IJBPAS, May, 2025, 14(5): 3014-3033

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL AND BIOACTIVE PARALLELS BETWEEN *MORINGA OLEIFERA* LEAVES AND THEIR NATIVE ENDOPHYTIC BACTERIA

SHONIMA GOVINDAN M[1], ANJANA M[2], SUJITHA A P[3] AND SHABANAMOL S[*4]

[1&2]Department of Biochemistry, SAFI Institute of Advanced Study Autonomous

[3]Department of Food Technology, SAFI Institute of Advanced Study Autonomous

[4]Department of Microbiology, SAFI Institute of Advanced Study Autonomous, Vazhayur East PO, Ramanattukara via, Malappuram, Kerala, India-673633

***Corresponding Author: Dr. Shabanamol S: E Mail: bioshabana@siasindia.org**

Received 16th Jan. 2025; Revised 20th Feb. 2025; Accepted 20th April 2025; Available online 1st May 2025

https://doi.org/10.31032/IJBPAS/2025/14.5.9064

ABSTRACT

This study presents a comparative evaluation of the antimicrobial, antioxidant, and phytochemical activities of *Moringa oleifera* leaves and their native endophytic bacterial isolates. Surface-sterilized leaves of *M. oleifera* were subjected to crude extract preparation using various organic solvents and distilled water, while endophytic bacteria were isolated using tryptic soy agar. The antimicrobial activities of both leaf extracts and bacterial isolates were assessed via the well diffusion assay against selected clinical pathogens. Phytochemical components in the leaf extracts and culture filtrates were screened using standard protocols, and antioxidant potential was evaluated through the DPPH free radical scavenging assay. Among the tested solvents, the ethyl acetate extract demonstrated the highest antimicrobial activity, indicating its effectiveness in extracting potent bioactive compounds. Five predominant endophytic isolates were identified based on morphological, microscopic, and biochemical characteristics, belonging to the genera *Bacillus*, *Pseudomonas*, and *Serratia*. Notably, the isolate MLEB4, identified as *Pseudomonas sp.*, exhibited the most pronounced antimicrobial activity. Phytochemical screening revealed the presence of alkaloids, phenols, and terpenoids in both the leaf extracts and the endophytic bacterial culture filtrates. The antioxidant analysis indicated that while the Moringa leaf extract showed strong radical scavenging activity, the endophytic extracts also demonstrated moderate antioxidant potential. These findings underscore the potential of endophytic bacteria, particularly *Pseudomonas sp*. MLEB4, as alternative sources of natural therapeutic agents. Moreover, the study reinforces the concept that endophytes associated with medicinal plants may share or mimic the host plant's bioactive traits, paving the way for novel pharmaceutical and agricultural applications.

Keywords: Antioxidant activity, endophytes, *Pseudomonas* sp. Phytochemical analysis, *Moringa oleifera,* Ethyl acetate, antimicrobial activity

INTRODUCTION

Traditional medicine embodies a comprehensive approach to health, focusing on promoting well-being, preventing diseases, modulating immunity, and offering personalized treatments. Notably, a significant portion of modern pharmaceuticals have their origins in plant-based compounds. In India, a substantial segment of the population relies on non-allopathic medicinal systems, such as Ayurveda, Unani, Siddha, Homeopathy, and Traditional Chinese Medicine (TCM). These traditional practices are valued for their gentle impact on the human body, minimal side effects, cost-effectiveness, and accessibility **[1]**. *Moringa oleifera,* native to the Indian subcontinent, is recognized for its rich phytochemical content, including flavonoids, alkaloids, and phenolic compounds. These bioactive molecules are reported to possess a wide range of antioxidant, antimicrobial, antiinflammatory and other therapeutic properties **[2]**. Because of its therapeutic qualities and health advantages, moringa has been utilized for generations. Additionally, it possesses anti-inflammatory, antiviral, antifungal, and depressive qualities **[3, 4]**.

Endophytes, microbial symbionts that inhabit the plant's tissues without causing harm, have emerged as significant sources of bioactive compounds. These microbes often confer beneficial traits to their host, including enhanced growth, stress tolerance, and disease resistance **[5]**.

Endophytes may include bacteria, fungi and a ctinomycetes. The entry and existence of endophytes depends on several factors like species of microorganisms, genotype of plants, life cycle of plants, nature of plant tissue where the endophyte colonize, soil type and climatic factors. It is commonly acknowledged that plant endophytic bacteria are a cost-effective source of essential and unique macromolecules and enzymes with prospective usage in food, pharmaceutical, agricultural industry and research **[6]**. Beyond the plant itself, the endophytic microorganisms residing within *M. oleifera* tissues have have been studied for their bioactive potentials in terms of medicinal value as well as agricultural purpose. The previous research from different authors have reported the isolation and characterization of endophytic bacteria, fungi, actinimycetes from *M. oleifera* plant parts. Most of these isolated microorganisms were evaluated for a ntibacterial, antifungal, antiviral and cytotoxi city and most of the studies have done from fungi isolated from moringa plants **[7-11]**.

Moringa plant contains a variety of antimicrobial secondary metabolites such as 4-(α-L-rhamnopyranosyloxy) benzylisothiocyanate, peterygospermine, 4 (rhamno-pyranosyloxy) benzylmustard oleosides **[12-14]**. Furthermore, phytochemical analysis by vari-ous authors showed that *Moringa* contains a group of steroids, flavonoids, alkaloids, phen olics and tannins **[15, 16]**.

The use of bioactive substances in medicinal plants is generally done through extraction of the plant **[17, 18]**. Investigating the native endophytes of medicinal plants is crucial, as these microorganisms often produce bioactive compounds mirroring those of their host plants, including antioxidant and antimicrobial agents. For instance, endophytes have been shown to synthesize alkaloids, flavonoids, terpenoids, and phenolics, compounds known for their medicinal properties **[19]**. By harnessing these endophytes, we can develop sustainable sources of valuable phytochemicals, thereby reducing the need to harvest the host plants themselves. This approach not only aids in the conservation of medicinal plant species but also ensures a continuous and eco-friendly supply of important medicinal compounds. The present study was designed to identify and compare the phytochemical, antimicrobial and antioxidant activities of *moringa* leaves and with that of native endophytic bacteria from leaves of moringa plants.

MATERIALS AND METHODS

Collection of plant samples and crude extract preparation

Moringa leaves were collected from healthy plants in the nearby housing area of Vazhayoor Panchayat. The collected leaf samples were immediately brought to the laboratory and were used within 24 hours and finally processed for crude extract preparation and isolation of endophytic bacteria. Initially, the leaves of *Moringa oleifera* underwent a comprehensive rinse to remove soil particles. This was followed by a thorough cleansing using the detergent Tween 20. The samples were subsequently rinsed multiple times with sterile tap water, after which they were blot dried with sterile blotting paper. Subsequently, the leaves were kept in sterile phosphate buffered saline at a pH of 7 for a duration of 10 minutes to balance osmotic pressure and inhibit the passive diffusion of sterilizing agents. The plant tissues were carefully processed keeping maximum aseptic conditions. Extreme care should be taken to avoid any damages to plant tissue which can happen during pre-treatment.

The crude extract preparation was done according to **[20, 28]** with major modifications. From the PSB, the leaves were blot dried using sterile filter paper and chopped into small pieces to increase the surface area for extraction. The chopped leaves were grouped into two randomly, one is for crude extract preparation, other is for surface sterilization prior to endophytic isolation. The first group of chopped leaves were placed into a conical flask and added different organic solvents like Iso propanol, Dimethyl sulfoxide (DMSO) and ethyl acetate in a ratio of 1:10 (w/v). An aqueous fraction of the leaves were also collected using distilled water in the same ratio. The flasks were securely sealed, and the mixture was left to macerate at ambient temperature for a period ranging from 24 to 48 hours, with occasional stirring to facilitate the extraction process. Following the maceration phase, the mixture was passed through Whatman No:1 filter paper to separate the liquid extract from the plant material. The filtrate, which contains the bioactive compounds dissolved in respective solvents was concentrated using a rotary evaporator set at 40°C. The resulting concentrated extract was used for further analysis. This approach enables the isolation of bioactive compounds directly from test plant leaves, eliminating the drying process and possibly retaining heat labile phytochemicals that might otherwise degrade.

Isolation of Endophytic bacteria

The second batch of chopped leaves was sterilized using a modified protocol based on the method outlined in **[21]**. The process began with immersing the leaves in sterile distilled water for 2–3 minutes. This was followed by surface sterilization using 1% sodium hypochlorite for 2 minutes. The leaves were then subjected to five sequential rinses with sterile distilled water. Next, they were treated with 70% ethanol for 30 seconds and again washed five times with sterile distilled water. The sterilized samples were gently dried using sterile filter paper, then excised under aseptic conditions with autoclaved scalpel and forceps inside a laminar airflow cabinet, and finally left to air-dry within the chamber.

To verify the effectiveness of the surface sterilization of plant tissues, 1 ml of the sterile distilled water used in the final rinse was placed on Tryptic Soy Agar (TSA) media and kept at room temperature. Bacterial colonies appeared after 24 hours. Additionally, surface sterilized leaves and roots were rolled on nutrient agar plates and incubated at room temperature for 24 h and checked for possible microbial growth. Colonies exhibiting distinct morphological characteristics were selected, purified, and subsequently maintained at 4°C on TSA slants.

Antibacterial activity test of the crude extracts of moringa leaves

Test organisms:

The test organisms used in this study included Gram negative pathogenic bacteria *Escherichia coli, Salmonella typhi, Acinetobacter baumenii, Klebsiella pneumoniae.* and Gram positive bacterial pathogens *Staphylococcus aureus* and *Bacillu*s *cereus* which were the kind gifts from the Microbiology lab of Prof. Dr Jyothis Mathews, School of Biosciences, M G University, Kottayam. The pathogenic test strains were kept in nutrient agar slants at 4^0C. The antimicrobial activity assay of the crude extracts from Moringa leaves, obtained using various solvents, was conducted utilizing the agar well diffusion method on Mueller Hinton agar (MHA). This procedure was executed as outlined by **[23]**. Bacterial strains were grown on nutrient agar (37ºC). Between one and three colonies of the clinical pathogens were mixed into a sterile normal saline solution (0.85% NaCl). A sterile, non-toxic cotton swab attached to a wooden applicator was used to spread 50uL of the inoculum onto 90-mm diameter Petri dishes containing 25ml of Mueller-Hinton Agar, employing the lawn culture technique. 5-mm diameter wells were punched in the agar using sterile cork borers and filled with 100µl of each of the crude extracts of moringa leaves. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zone observed, with the plates incubated for 24 hours at 37°C.

The antimicrobial activities of the isolated endophytic bacteria were also determined adopting well diffusion assay on MHA. For that, 24 hours old bacterial cultures were centrifuged to obtain cell free culture filtrate and 100uL of each culture filtrate was inoculated to MHA agar paltes wells pre swabed with clinical bacterial pathogens. The plates were kept in an incubator for 24h at 37ºC and observed for inhibition zones around the wells.

Extraction of crude secondary metabolic compounds from endophytic bacteria

The bacterial endophytes chosen for their antimicrobial properties were grown in 100 ml of Tryptic Soy broth (Himedia) and kept as a static culture at a temperature of 26 ± 2°C for a duration of one week. After the incubation period, cultures were centrifuged and filtered. Ethyl acetate was used in a 1:1 ratio to perform solvent extraction on the filtrate. All the solvent was evaporated by keeping open in laminar air flow chamber and the resultant compound was dried and redissolved in ethyl acetate for obtaining the bacterial crude extract **[22]**.

Comparative analysis of phytochemical activities of moringa leaves its and endophytic bacterial extract

The crude ethyl acetate extract derived from both Moringa leaves and endophytic bacterial sources was employed for phytochemical screening to assess the presence of various secondary metabolites, as briefed below following the procedures of Bharadwaj *et al* .,2015 with minor modifications **[23]**. The presence of alkaloids in both extracts were detected using 2NHCl solution followed by addition of Mayer's reagent and checked for the formation of cream coloured precipitate. Flavonoids were detected by observing the presence of yellow colour on addition of 20% sodium hydroxide solution. Ferric chloride solution was added to both extracts to detect the presence of phenolic compounds which produces a dark green color if present. Saponins were detected by frothing test and presence of steroids were experimented by Libermann-Burchard reaction. Addition of alcoholic ferric chloride followed by dilute H_2SO_4 was performed for the detection of tannins to observe a yellowish brown precipitate and terpenoids were detected using chloroform and concentrated H_2SO_4 and observed for reddish brown hue interface.

***In vitro* Anti-oxidant activity (DPPH(2,2-diphenyl-1-picrylhydrazyl) Assay)**

The potential of moringa leaves and their associated endophytic bacteria to neutralize free radicals was evaluated using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (24). The bacteria were cultured in Tryptic Soy Broth (TSB) for a duration of 24 hours at a temperature of 26 ± 2°C, followed by centrifugation at 6000 g for 5 minutes. The resulting supernatants were mixed with an equal volume of 98% methanol to reach a final concentration of (5 mg mL−1). This same method was applied to prepare moringa leaves for the DPPH assay. Subsequently, equal volumes of 1 ml of DPPH (200μM in methanol) and crude metabolites from moringa leaves and selected endophytic isolates were incubated for 30 minutes at room temperature (25 ± 2°C). Absorbance was recorded at 517 nm using a UV

Spectrophotometer. Ascorbic acid was used as the positive control.

The results are presented as a percentage of radical scavenging capacity, calculated by:

% DPPH radical scavenging = [(AC−AS)/AC] × 100, where (AS) represents sample values and (AC) represents positive control values.

RESULTS AND DISCUSSION

Moringa oleifera, commonly known as the drumstick or miracle tree, has gained widespread attention in research due to its nutritional and medicinal properties **[26]**. In this study, healthy leaves of *Moringa oleifera* plants were used for preparation of crude extract using solvent extraction and isolation of endophytic bacteria. Solvent extraction is a fundamental technique for isolating bioactive compounds from medicinal plants and the most common method is the maceration technique. Many authors have reported the use of maceration using various solvents to extract bioactive compounds from different medicinal plants **[27, 28]**.

The results of isolation of endophytic bacteria from surface sterilized moringa plants are discussed as follows. The epiphytic microorganisms were partially removed by the pretreatment procedure as described. Validation of the surface sterilization procedure was done and bacterial growth was never detected on sterility control plates indicating the efficiency of the developed procedure. Five morphologically different bacteria were obtained after purification of the endophytic isolates on TSA plates from surface sterilized leaves of *Moringa oleifera* test plants **(Figure 1)**. The bacterial isolates were sub cultured and stored respectively on TSA slants at refrigerated condition. The detailed list of the isolates along with colony morphology are illustrated in **Table 1**. The endophytic bacterial isolates were partially identified based on colony morphology, motility, grams reaction, pigmentation and biochemical characters **(Table 2)**. Out of the 5 endophytic isolates from leaves, 3 isolates labelled MLEB1, MLEB2 and MLEB3 found belonging to *Bacillus sp.* MLEB4 was identified as *Pseudomonas sp. Amd i*solate MLEB5 as *Serratia sp.* There are reports on the isolation of endophytic bacteria specifically belonging to *Bacillus sp* and *Pseudomonas sp*. from different medicinal plants **[29, 30]**. *Serratia marcescens* has been isolated from the leaves of *Bryophyllum pinnatum*, exhibiting notable antibacterial activity **[31]** and *Serratia marcescens* AL2-16, an endophytic bacterium from *Achyranthes aspera*, demonstrated plant growth-promoting properties **[32]**. But there are no reports on the isolation of *Serratia sp.*

from *Moringa oelifera* plants. Meanwhile, *Bacillus sp.* like *Bacillus pumilus* MPE1*,* *Paenibacillus glucanolyticus* MPE3 has been reported as endophytic isolates with biocontrol activities from *moringa* plants **[33]** and *Bacillus licheniforrmis, Bacillus subtilis sub sp. inaquasorum* and *Bacillus pumilus*, from *moringa* plants which showed antimicrobial activities against human pathogens **[34]**. The results of our study is validated by these diverse reports.

(A)

(B)

Figure 1: Emergence of endophytic bacteria from surface sterilized leaves of *Moringa oleifera* plants on TSA plates after 4th day (A) and 7thday (B) of incubation

Table 1: Colony morphology and microscopic observations of endophytic bacteria isolated from surface sterilized leaves of *Moringa oleifera*

S. No	Endophytic Isolate	Colony Morphology on TSA	Gram staining	Motility
1	MLEB1	Opaque,medium irregular cream colonies with thick center and fimbriated or irregular margins	Gram positive rods	+
2	MLEB 2	Medium, low convex, opaque, smooth, regular circular, creamy colony	Gram positive rods	+
3	MLEB 3	Medium to large opaque colourless shiny flat colonies with wrinkled irregular margin	Gram positive rods	+
4	MLEB 4	Small to medium circular convex smooth shiny opaque green coloured colonies with entire margin	Gram negative rods	+
5	MLEB 5	Pin point circular convex opaque red pigmented colonies with entire margin	Gram negative rods	+

MLEB- Endophytic bacteria from Moringa leaves; - Non Motile Bacteria; + Motile Bacteria

Table 2: Biochemical reactions of the endophytic bacteria

ISOLATE	I	MR	VP	C	U	TSI	G	L	S	M	Cat	Oxi
MLEB1	-VE	-VE	+VE	+VE	+VE	A/A	A	A	A	A	+VE	-VE
MLEB2	-VE	+VE	-VE	+VE	+VE	K/K	A/ G	A/ G	A/ G	A/G	+VE	+VE
MLEB3	-VE	+VE	+VE	+VE	+VE	K/A	A	A	A	A	+VE	+VE
MLEB4	-VE	-VE	+VE	+VE	+VE	K/K	-VE	-VE	-VE	A/G	+VE	+VE
MLEB5	-VE	-VE	-VE	+VE	-VE	A/A	A/ G	-VE	-VE	A/G	+VE	-VE

I-	**Indole Test**	**G-**	**Glucose**
MR-	**Methyl Red Test**	**L-**	**Lactose**
VP-	**Voges Proskauer Test**	**S-**	**Sucrose**
C-	**Citrate Test**	**M-**	**Mannitol**
U-	**Urease Test**	**A-**	**Acid production only**
TSI-	**Triple Sugar Iron Agar Test**	**A/G-**	**Acid and Gas production**
Cat-	**Catalase Test -**	**+VE-**	**Positive Reaction**
Oxi-	**Oxidase Test**	**-VE-**	**Negative reaction**
	A/A- Acid slant Acid butt		**K/A- Alkaline slant Acid butt**
	A/K- Acid slant Alkaline butt		**K/K- Alkaline slant/Alkaline butt**

The rise of antimicrobial resistance by clinical pathogens implies a significant challenge to global public health. This crisis seeks for alternate antimicrobial agents and there is a surge in investigating the role of bioactive compounds from traditionally known medicinal plants for the same **[36]**. In our study, we did a comparative analysis of the antimicrobial activities of moringa leaves and its native endophytic bacteria using different solvent systems. Aligning with the existing reports, the ethyl acetate extract of *moringa* leaves showed highest antimicrobial activities against the clinical pathogens tested **[37, 38]**. No zone of inhibition was found with Di methyl sulfoxide against all the pathogens tested and the iso propanol fractions showed absence of antimicrobial activities against *E.coli* and *Salmonella typhi.* However, aquoeus extract of moringa leaves prepared using distilled water showed zones of clearance to *Staphylococus aureus* and *Bacillus cereus* **(Figure 2, Table 3)**. Prabakaran *et al* **[28]** reported highest zone of inhibition by ethyl acetate extract of moringa leaves against pathogen *Pseudomonas aeruginosa* compared to *Erwinia chrysanthami. V*arious authors have reported the antimicrobial activities of other parts of moringa plants using ethyl acetate as solvent. Zaffer M *et al* (39) evaluated the antibacterial activity of *Moringa oliefera* bark extracts using different solvent systems. Their reports also showed that ethyl acetate extract of moringa bark showed highest antimicrobial activities against *Staphylococcus aureus, Citrobacter fruendii, Bacillus megaterium* and *Pseudomonas fluorescence*. Two different studies using seed and root extracts of *Moringa oleifera* **[40, 41]**, extracted using various organic solvent system reported that, ethyl acetate extracts of the plant parts showed highest antibacterial activities against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans* compared to other organic solvents like methanol, chloroform and aqueous extracts. Our findings are also found to be validated by these reports about the ability of ethyl acetate extracts of *Moringa oleifera* leaves to perform maximum antimicrobial activities against tested pathogens.

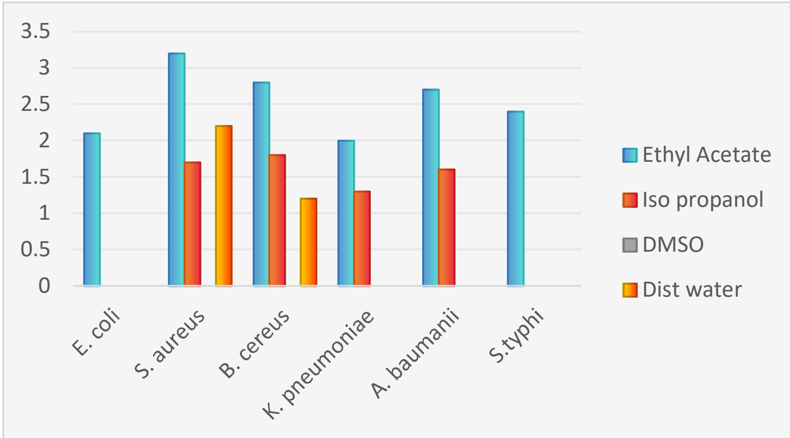


Figure 2: Antimicrobial activities of *Moringa oleifera* leaves using different solvents

Table 3: Antimicrobial activities of *Moringa oleifera* leaves using different solvents

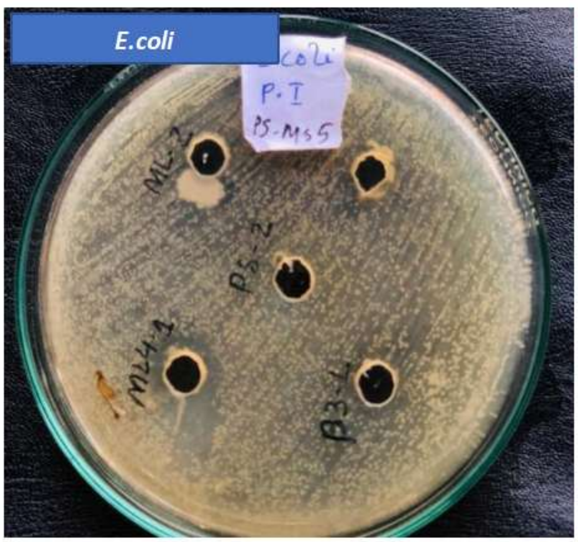
Clinical Pathogen	Ethyl acetate	Isopropanol	DMSO	Distilled water
	Zone of clearance (cm)			
E. coli	2.1	0	0	0
S. aureus	3.2	1.7	0	2.2
B. cereus	2.8	1.8	0	1.2
K. pneumoniae	2.0	1.3	0	0
A. baumanii	2.7	1.6	0	0
S.typhi	2.4	0	0	0

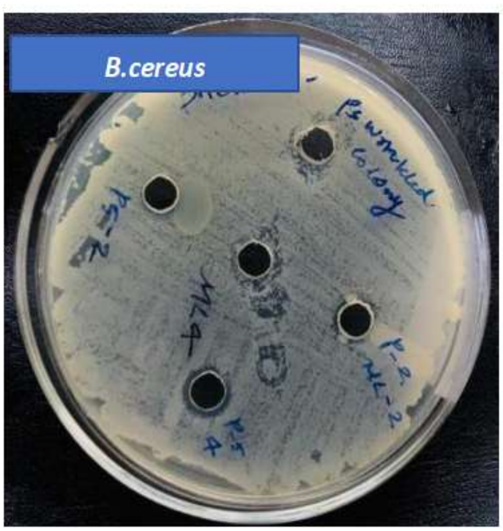
Meanwhile, the antimicrobial activities of the cell free culture filtrates of native endophytes against the same clinical pathogens using well diffusion technique, the results showed variations. The endophytic isolates MLEB 4 which was partially identified as *Pseudomonas sp.* showed zone of clearance to all the pathogens studied except *Salmonella typhi.* All the isolates showed antimicrobial activities against Gram positive pathogens *Bacillus cereus* and *Staphylococcus aureus.* The antimicrobial activities of the endophytic isolates to all gram negative pathogens shown variation in results and are detailed in **Table 4 and Figure 3**.

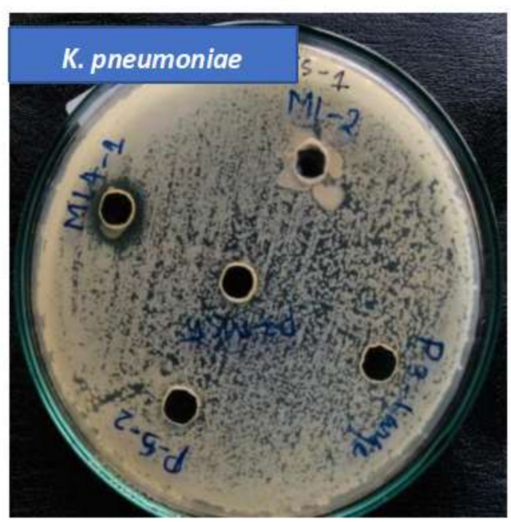
Although the native endophytic bacteria of *Moringa oleifera leaves* showed antimicrobial activities similar to its host plant, their antimicrobial spectrum showed variation. Notably, the absence of inhibition against *Salmonella typhi* could be due to the differences in metabolite complexity, concentration and specificity. The bacterial endophtes in this study might not be able to produce specific antimicrobial compounds against *Salmonella typhi* probably due to the absence of relevant biosynthetic gene clusters. Another possibility is inability of the antimicrobial compounds by endophtic bacteria to cross the cell wall of the pathogen and also might be in a low concentration to exert inhibition. Moreover, it can also be assumed that, the ethyl acetate extracts of the moringa leaves may contain an array of antimicrobial compounds that may act more synergistically, when compared to that of the native endophytic isolates from *moringa* leaves **[42, 43]**.

Table 4: Antimicrobial activities of the endophytic isolates of *Moringa oleifera leaves* against clinical pathogens

Clinical Pathogen	MLEB 1	MLEB 2	MLEB 3	MLEB 4	MLEB 5
	Zone of clearance (cm)				
E. coli	0	0	0	1.0	0
S. aureus	1.2	1.0	1.0	1.7	1.0
B. cereus	1.2	1.0	0.8	1.3	1.0
K. pneumoniae	0	0.5	0.4	1.2	0
A. baumanii	0	0	0	0.9	0
S.typhi	0	0	0	0	0







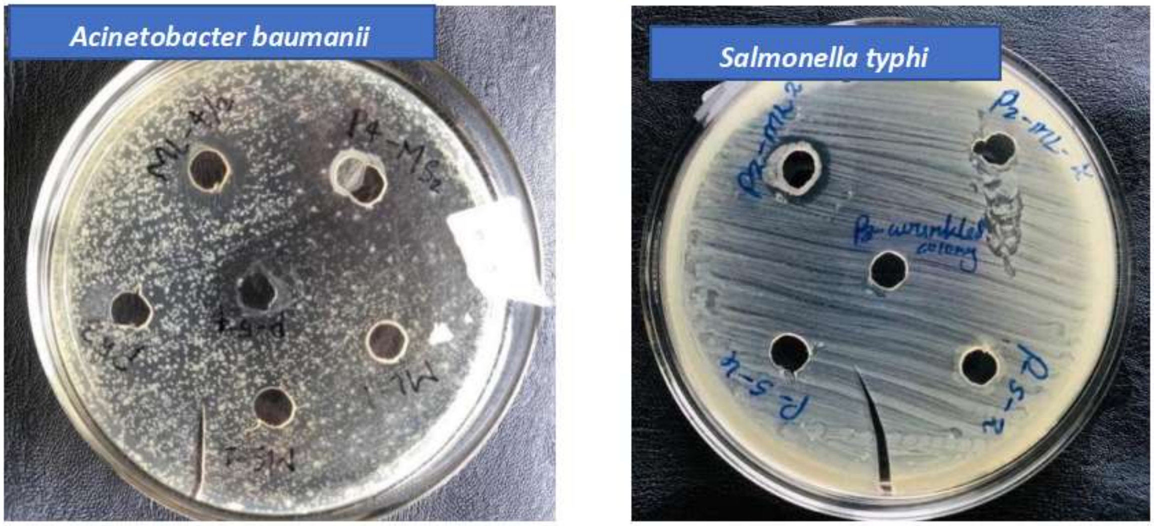


Figure 3: Antimicrobial activities of endophytic bacteria against clinical pathogens on MHA

Based on the highest antimicrobial activities given by ethyl acetate extracts of both moringa leaves and its native endophytic bacteria, ethyl acetate was selected for phytochemical screening and antioxidant activities with an assumption that this solvent has been effective in releasing the bioactive compounds from the leaves and bacterial cells. The ethyl acetate crude extracts of moringa leaves showed positive for all the phytochemicals tested except saponins.

The ethyl acetate crude extracts of the isolates showed various reactions to the screening for phytochemicals. All the isolates showed positive reactions to the presence of phenolic compounds. Endophytic isolate, MLEB4, identified as *Pseudomonas sp* showed positve reactions to the production of flavonoids and alkaloids in addition to phenolics. The isolate MLEB3 showed positive reaction to terpenoid production in addition to the phenolic compound production. Any isolates did not show positive reactions for the production of saponins, steroids and tannins. The results are listed in **Table 5**.

Table 5: Screening of endophytic isolates for the production of phytochemicals

	Phytochemicals	MLEB1	MLEB2	MLEB3	MLEB4	MLEB5
1	Alkaloids	Negative	Negative	Negative	Positive	Negative
2	Flavonoids	Negative	Negative	Negative	Positive	Negative
3	Phenols	Positive	Positive	Positive	Positive	Positive
4	Saponins	Negative	Negative	Negative	Negative	Negative
5	Steroids	Negative	Negative	Negative	Negative	Negative
6	Tannins	Negative	Negative	Negative	Negative	Negative
7	Terpenoids	Negative	Negative	Positive	Negative	Negative

The endophytic isolate MLEB4 only were found to have highest free radical scavengers in DPPH antioxidant assay **(Figure 4)**. The free radical scavenging activity of ethyl acetate extracts of moringa leaves was higher (72.68%) when compared to that of MLEB4, *Pseudomonas sp* (57.75%) the highest %RSA among endophytic isolates. For other endophytic isolates, the DPPH reducing activity was found to be comparatively lower 47.36%, 40.95%, 40.79% and 25.99% for MLEB5, MLEB3, MLEB1 and MLEB2 respectively. The highest activity was shown by the positive control Ascorbic acid (92.59%).

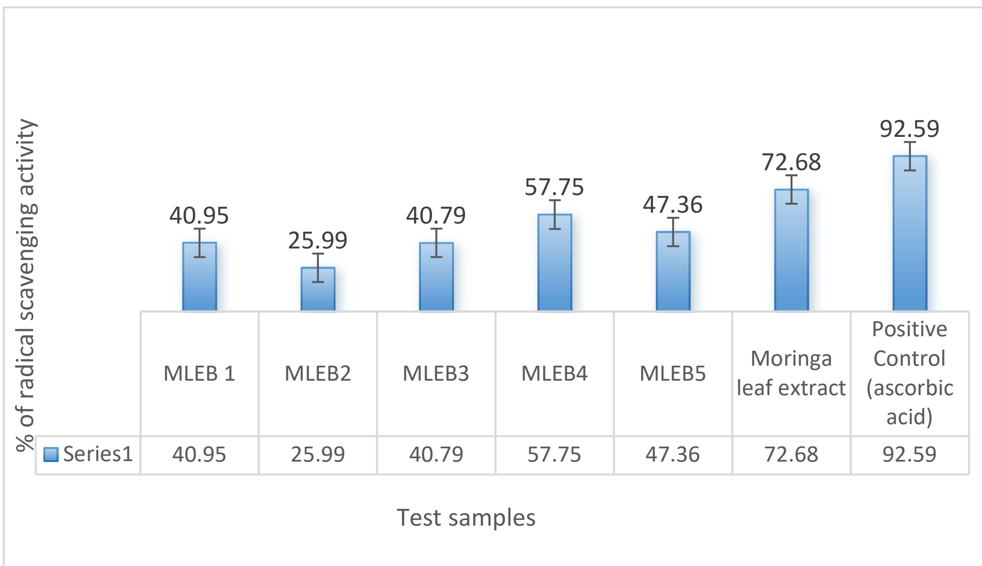


Figure 4: % of free radical scavenging activities of the endophytic isolates and moringa leaf extracts in the DPPH antioxidant assay

The preliminary screening of endophytic isolates from *Moringa oleifera* leaves in this study aligns with the findings of research on endophytes from various medicinal plants **[44]**. Production of phenolic compounds by the moringa leaf endophytic isolates is consistent with studies indicating that endophytic bacteria commonly synthesize phenolics, contributing to their host plants' antioxidant and antimicrobial properties. For example, endophytes from *Urtica dioica* were found to produce phenolic compounds, including caffeic and chlorogenic acids, enhancing the plant's antioxidant capacity **[45]**. In our study, production of phenolic compounds by the endophytic bacteria substantiates the antimicrobial and antioxidant activities of the isolates. The ability of isolate MLEB 4 (*Pseudomonas* sp.) to produce flavonoids and alkaloids is noteworthy. Endophytic *Pseudomonas* species have been reported to enhance alkaloid content in host plants. For instance, *Pseudomonas* sp. isolated from *Catharanthus roseus* roots increased the production of alkaloids like ajmalicine and serpentine by upregulating key biosynthetic genes **[46]**. It has been reported that, flavonoids and alakloids production by *Pseudomonas sp.* contributes to their antioxidant activities **[47]**. In our study,the highest antimicrobial activities and antioxidant activities by the isolate MLEB4, *Pseudomonas sp.*may be the cumulative effect of production of phenolic compounds, flavanoids and alkaloids.

The detection of terpenoid production in isolate MLEB 3 (*Bacillus* sp.) aligns with findings that endophytic bacteria can influence terpenoid biosynthesis. Research indicates that endophytes can either produce terpenoids directly or modulate their biosynthesis in host plants, and the endophytic isolates *Bradyrhizobium* and *Dugganella* showed gene clusters for terpene synthesis indicating their colaborative role in plant metabolite production **[49]**. Other than endophytic bacteria, endophytic fungi from different medicinal plants shown to produce terpenoids. In the detailed review by **[50]**, it is reported that 516 classes of terpenoids were obtained from different plant endophytic fungal species from 2011 to 2020. When a terpenoid producing endophytic fungus *Cladosporium cladosporioides,* cocultured with extracts of host medicinal plant *Cymbopogon martinii* showed enhanced production of plant secondary metabolites including terpenoids **[51]**. This shows the influence of host-endophyte interactions in regulating plant metabolite production. The lack of saponin, steroid, and tannin production among the isolates may be due to the specific metabolic capacities of the endophytic community in *Moringa oleifera*. Endophyte metabolite production varies widely among host plants and microbial species. While some endophytes produce a broad range of secondary metabolites, others are more specialized. It is reported that, endophytes from *Ephedra foliata* were found to produce alkaloids, flavonoids, and terpenoids but not saponins or tannins **[52]**.

CONCLUSION

Findings of the present study contribute to the growing body of evidence that endophytic bacteria from medicinal plants produce bioactive compounds mirroring those of their hosts. The investigation successfully demonstrates the comparable medicinal potential of native endophytes from *Moringa oleifera* leaves to that of the plant itself. This significantly put forwards the potential of endophytes as alternative sources for valuable phytochemicals, which could reduce the need for extensive plant harvesting. The antimicrobial, antioxidant activities and the phytochemical production by the endophytic isolates place them as promising area of research for applications in agriculture, medicine, and beyond. These bacteria produce bioactive compounds capable of scavenging free radicals, reduce oxidative stress, and mitigate overall health.

REFERENCES

[1] Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of traditional and complementary medicine. 2017 Apr 1;7(2):234-44.

[2] Martínez-González CL, Martínez L, Martínez-Ortiz EJ, González-Trujano ME, Déciga-Campos M, Ventura-Martínez R, Díaz-Reval I. *Moringa oleifera*, a species with potential analgesic and anti-inflammatory activities. Biomedicine & Pharmacotherapy. 2017 Mar 1;87:482-8.

[3] Divya S, Pandey VK, Dixit R, Rustagi S, Suthar T, Atuahene D, Nagy V, Ungai D, Ahmed AE, Kovács B, Shaikh AM. Exploring the Phytochemical, Pharmacological and Nutritional Properties of *Moringa oleifera*: A Comprehensive Review. Nutrients. 2024 Oct 9;16(19):3423.

[4] Mishra G, Singh P, Verma R, Kumar S, Srivastav S, Jha KK, Khosa RL. Traditional uses, phytochemistry and pharmacological properties of *Moringa oleifera* plant: An overview. Der Pharmacia Lettre. 2011;3(2):141-64.

[5] Attia MS, Salem MS, Abdelaziz AM. Endophytic fungi *Aspergillus* spp. reduce fusarial wilt disease severity, enhance growth, metabolism and stimulate the plant defense system in pepper plants. Biomass Conversion and Biorefinery. 2024 Jul;14(14):16603-13.

[6] Hashem AH, Al-Askar AA, Abd Elgawad H, Abdelaziz AM. Bacterial endophytes from *Moringa oleifera* leaves as a promising source for bioactive compounds. Separations. 2023 Jul 6;10(7):395.

[7] Akinsanya MA, Goh JK, Lim SP, Ting AS. Diversity, antimicrobial and antioxidant activities of culturable bacterial endophyte communities in Aloe vera. FEMS microbiology letters. 2015 Dec 1;362(23):fnv184.

[8] Enyi E, Ekpunobi N. Secondary metabolites from endophytic fungi of

Moringa oleifera: Antimicrobial and antioxidant properties. J Microbiol Exp. 2022;10(5):150-4.

[9] Ebabhi A, Samuel T, Adekunle A. Investigation of endophytic fungi in *Moringa oleifera* Lam. and determination of the phytochemical and proximate contents of its leaves and seeds. CaJoST. 2023 Aug 23;5(2):184-92.

[10] Ebabhi A, Samuel T, Adekunle A. Investigation of endophytic fungi in *Moringa oleifera* Lam. and determination of the phytochemical and proximate contents of its leaves and seeds. CaJoST. 2023 Aug 23;5(2):184-92.

[11] Reda F, Borjac J, Fakhouri R, Usta J. Cytotoxic effect of *Moringa oleifera* on colon cancer cell lines. Acta Hortic. 2017;1158:45-54.

[12] Oluduro OA, Aderiye BI, Connolly JD, Akintayo ET, Famurewa O. Characterization and antimicrobial activity of 4-([beta]-d-glucopyranosyl-1 [arrow right] 4-[alpha]-l-rhamnopyranosyloxy)-benzyl thiocarboxamide; a novel bioactive compound from *Moringa oleifera* seed extract. Folia Microbiologica. 2010 Sep 1;55(5):422.

[13] Rao RR, George M, Pandalai KM. Pterygospermin: the antibacterial principle of *Moringa pterygosperma*, Gaertn. Nature. 1946 Nov 23;158(4021):745-6.

[14] Giacoppo S, Galuppo M, De Nicola GR, Iori R, Bramanti P, Mazzon E. 4 (α-l-rhamnosyloxy)-benzyl isothiocyanate, a bioactive phytochemical that attenuates secondary damage in an experimental model of spinal cord injury. Bioorganic & medicinal chemistry. 2015 Jan 1;23(1):80-8.

[15] Hassan MA, Xu T, Tian Y, Zhong Y, Ali FA, Yang X, Lu B. Health benefits and phenolic compounds of *Moringa oleifera* leaves: A comprehensive review. Phytomedicine. 2021 Dec 1;93:153771.

[16] Setyani W, Murwanti R, Sulaiman TN, Hertiani T. Flavonoid from *Moringa oleifera* leaves revisited: A review article on in vitro, in vivo, and in silico studies of antidiabetic insulin-resistant activity. Journal of Advanced Pharmaceutical Technology & Research. 2023 Oct 1;14(4):283-8.

[17] Sivapragasam G, Devi TG, Ragavan ND, Suryadevara N, Sridewi N, Al Obaid S, Alharbi SA, Arulselvan P. A Comparative Phytochemical Characterization of *Moringa oleifera* Plant Parts by Different Solvent Extraction. Indian Journal of Pharmaceutical Education and Research.

2024 May 27;58(2s): s648-59.

[18] Mehmood A, Javid S, Khan MF, Ahmad KS, Mustafa A. In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC chemistry. 2022 Aug 27;16(1):64.

[19] Strobel G, Daisy B. Bioprospecting for microbial endophytes and their natural products. Microbiology and molecular biology reviews. 2003 Dec;67(4):491-502.

[20] Azra WN, Susilo B, Izza NM, Rohim A, Samudra RP. Extraction of Bioactive Compounds in Moringa Leaves (*Moringa oleifera* Lam.) using Modified Sonication Technique. In BIO Web of Conferences 2024 (Vol. 123, p. 02003). EDP Sciences.

[21] Shabanamol S, Divya K, George TK, Rishad KS, Sreekumar TS, Jisha MS. Characterization and in planta nitrogen fixation of plant growth promoting endophytic diazotrophic *Lysinibacillus sphaericus* isolated from rice (*Oryza sativa*). Physiological and Molecular Plant Pathology. 2018 Apr 1;102:46-54.

[22] Gunasekaran S, Sathiavelu M, Arunachalam S. In vitro antioxidant and antibacterial activity of endophytic fungi isolated from *Mussaenda luteola*. Journal of applied pharmaceutical science. 2017 Aug 30;7(8):234-8.

[23] Bhardwaj A, Sharma D, Jadon N, Agrawal PK. Antimicrobial and phytochemical screening of endophytic fungi isolated from spikes of *Pinus roxburghii*. Arch clin microbiol. 2015;6(3):1-9.

[24] Sulistiyani S, Ardyati T, Winarsih S. Antimicrobial and antioxidant activity of endophyte bacteria associated with *Curcuma longa* rhizome. The Journal of Experimental Life Science. 2016;6(1):45-51.

[25] Pérez MJ, Falqué E, Domínguez H. Antimicrobial action of compounds from marine seaweed. Marine drugs. 2016 Mar 9;14(3):52.

[26] Kashyap P, Kumar S, Riar CS, Jindal N, Baniwal P, Guiné RP, Correia PM, Mehra R, Kumar H. Recent advances in Drumstick (*Moringa oleifera*) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. Antioxidants. 2022 Feb 16;11(2):402.

[27] Madhu M, Sailaja V, Satyadev TN, Satyanarayana MV. Quantitative phytochemical analysis of selected medicinal plant species by using various organic solvents. Journal of Pharmacognosy and Phytochemistry.

2016 Mar 1;5(2):25-9.

[28] Prabakaran M, Kim SH, Sasireka A, Chandrasekaran M, Chung IM. Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of *Moringa oleifera*. Food bioscience. 2018 Dec 1;26:23-9.

[29] Duhan P, Bansal P, Rani S. Isolation, identification and characterization of endophytic bacteria from medicinal plant *Tinospora cordifolia*. South African journal of botany. 2020 Nov 1;134:43-9.

[30] Rustamova N, Wubulikasimu A, Mukhamedov N, Gao Y, Egamberdieva D, Yili A. Endophytic bacteria associated with medicinal plant *Vernonia anthelmintica*: diversity and characterization. Current Microbiology. 2020 Aug;77:1457-65.

[31] Idris HA, Adetunji CO. Effect of Endophytic Serratia marcescens Isolated from *Bryophyllum pinnatum* against Clinical Bacterial Isolates. Traektoriâ Nauki Path of Science. 2024;10(4):11001-6.

[32] Devi KA, Pandey P, Sharma GD. Plant growth-promoting endophyte Serratia marcescens AL2-16 enhances the growth of *Achyranthes aspera* L., a medicinal plant. HAYATI Journal of Biosciences. 2016 Oct 1;23(4):173-80.

[33] Al-Rahbi BA, Al-Sadi AM, Al-Harrasi MM, Al-Sabahi JN, Al-Mahmooli IH, Blackburn D, Velazhahan R. Effectiveness of endophytic and rhizospheric bacteria from Moringa spp. in controlling *Pythium aphanidermatum* damping-off of cabbage. Plants. 2023 Feb 2;12(3):668.

[34] Aljuraifani A, Aldosary S, Ababutain I. In vitro antimicrobial activity of endophytes, isolated from *Moringa peregrina* growing in eastern region of Saudi Arabia. National Academy Science Letters. 2019 Feb 4;42:75-80.

[35] Hashem AH, Al-Askar AA, Abd Elgawad H, Abdelaziz AM. Bacterial endophytes from *Moringa oleifera* leaves as a promising source for bioactive compounds. Separations. 2023 Jul 6;10(7):395.

[36] Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou E. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms. 2021 Sep 27;9(10):2041.

[37] Abalaka ME, Daniyan SY, Oyeleke SB, Adeyemo SO. The antibacterial evaluation of *Moringa oleifera* leaf extracts on selected bacterial pathogens.

Journal of Microbiology research. 2012;2(2):1-4.

[38] Ezugwu RI, Chukwubike C. Evaluation of the antimicrobial activity of *Moringa oleifera* leaves extract on Helicobacter pylori. IOSR J. Pharm. Biol. Sci. 2014;9:57-60.

[39] Zaffer M, Ahmad S, Sharma R, Mahajan S, Gupta A, Agnihotri RK. Antibacterial activity of bark extracts of *Moringa oleifera* Lam. against some selected bacteria. Pak J Pharm Sci. 2014 Nov 1;27(6):1857-62.

[40] Emmanuel SA, Olajide OO, Abubakar S, Idowu ID, Orishadipe AT, Thomas SA. Phytochemical and antimicrobial studies of methanol, ethyl acetate, and aqueous extracts of *Moringa oleifera* seeds. American Journal of Ethnomedicine. 2014;1(5):346-54.

[41] Raj AJ, Gopalakrishnan VK, Yadav SA, Dorairaj S. Antimicrobial activity of *Moringa oleifera* (Lam.) root extract. Journal of Pharmacy Research. 2011 May;4(5):1426-7.

[42] Ahmed, M., Marrez, D.A., Abdelmoeen, N.M., Mahmoud, E.A., Abdel-Shakur Ali, M., Decsi, K. and Tóth, Z., 2023. Proximate analysis of *Moringa oleifera* leaves and the antimicrobial activities of successive leaf ethanolic and aqueous extracts compared with green chemically synthesized Ag-NPs and crude aqueous extract against some pathogens. *International Journal of Molecular Sciences*, *24*(4), p.3529.

[43] Sharma M, Mallubhotla S. Diversity, antimicrobial activity, and antibiotic susceptibility pattern of endophytic bacteria sourced from *Cordia dichotoma* L. Frontiers in microbiology. 2022 May 13;13:879386.

[44] Semenzato G, Fani R. Endophytic bacteria: a sustainable strategy for enhancing medicinal plant cultivation and preserving microbial diversity. Frontiers in Microbiology. 2024 Nov 18;15:1477465.

[45] Chen Y, Hu B, Xing J, Li C. Endophytes: the novel sources for plant terpenoid biosynthesis. Applied microbiology and biotechnology. 2021 Jun;105(11):4501-13.

[46] Singh S, Pandey SS, Shanker K, Kalra A. Endophytes enhance the production of root alkaloids ajmalicine and serpentine by modulating the terpenoid indole alkaloid pathway in *Catharanthus roseus* roots. Journal of Applied Microbiology. 2020 Apr;128(4):1128-42.

[47] Urumbil SK, Anilkumar MN. Anti-inflammatory activity of endophytic bacterial isolates from *Emilia sonchifolia* (Linn.) DC. Journal of Ethnopharmacology. 2021 Dec 5;281:114517.

[48] Dholakiya RN, Kumar R, Mishra A, Mody KH, Jha B. Antibacterial and antioxidant activities of novel actinobacteria strain isolated from Gulf of Khambhat, Gujarat. Frontiers in microbiology. 2017 Dec 7;8:2420.

[49] Tamang A, Swarnkar M, Kumar P, Kumar D, Pandey SS, Hallan V. Endomicrobiome of in vitro and natural plants deciphering the endophytes-associated secondary metabolite biosynthesis in *Picrorhiza kurrooa*, a Himalayan medicinal herb. Microbiology Spectrum. 2023 Dec 12;11(6):e02279-23.

[50] Amirzakariya BZ, Shakeri A. Bioactive terpenoids derived from plant endophytic fungi: an updated review (2011–2020). Phytochemistry. 2022 May 1;197:113130.

[51] Jayaram H, Marigowda V, Thara Saraswathi KJ. Secondary metabolite production and terpenoid biosynthesis in endophytic fungi *Cladosporium cladosporioides* isolated from wild *Cymbopogon martinii* (Roxb.) Wats. Microbiology research. 2021 Nov 1;12(4):812-28.

[52] Ghiasvand M, Makhdoumi A, Matin MM, Vaezi J. Exploring the bioactive compounds from endophytic bacteria of a medicinal plant: *Ephedra foliata* (Ephedrales: Ephedraceae). Advances in Traditional Medicine. 2020 Mar; 20: 61-70.