungal activity against all tested *Candida* strains, as depicted in Figures 5 and 6. Minimum inhibitory concentrations of crude extracts are summarized in Table 6. Ethyl acetate extract of endophytic extract A (2111) exhibited antifungal activity against *C. albicans* at 20.84 ± 9.1 µg/mL. Whereas, endophytic extract B ethyl acetate (2211) showed potent activity against *C. albicans* and *C. parapsilosis* with MIC of 13.02 ± 4.5, 13.02 ± 4.5 to 20.84 ± 9.1 µg/mL, respectively, compared to control. Endophytic extract C extract (2311) demonstrated the highest antifungal activity against *C. albicans* with an MIC of 6.51 ± 2.25 µg/mL to the control MIC of 20.84 ± 9.1 µg/mL.

3.4. Antioxidant Property

The antioxidant activity varied significantly among ethyl acetate solvent extracts and their concentrations. The percentage inhibition increased with the concentration of the extracts. Among all the extracts, those from *Staphylococcus pasteuri* exhibited the highest inhibition as depicted in Figure 7.

Antioxidants are metabolites that scavenge reactive oxygen species. In our study, the antioxidant activity of crude extracts of endophytic bacteria in ethyl acetate solvent, along with a standard antioxidant, ascorbic acid, was investigated at concentrations ranging from 20 µg/mL to 100 µg/mL and their ability to scavenge free radicals was assessed, and the IC_{50} values were calculated, as shown in Figure 8.

The results indicated that extract B (2211) has the highest IC_{50} values of 186.73 ± 1.30 while extract A (2111) has the lowest IC_{50} values of 94.01 ± 1.40, compared to the standard with an IC_{50} of 41.60 ± 1.13. Interestingly, the phenolic content in the crude extracts correlates with significant antioxidant activity in the DPPH assay, supported by our GC/ MS results, which identified various phenolic compounds. Previous research has demonstrated that phenolic compounds possess optimal structural chemistry for radical scavenging activity [12].

Furthermore, this property aligns with the presence of flavonoid compounds identified in the biochemical analysis in Figure 4. Flavonoids have also been shown to play a significant role in minimizing the rate of lipid peroxidation, acting as both primary and secondary antioxidants [19]. Extracts from the bacterial endophyte *Methylobacterium radiotolerans* MAMP 4754 have similarly exhibited radical scavenging activity, attributed to the presence of flavonoids [12].

3.5. Bioactive Metabolite Analysis

3.5.1. FTIR analysis

From FTIR results, all three endophytic extracts 9-10 peaks, with prominent peaks observed at 1704-1709, 1367-1376, and 1249-1258 cm^{-1} as illustrated in Supplementary Figures 1-3. The peak at 3396 cm^{-1} corresponds to the N-H stretch of the amines. The O-H stretch and the H-bond of the phosphorus were identified at 2607 cm^{-1}. Peaks at 1709 cm^{-1} are attributed to the C=O stretching of ketones. The peaks at 879 and 1379 cm^{-1}, correspond to C-C stretching and symmetrical C-H bending in alkane, respectively. The C-O stretching of alcohols and phenols was detected at 1005 cm^{-1}. Peaks at 1249 cm^{-1} and 1047 cm^{-1} arise from asymmetrical C-O-C stretching and symmetrical C-O-C stretching in ether and epoxides, respectively. The peak at 604.102 cm^{-1} corresponds to C-Cl stretching in alkyl halide.

3.5.2. GC-MS analysis

GCMS chromatogram analysis revealed the identification of more than fifty compounds in each endophytic extract, with details of endophytic metabolite name, retention times, and area percentage described in Supplementary Figures 4-6, and Supplementary Tables 1-3. The analysis of volatile compounds in bacterial endophyte extracts

indicates the presence of a variety of metabolites responsible for various bioactivities, including antifungal and antioxidant properties. Common metabolites found across all endophytic extracts included Pyrrolo [1, 2-a] pyrazine-1, 4-dione, hexahydro-3-(phenylmethyl) ($C_{14}H_{16}N_2O_2$) and Uric acid ($C_5H_4N_4O_3$), which exhibited antifungal and antioxidant potential.

While the endophytic extracts contained numerous bioactive metabolites, a table listing active metabolites with bioactive potential based on previous data is provided in Table 7. The identification of volatile metabolites responsible for these activities was validated based on their retention time, molecular weight, molecular formula, and peak area.

In our study, the major volatile compounds such as ageratochromene (1.06%), 13-Docosenamide, (Z) (1.88%), Diethyl Phthalate (1.06%), Pyrrolo [1,2-a] pyrazine-1, 4-dione, hexahydro (2.74%),4-Aminobenzoic Acid (1.47%), n-Nonadecanol-1(1.46%),3-Isobutylhexahydropyrrolo [1,2-a] pyrazine-1, 4-dione (8.57%), Pyrrolo[1,2-a]pyrazine-1, 4-dione, hexahydro (6.44%), 9-Octadecenamide (3.11%) were identified. These compounds are responsible for the observed bioactive properties, such as antifungal and antioxidant activities.

4. DISCUSSION

Capparis decidua (Forssk) Edgew has a rich history in traditional medicine, supported by documented pharmacological activities [9], highlighting its significant potential for exploration in the pharmaceutical, agricultural, and industrial sectors. Dos Reis et al. (2019) investigated the antifungal properties of endophytic extracts from *Solanum americanum* Mill against Candida strains, with MICs ranging from 3.9 to 250 µg/mL across different solvents [27]. Similarly, Photolo et al. (2020) examined the efficacy of an endophytic crude extract (*M. radiotolerans* MAMP 4754) from *Combretum erythrophyllum* seeds against *Candida albicans*, reporting an MIC of 125 µg/mL [12]. In contrast, Das et al. (2018) studied endophytic bacteria extracts from *Dryopteris uniformis*, noting an MIC of 252 µg/mL against *Candida albicans* [5], whereas our study observed a significantly lower MIC of 6.51 ± 166 µg/mL against Candida strains.

Endophytes are valuable sources of bioactive compounds, producing secondary metabolites similar to those found in their host plants without causing harm. These metabolites include antimicrobial and antioxidant agents essential for the endophytes' survival within the host plant [20]. Numerous studies have focused on extracting endophytic bacteria from medicinal plants to explore their antimicrobial and antioxidant properties. Additionally, endophytes play crucial roles in agriculture by enhancing plant growth, boosting immunity, and outcompeting plant pathogens through niche competition and phenylpropanoid metabolism [21].Research highlights the abundance of bioactive secondary metabolites in microbes, such as alkaloids, flavonoids, terpenoids, phenols, or indoles, which can penetrate cell membranes and interfere with specific signal transduction pathways in host organisms, affecting their physiology [22]. For instance, Peng et al., 2021, isolated *Streptomyces typhae* from *Typha angustifolia* L. and demonstrated its antifungal activity [23]. Similarly, various endophytic bacteria isolated from *Drypteris uniformis* (Makino) exhibited anti-candidal activity against *Candida saitoana (KACC 41238), C. albicans (KACC 30003), C. albicans (KACC 30062), C. glabrata (KBNO6P00368) and C. glochares (KACC 30061)* [24]. Moreover, the choice of solvent system significantly influences the antifungal activity of crude extracts from isolated endophytes, necessitating the use of different solvents with varying polarities for effective extraction [25]. Previous studies have documented the antifungal properties of endophytic bacteria from diverse plant species such as *Calotropis procera*, *Datura stramonium*, and *Morus macroura* Miq. [24,26,27]. In our study, members of the *Staphylococcus* genus showed substantial antifungal activity, with compounds like Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), Ageratochromene,9-Octadecenamide and uric acid identified as responsible for this activity. Furthermore, Photolo et al, 2020, also reported on the antimicrobial and antioxidant potential of secondary metabolites from *Methylobacterium radiotolerans MAMP 4754,* corroborating our findings [12]. These consistent results support the promising development of endophytic bacterial extracts for pharmaceutical applications. However, further research is needed to fully understand endophytes, their secondary metabolites, and their mechanisms of action.

5. CONCLUSIONS

Endophytic bacteria, namely *Staphylococcus pasteuri*, *Staphylococcus warneri* and *Staphylococcus* sp. were isolated from fresh seeds of the plant *Capparis decidua* (Forssk.) Edgew. and demonstrated significant antifungal and antioxidant activities. This underscores the potential of endophytes to contribute to the therapeutic effects observed in medicinal plants, potentially through their own metabolites [28]. The ethyl acetate extract of the endophyte exhibited robust in vitro inhibitory activity against *Candida* strains. Moreover, the bacterial extracts displayed notable free radical scavenging activity, further highlighting their potential in antioxidant therapy. Analysis of the bioactive compounds in these bacterial extracts identified alkaloids, flavonoids, steroids, and saponins, which are known for their diverse agricultural and pharmaceutical applications. Screening of the ethyl acetate extract also revealed a variety of metabolites previously reported for their beneficial properties. Continued research on bacterial endophytes holds promise for elucidating their biosynthetic pathways and understanding the mechanisms of action of these bioactive compounds. This knowledge could pave the way for the development of new therapeutic drugs with enhanced efficacy and safety profiles.

6. ABBREVIATIONS

LB: Luria Bertani; rRNA: Ribosomal Ribonucleic acid; DNA: Deoxyribonucleic acid; PCR: Polymerase Chain Reation; FESEM: Field emission scanning electron microscopy; DMSO: Dimethyl Sulfoxide; DPPH: 2,2-diphenyl-1-picryl-hydrazyl-hydrate; FTIR: Fourier Transform Infrare; GCMS: Gas chromatography-mass spectroscopy; *C.*: *Candida*; MIC: Minimum Inhibitory Concentration.

7. ACKNOWLEDGMENTS

Authors also acknowledged the "DBT Builder Maharshi Dayanand University Interdisciplinary Life Science program for Advance Research and Education" for infrastructure facilities. Authors are thankful to AIRF, JNU for GC-MS instrument facility.

8. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

9. FINANCIAL SUPPORT AND SPONSORSHIP

DBT grant (no. BT/INF/22/SP2715/2018, 28/09/2018) and Indian Council of Medical Research (ICMR) for providing the financial assistance [3/1/3/JRF- 2019/HRD–062(25108)].

10. CONFLICTS OF INTEREST

The authors declare no conflicts of interest associated with this study.

11. ETHICS APPROVAL

This study, not involving human or animal subjects, does not require ethical approval.

12. CONSENT TO PARTICIPATE

All individual participants included in the study provided informed consent.

13. CONSENT TO PUBLISH

The absence of personal or individual data in the present study obviates the need for consent to publish.

14. DATA AVAILABILITY

Endophyte sequences from isolated bacteria have been submitted at the Genbank, NCBI and all the data is available with the authors and shall be provided upon request.

15. SUPPLEMENTARY MATERIAL

The supplementary material can be accessed at the journal's website: [https://jabonline.in/admin/php/uploadss/1249_pdf.pdf]

16. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

17. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

1. Nnadi, NE. Carter DA. Climate change and the emergence of fungal pathogens. PLoS Pathogens. 2021;17:e1009503. https://doi.org/10.1371/journal.ppat.1009503.
2. Ngo HX, Garneau-Tsodikova S, Green KD. A complex game of hide and seek: the search for new a ntifungals. Medchemcomm. 2016;7(7):1285-306. https://doi.org/10.1039/C6MD00222F.
3. Fisher MC, Alastruey-Izquierdo A, Berman J, Bicanic T, Bignell EM, Bowyer P, et al. Tackling the emerging threat of antifungal resistance to human health. Nat Rev Microbiol. 2022:20(9):557-71. https://doi.org/10.1038/s41579-022-00720-1
4. Mani Chandrika KVS, Sharma S. Promising antifungal agents: a minireview. Bioorganic Med Chem. 2020;28(7):115398. https://doi.org/10.1016/j.bmc.2020.115398
5. Das G, Park S, Choi J, Baek KH. Anticandidal potential of endophytic bacteria isolated from dryopteris uniformis (Makino). Jundishapur J Microbiol. 2019;12(1):e69878. https://doi.org/10.5812/jjm.69878
6. Mehmood Z, Ahmad I, Mohammad F, Ahmad S. Indian medicinal plants: a potential source for anticandidal drugs. Pharm Biol. 1999;37(3):237-42. https://doi.org/10.1076/phbi.37.3.237.6296
7. Tyagi J, Chaudhary P, Mishra A, Khatwani M, Dey S, Varma A. Role of endophytes in abiotic stress tolerance: with special emphasis on serendipita indica. Int J Environ Res. 2022;16(4):62. https://doi.org/10.1007/s41742-022-00439-0
8. Chaudhary P, Agri U, Chaudhary A, Kumar A, Kumar G. Endophytes and their potential in biotic stress management and crop production. Front Microbiol. 2022;13(October):933017. https://doi.org/10.3389/fmicb.2022.933017
9. Singh P, Mishra G, Sangeeta, Srivastava S, Jha KK, Khosa RL. Traditional uses, phytochemistry and pharmacological properties of capparis decidua: an overview. Der Pharm Lett. 2011;3(2):71-82.
10. Upadhyay RK, Ahmad S, Tripathi R, Rohtagi L, Jain SC. Screening of antimicrobial potential of extracts and pure compounds isolated from capparis decidua. J Med Plants Res. 2010;4(6):439-45.
11. Duhan P, Bansal P, Rani S. Isolation, identification and characterization of endophytic bacteria from medicinal plant tinospora cordifolia. South African J Bot. 2020;134:43-9. https://doi.org/10.1016/j.sajb.2020.01.047
12. Photolo MM, Mavumengwana V, Sitole L, Tlou MG. Antimicrobial and antioxidant properties of a bacterial endophyte, methylobacterium radiotolerans MAMP 4754, isolated from combretum erythrophyllum seeds. Int J Microbiol. 2020;2020:9483670. https://doi.org/10.1155/2020/9483670
13. Rahman L, Shinwari ZK, Iqrar I, Rahman L, Tanveer F. An assessment on the role of endophytic microbes in the therapeutic potential of fagonia indica. Ann Clin Microbiol Antimicrob. 2017;16(1):53. https://doi.org/10.1186/s12941-017-0228-7
14. Bertini L, Perazzolli M, Proietti S, Capaldi G, Savatin DV, Bigini V, et al. Biodiversity and bioprospecting of fungal endophytes from the Antarctic plant colobanthus quitensis. J Fungi. 2022;8(9):979. https://doi.org/10.3390/jof8090979
15. Aiyegoro OA, Okoh AI. Research article preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of helichrysum longifolium DC. BMC Complement Altern Med. 2010;10(1):21. https://doi.org/10.1186/1472-6882-10-21
16. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 1998;64(8):711-3. https://doi.org/10.1055/s-2006-957563
17. Chhillar AK, Yadav V, Kumar A, Kumar M, Parmar VS, Prasad A, Sharma GL. Differential expression of aspergillus fumigatus protein in response to treatment with a novel antifungal compound, diethyl 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarboxylate. Mycoses. 2009;52(3):223-7. https://doi.org/10.1111/j.1439-0507.2008.01563.x
18. Takao LK, Imatomi M, Gualtieri SCJ. Antioxidant activity and phenolic content of leaf infusions of myrtaceae species from cerrado (Brazilian Savanna). Brazilian J Biol. 2015;75(4):948-52. https://doi.org/10.1590/1519-6984.03314
19. Pawle G, Singh SK. Antioxidant potential of endophytic fungus colletotrichum species isolated from polygala elongata. Int J Pharma Bio Sci. 2014;5:B313-9.
20. Ek-Ramos MJ, Gomez-Flores R, Orozco-Flores AA, Rodríguez-Padilla C, González-Ochoa G, Tamez-Guerra P. Bioactive products from plant-endophytic gram-positive bacteria. Front Microbiol. 2019;10(March):463. https://doi.org/10.3389/fmicb.2019.00463
21. Pandey SS, Singh S, Pandey H, Srivastava M, Ray T, Soni S, et al. Endophytes of withania somnifera modulate in planta content and the site of withanolide biosynthesis. Sci Rep. 2018;8(1):5450. https://doi.org/10.1038/s41598-018-23716-5

22. Matuszewska A, Jaszek M, Stefaniuk D, Ciszewski T, Matuszewski Ł. Anticancer, antioxidant, and antibacterial activities of low molecular weight bioactive subfractions isolated from cultures of wood degrading fungus cerrena unicolor. PLoS One. 2018:13(6):e0197044. https://doi.org/10.1371/journal.pone.0197044
23. Peng C, Zhuang X, Gao C, Wang Z, Zhao J, Huang SX, et al. Streptomyces typhae Sp. Nov., a novel endophytic actinomycete with antifungal activity isolated the root of cattail (typha angustifolia L.). *Antonie van Leeuwenhoek*. Int J Gen Mol Microbiol. 2021;114(6):823-33. https://doi.org/10.1007/s10482-021-01561-3
24. Devi R, Nath T, Boruah RR, Darphang B, Nath PK, Das P, et al. Antimicrobial activity of bacterial endophytes from chirata (swertia chirata wall.) and datura (datura stramonium L.). Egypt J Biol Pest Control. 2021;31(1):69. https://doi.org/10.1186/s41938-021-00410-9
25. Khan W, Subhan S, Shams DF, Afridi SG, Khan AJ, Iqbal A. In vitro assessment of the antibacterial activity of datura alba with different solvents. Fresenius Environ Bull. 2019;28(10):7333-9.
26. Hamayun M, Khan N, Khan MN, Qadir M, Hussain A, Iqbal A, et al. Antimicrobial and plant growth-promoting activities of bacterial endophytes isolated from calotropis procera (Ait.) W.T. Aiton. Biocell. 2021;45(2):363-9. https://doi.org/10.32604/BIOCELL.2021.013907
27. Putri DH, Violita V, Irdawati, Fifendy M, Nurhasnah N. Production of antifungal compounds by andalas endophytic bacteria (Morus Macroura Miq.) isolate ATB 10-6 at fermentation medium with optimum carbon and organic nitrogen source. J Phys Conf Ser. 2021;1940(1):012076. https://doi.org/10.1088/1742-6596/1940/1/012076
28. Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev. 2003;67(4):491-502. https://doi.org/10.1128/MMBR.67.4.491-502.2003
29. Davis-Hanna A, Piispanen AE, Stateva LI, Hogan DA. Farnesol and dodecanol effects on the candida albicans Ras1-CAMP signalling pathway and the regulation of morphogenesis. Mol Microbiol. 2008;67(1):47-62. https://doi.org/10.1111/j.1365-2958.2007.06013.x
30. Rioba NB, Stevenson PC. Ageratum conyzoides L. for the management of pests and diseases by small holder farmers. Ind Crops Prod. 2017;110(July):22-9. https://doi.org/10.1016/j.indcrop.2017.06.068
31. Gushiken LFS, Beserra FP, Hussni MF, Gonzaga MT, Ribeiro VP, De Souza PF, et al. Beta-caryophyllene as an antioxidant, anti-inflammatory and re-epithelialization activities in a rat skin wound excision model. Oxid Med Cell Longev. 2022;2022:9004014. https://doi.org/10.1155/2022/9004014
32. Hilgers F, Habash SS, Loeschcke A, Yannic Sebastian Ackermann, Neumann S, Heck A, et al. 2021 heterologous production of β-caryophyllene microorganisms-09-00168 (1).Pdf. Microorganisms. 2021;9:168.
33. Garnier L, Penland M, Thierry A, Maillard MB, Jardin J, Coton M, et al. Antifungal activity of fermented dairy ingredients: identification of antifungal compounds. Int J Food Microbiol. 2020;322(November 2019):108574. https://doi.org/10.1016/j.ijfoodmicro.2020.108574
34. Aneja M, Gianfagna TJ, Hebbar PK. Trichoderma harzianum produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. Physiol Mol Plant Pathol. 2006;67(6):304-7. https://doi.org/10.1016/j.pmpp.2006.05.002
35. Sholkamy EN, Muthukrishnan P, Abdel-Raouf N, Nandhini X, Ibraheem IBM, Mostafa AA. Antimicrobial and antinematicidal metabolites from streptomyces cuspidosporus strain SA4 against selected pathogenic bacteria, fungi and nematode. Saudi J Biol Sci. 2020;27(12):3208-20. https://doi.org/10.1016/j.sjbs.2020.08.043
36. Dos Reis CM, da Rosa BV, da Rosa GP, do Carmo G, Morandini LMB, Ugalde GA, et al. Antifungal and antibacterial activity of extracts produced from diaporthe schini. J Biotechnol. 2019;294:30-7. https://doi.org/10.1016/j.jbiotec.2019.01.022
37. Roy RN. Bioactive natural derivatives of phthalate ester. Crit Rev Biotechnol. 2020;40(7):913-29. https://doi.org/10.1080/07388551.2020.1789838
38. Kiran GS, Priyadharsini S, Sajayan A, Ravindran A, Selvin J. An antibiotic agent pyrrolo[1,2-: A] pyrazine-1,4-dione,hexahydro isolated from a marine bacteria bacillus tequilensis MSI45 effectively controls multi-drug resistant staphylococcus aureus. RSC Adv. 2018;8(32):17837-46. https://doi.org/10.1039/c8ra00820e
39. Ser HL, Palanisamy UD, Yin WF, Abd Malek SN, Chan KG, Goh BH, et al. Presence of antioxidative agent, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro- in newly isolated streptomyces mangrovisoli Sp. Nov. Front Microbiol. 2015;6(August):854. https://doi.org/10.3389/fmicb.2015.00854
40. Zhao F, Wang P, Lucardi RD, Su Z, Li S. Natural sources and bioactivities of 2,4-di-tert-butylphenol and its analogs. Toxins (Basel). 2020;12(1):35. https://doi.org/10.3390/toxins12010035
41. Zhu F, Yuan C, Gang F, Yang C, Wu W, Zhang J. Bioassay-guided isolation of antifungal compounds from disporopsis aspersa (hua) engl. ex diels against pseudoperonospora cubensis and phytophthora infestans. Chem Biodivers. 2018;15(7):e1800090. https://doi.org/10.1002/cbdv.201800090
42. Krátký M, Konečná K, Janoušek J, Brablíková M, Janďourek O, Trejtnar F, et al. 4-aminobenzoic acid derivatives: converting folate precursor to antimicrobial and cytotoxic agents. Biomolecules. 2020;10(1):9. https://doi.org/10.3390/biom10010009
43. Sahin N, Kula I, Erdogan Y. Investigation of antimicrobial activities of nonanoic acid derivatives. Fresenius Environ Bull. 2006;15(2):141-3.
44. Mangamuri UK, Muvva V, Poda S, Manavathi B, Bhujangarao C, Yenamandra V. Chemical characterization & bioactivity of diketopiperazine derivatives from the mangrove derived pseudonocardia endophytica. Egypt J Aquat Res. 2016;42(2):169-75. https://doi.org/10.1016/j.ejar.2016.03.001
45. Amudha P, Jayalakshmi M, Pushpabharathi N, Vanitha V. Identification of bioactive components in enhalus acoroides seagrass extract by gas chromatography–mass spectrometry. Asian J Pharm Clin Res. 2018;11(10):313-7. https://doi.org/10.22159/ajpcr.2018.v11i10.25577
46. Druzian SP, Pinheiro LN, Susin NMB, Dal Prá V, Mazutti MA, Kuhn RC, Terra LdM. Production of metabolites with antioxidant activity by botryosphaeria dothidea in submerged fermentation. Bioprocess Biosyst Eng. 2020;43(1):13-20. https://doi.org/10.1007/s00449-019-02200-y
47. Cheng MC, Ker YB, Yu TH, Lin LIY, Peng RY, Peng CH. Chemical synthesis of 9(z)-octadecenamide and its hypolipidemic effect: a bioactive agent found in the essential oil of mountain celery seeds. J Agric Food Chem. 2010;58(3):1502-8. https://doi.org/10.1021/jf903573g
48. Sanjenbam P, Krishnan K. Bioactivity of pyrrolo[1,2-A]pyrazine-1,4-dione,hexahydro-3-(phenylmethyl)- extracted from streptomyces Sp. VITPK9 isolated from the salt spring habitat of Manipur, India. Asian J Pharm. 2016;10(4):265-70.
49. El Ridi R, Tallima H. Physiological functions and pathogenic potential of uric acid: a review. J Adv Res. 2017;8(5):487-93. https://doi.org/10.1016/j.jare.2017.03.003

How to cite this article:
Kumari S, Gulia P, Choudhary P, Pasrija R, Dangi M, Chhillar AK. Evaluation and characterization of endophytic bacteria from *Capparis decidua* (Forssk.) Edgew. for their antifungal and antioxidant activities. J App Biol Biotech. 2025;13(1):192-201.
DOI: 10.7324/JABB.2024.182980