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Research Article

In Vitro Screening of Bioactive Endophytic Microorganisms Isolated from Medicinal Flora of the Himalayan Region

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Abstract

Plant growth and its defence mechanism have been facilitated by endophytic microorganism, in symbiotic association with bacteria found in various plant sections. This study, focuses on the isolation and characterization of endophytes utilizing different medicinal herbs like *Achyranthes bidentata, Asparagus racemosus, Centella asiatica, Withania somnifera*, and *Viola odorata, Urtica dioica, Eupatorium adenophorum, Carica papaya, Thuja occidentalis,* and *Cinnamonum tamala* that are well known for their therapeutic efficacy. Endophytic microorganisms were isolated, out of which some were bacterial and some were fungal species. These endophytic organisms were further screened for their antimicrobial, antioxidant, and plant growth-promoting activities. Among fungi, *Arthrinium* sp. from *Cinnamonum tamala* exhibited maximum free radical scavenging activity of 93.95%, while Alternaria sp. showed the minimum of 77.32% free radical scavenging activity. In antifungal assays, *Alternaria sp. and Fusarium sp.* inhibited *Aspergillus niger* and *Penicillium notatum*, while *Ulocladium* sp. inhibited *Rhizopus* sp. In antibacterial tests, *Bipolaris* sp. from Thuja occidentalis showed the largest zone of inhibition (32mm), while *Alternaria* sp. showed the smallest (9mm). Minimum inhibitory concentration (MIC) assays revealed that the fungi ethyl acetate extracts of the effectively inhibited pathogenic bacteria, with MIC values as low as 0.625mg/100µl. These insights reveal the potential of endophytic microorganisms in sustainable agriculture and therapeutic development, offering promising leads for combating antibiotic-resistant pathogens.

Keywords: Antibiotic Resistance; Endophytes; Medicinal Plants; Antioxidant

Introduction

Numerous microbial populations, including bacteria and fungus, have been identified as endophytes and linked to the interior tissues of plants. De Bary (1866) first used the word "endophyte" to describe the presence of microorganisms within infected plant tissues that do not negatively impact the host plant [1]. Almost all the plants have been found to be infested with one or more endophytes [2]. By releasing beneficial secondary metabolites, these endophytes shield their hosts against pathogens and unfavorable circumstances [2-4].

Endophytic fungi, in particular, are recognized for their critical physiological [5] and ecological [6] contributions to plant life. They assist in nutrient acquisition, enhance the plant's stress tolerance, and modulate the host's defence mechanisms. These fungi and bacteria synthesize a diverse range of bioactive compounds, including antimicrobial agents effective against plant pathogens, insect- and pest-repellent molecules, phytohormones that promote plant growth, and metabolites that enable plants to withstand abiotic stresses such as drought, salinity, and temperature extremes [7]. Moreover, endophytes possess the ability to produce unique secondary metabolites with significant potential for application in various fields, including agriculture, pharmaceuticals, and biotechnology [8]. For instance, in agriculture, these metabolites can be utilized as natural biopesticides and biofertilizers to promote

sustainable farming practices. In the pharmaceutical sector, endophytes have emerged as a promising source of novel antibiotics, anticancer compounds, and other therapeutic agents [9]. The ecological importance of endophytes extends to their role in maintaining biodiversity, improving soil health, and facilitating ecosystem stability. Endophytes represent a diverse group of microorganisms with immense potential for contributing to plant health and productivity, as well as offering innovative solutions for challenges in agriculture, medicine, and environmental sustainability [10]. The ongoing exploration of endophytic diversity and their metabolic capabilities is crucial for unlocking their full potential. In the present study, *Urtica dioica*, *Eupatorium adenophorum* (also known as *Ageratina adenophora*), *Carica papaya, Thuja occidentalis*, *Cinnamonum tamala, Achyranthes bidentata, Asparagus racemosus, Centella asiatica, Withania somnifera* and *Viola odorata,* are some of the valuable curative herbs that were screened. The main objectives are the isolation and identification of endophytes (fungi and bacteria), fermentation and extraction of metabolites (ethyl acetate) from isolated fungal isolates and to investigate *in vitro* antioxidant efficacy of endophytic fungal ethyl acetate extracts by DPPH free radical scavenging method and antimicrobial efficacy of endophytic fungal and bacterial isolates by dual culture, agar well diffusion and minimum inhibitory concentration method.

Material and Methods

Chemical and reagent

All the chemical and reagent utilized in the current analysis were of high purity and analytical grade (AR/GR), and they were collected from Hi-Media Pvt. Ltd. Mumbai, E-Merck, and others companies.

Test organisms

In the present work, test microorganisms were used *i.e.,* Bacterial (*P. aeruginosa, S. aures, B. cereus, E. coli, S. typi, S. flexneri, K. pneumoniae)* and fungal strains (*Aspergillus* sp., *Penicillium* sp.*, Rhizopus* sp.*,*).

Sample collection

In this study,the roots,stem and leaves of the plants viz., *Urtica dioica*, *Eupatorium adenophorum* (also known as *Ageratina adenophora*), *Carica* papaya*,* Thuja *occidentalis*, Cinnamonum *tamala, Achyranthes bidentata ,Asparagus racemosus*, *Centella asiatica*, *Withania somnifera* and *Viola odorata* were collected from different areas of Himachal Pradesh and Uttarakhand.

Isolation of endophytic microorganisms

For the isolation of endophytes, healthy plant parts were washed in running tap water. A sterile cutter and forceps were used to chop the leaves into little pieces. Following that, each segment was put on nutrient agar (NA) and potato dextrose agar (PDA) media and then the plates were incubated at 37°C. After inoculation period, different fungal and bacterial strains were isolated by subculturing the hyphal tips onto a fresh PDA and nutrient agar media [11].

Identification of isolated endophytic microorganisms

Isolated endophytic bacterial isolates were identified by gram staining and different biochemical tests viz., methyl red, indole test, Voges Proskauer, oxidase test, citrate test catalase test, motility test and fermentation of different sugars for the confirmation of isolate [12,13] and for fungal isolates by Lactophenol picric acid [14].

Preservation of isolated endophytic microorganisms

Pure endophytic fungal and bacterial isolates were then transferred separately to PDA and nutrient agar slants [15]. Growths of endophytic bacterial and fungal strains were observed for 24 hours and 72 hours respectively and were maintained at 4°C till further use [16].

Fermentation and extraction of metabolites from endophytic microorganisms i.e., bacterial extract and fungal ethyl acetate extract

The cultivation of isolated fungal endophytes was conducted in conical flasks containing a sterilized rice medium. The rice was pre-soaked in sterile water within a glass container [17]. After cooling, the solidified rice medium was aseptically inoculated with pure mycelial cultures of the isolated endophytic fungi. The resulting supernatant was evaporated to dryness, yielding crude fungal extracts [18]. Similarly, all endophytic bacterial strains were cultured in nutrient broth, followed by centrifugation of each bacterial culture. The resulting supernatant was collected and stored in collection tubes as bacterial extracts [19].

In vitro antioxidant activity of endophytic fungal ethyl acetate extract

The free radical scavenging activity of fungal extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Fungal extracts and a positive control (DPPH dissolved in distilled water) were prepared in methanol and then combined with DPPH in methanol [20]. A mixture of methanol and DPPH without the fungal extract was used as the control. The experiment was conducted in triplicate, and the inhibition of DPPH was expressed as a percentage of radical scavenging activity at different extract concentrations [21].

In Vitro antifungal screening of endophytic fungal isolates by dual culture method

Ethyl acetate extracts of endophytic fungal isolates were evaluated for antifungal activity against standard pathogenic fungal strains, including *Aspergillus* sp., *Penicillium* sp., and *Rhizopus* sp., using the dual culture method. Potato dextrose agar (PDA) was utilized as the culture medium [22]. Mycelial plugs of both the pathogens and endophytic fungi were placed at a specific distance from each other on PDA plates. The incubation of each endophytic fungal isolate against pathogenic strains were observed by the growth pattern of fungus by comparing with controls [23].

In vitro Antibacterial Activity of endophytes

The activity of endophytic ethyl acetate fungal extracts against various pathogenic bacteria was evaluated using the agar well diffusion method. Additionally, the minimum inhibitory concentration (MIC) of the endophytic bacterial extracts was determined using the resazurin dye assay [24].

Antibacterial activity of endophytic fungal ethyl acetate extract by agar well diffusion method

The antimicrobial compounds in the ethyl acetate extract of endophytic fungi were allowed to diffuse into the agar medium, interacting with freshly inoculated test bacteria, including *P. aeruginosa*, *S. aureus*, *B. cereus*, *S. typhi*, *S. flexneri*, and *E. coli*. The inhibition zones formed around the wells were measured using a diameter scale, as described by [25]. Each extract was tested against these pathogenic bacterial strains, along with positive controls, including chloramphenicol, amoxicillin, clindamycin, azithromycin, ciprofloxacin, azlocillin, and imipenem. The diameter of the inhibition zones was recorded following the method of [26,27].

Evaluation of minimum inhibitory concentration (MIC) using the resazurin dye assay

MIC is the concentration of the higher dilution in which the absence of bacterial growth occurs. Sterile microtitre plates (Tarson) were used. Rows were filled with Muller Hinton broth. Fungal extracts showing maximum activity in first column and further 2-fold serial dilution was achieved Now 10µl of different bacterial suspension were added in each row separately of the microtitre plate [28,29]. Now 1µl of Resazurin dye was added in the entire plate as an indicator dye. Positive and negative controls were also used. Furthermore, a shift in color from purple to pink or a loss of color (colorless) was interpreted as a negative outcome [30].

Result and Discussion

Isolation of endophytes from various plants

Different endophytic microorganisms were isolated from stem, leaves and roots of different plants (table 1) viz., Urtica *dioica, Eupatorium adenophorum* (leaf, stem, root), *Carica papaya, Thuja occidentalis,* and *Cinnamonum tamala* which were collected from different district of Himachal Pradesh (Shimla, Solan, Bilaspur), and Uttarakhand (Dehradun, Rudraprayag), *Achyranthes bidentata, Asparagus racemosus, Centella asiatica, Withania somnifera* and *Viola odorata* from H.P.

Identification of Endophytic microorganism isolated from different medicinal plants

The identification of endophytic fungi was done by Lactophenol picric acid and bacteria by gram staining, further isolated bacterium was identified by using different biochemical tests viz., indole test, methyl red, Voges Proskauer, citrate test, oxidase, catalase, motility test and fermentation of different sugars for the identification of isolates. In present study the isolated endophytic fungi were identified on the basis of colony morphology, microscopic examination-like shapes of conidia, mycelium and hyphae and endophytic bacteria were identified by gram staining and by different sugar tests.

Preservation of isolated endophytic microorganisms

The isolated endophytic isolates were preserved by slant preparation method in refrigerator at 4°C on PDA for fungal isolates and Nutrient agar for bacterial isolates (figure 2).

Sr. No.	Medicinal plant	Location	Types of isolates	No. of isolates
1.	*Urtica dioica*	Himachal Pradesh	Bacterial	3
			Fungal	1
		Uttarakhand	Bacterial	1
			Fungal	1
2.	*Eupatorium adenophorum*	Himachal Pradesh	Bacterial	2
			Fungal	1
		Uttarakhand	Bacterial	2
			Fungal	1
3.	*Carica papaya*	Himachal Pradesh	Bacterial	-
			Fungal	-
		Uttarakhand	Bacterial	1
			Fungal	-
4.	*Thuja occidentalis*	Himachal Pradesh	Bacterial	1
			Fungal	1
		Uttarakhand	Bacterial	1
			Fungal	2
5.	*Cinnamomum tamala*	Himachal Pradesh	Bacterial	2
			Fungal	1
		Uttarakhand	Bacterial	1
			Fungal	-
6.	*A. bidentata*	Himachal Pradesh	Bacterial	3
			Fungal	2
7.	*A. racemosus*	Himachal Pradesh	Bacterial	2
			Fungal	-
8.	*C. asiatica*	Himachal Pradesh	Bacterial	2
			Fungal	-
9.	*W. somnifera*	Himachal Pradesh	Bacterial	2
			Fungal	2
10.	*V. odorata*	Himachal Pradesh	Bacterial	1
			Fungal	1
Total			Bacterial	24
			Fungal	13

Table 1: Endophytic microorganisms isolated from different medicinal plants.

Fermentation and extraction of metabolites from isolated endophytic fungal ethyl acetate extract

All the isolated endophytic fungal isolates were used for the extraction of fungal metabolites which were extracted by using ethyl acetate. Whole process took 9-10 days. The endophytic ethyl acetate extract was then dissolved in Dimethyl sulfoxide (DMSO) (figure 3).

In vitro Antioxidant Activity of isolated Endophytic Fungal ethyl acetate extracts

All the isolated endophytic fungal ethyl acetate extract were having antioxidant activity where they showed ability to reduce free radicals. In contrast with the current investigation, Selim and coworkers reported that *Ulocladium* sp. isolated from *Pulicaria undulate* showed 14% antioxidant activity and *Ulocladium* sp. isolated from *Galium sanaicum* don't showed any activity [31].

Figure 1: Results of different biochemical tests of isolated Endophytic bacterial isolates.

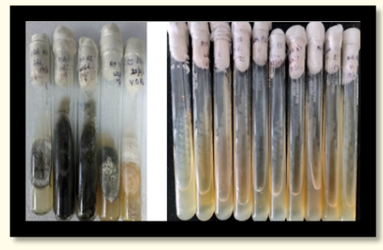

Figure 2: Slants of isolated endophytic bacterial and fungal isolates.

Figure 3: (a) Growth of isolated endophytic fungal isolates in solid rice media
(b) different isolated endophytic fungal ethyl acetate extracts.

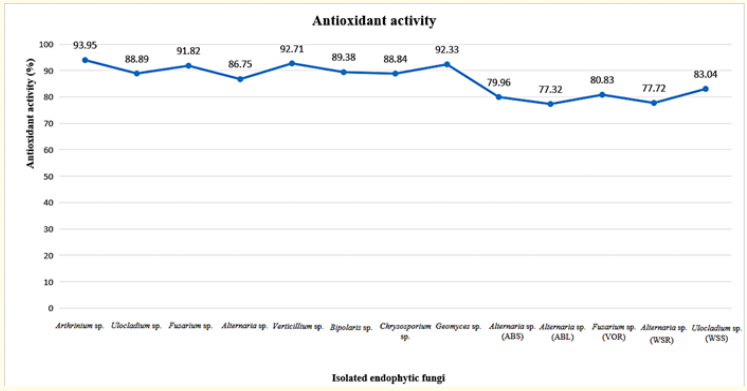

Figure 4: DPPH Radical Scavenging Activity of isolated endophytic fungal ethyl acetate extracts.

Table 2: Antioxidant activity (%) of different isolated endophytic fungal ethyl acetate extracts.

Sr. No.	Sample (Endophytic fungal ethyl acetate extract)	Antioxidant Activity (%)
1.	*Arthrinium* sp.	93.95
2.	*Ulocladium* sp.	88.89
3.	*Fusarium* sp.	91.82
4.	*Alternaria* sp.	86.75
5.	*Verticillium* sp.	92.71
6.	*Bipolaris* sp.	89.38
7.	*Chrysosporium* sp.	88.84
8.	*Geomyces* sp.	92.33
9.	*Alternaria* sp. (ABS)	79.96
10.	*Alternaria* sp. (ABL	77.32
11.	*Fusarium* sp. (VOR)	80.83
12.	*Alternaria* sp. (WSR)	77.72
13	*Ulocladium* sp. (WSS)	83.04

Anti-fungal screening of endophytic fungal isolates by dual culture method

The isolated endophytic fungal isolates were tested against 3 pathogenic fungi viz. *Penicillium notatum, Rhizopus stolonifer* and *Aspergillus niger*. In the present study isolated endophytic fungi *Verticillium* sp., *Chrysosporium* sp., *Bipolaris* sp., and *Fusarium* sp. showed antagonism against pathogenic fungus *Aspergillus niger* and *Penicillium notatum* were as *Arthrinium* sp., *Alternaria* sp. showed antagonism against *Aspergillus niger* and *Ulocladium* sp. against *Penicillium notatum. Alternaria* sp. from *A. bidentata* and *W. somnifera, Fusarium* sp. from *V. odorata* showed antifungal activity against *Aspergillus niger* but *Ulocladium* sp. from *W. somnifera* was unable to inhibit *Aspergillus niger*. In case of *Penicillium notatum, Alternaria* sp. from *A. bidentata* and *W. somnifera, Fusarium* sp. from *V. odorata* but *Ulocladium* sp. from *W. somnifera* donot showed any inhibitory activity. In case of *Rhizopus stolonifer*, *Alternaria* sp. from *W. somnifera*, *Ulocladium* sp. of *W. somnifera* and *Fusarium* sp. of *V. odorata* showed antagonistic effect but *Alternaria* sp. of *A. bidentata* does not.

Antibacterial activity of endophytic microorganisms:

Antibacterial activity of endophytic fungal ethyl acetate extract by agar well diffusion method

In the present study antibacterial activity of isolated fungal ethyl acetate extracts and endophytic bacterial isolate were checked against various pathogenic microorganisms viz., *Bacillus cereus, E.*

coli, Staphylococcus aureus, Shigella flexneri, Salmonella typhi and Pseudomonas aeruginosa by agar well diffusion method on Muller Hinton Agar. Positive controls were also run (Chloramphenicol, Amoxicillin, Clindamycin, Azithromycin, Ciproflaxin, Azlocillin and Imipenem). *Bacillus cereus, E. coli, Staphylococcus aureus, Shigella flexneri, Salmonella typhi* and *Pseudomonas aeruginosa* on Muller Hinton Agar along with positive controls (Chloramphenicol, Amoxicillin, Clindamycin, Azithromycin, Ciprofaxin, Azlocillin and imipenem) and negative control (Sterile nutrient broth).The maximum zone of inhibition was shown by *Bipolaris* sp. isolated from *Thuja occidentalis i.e.* 32mm against *Shigella flexneri* and minimum zone of inhibition was shown by *Ulocladium* sp. and *Fusarium* sp. isolated from *Urtica dioica* (Uk) and *Eupatorium adenophorum* (UK and HP) i.e. 10mm against *S. typhi* and *P. areuginosa*. (Figure 5, 6 and 7 and table 3 and 4). Similar type of investigation was done where Enany and coworkers reported that *Ulocladium* sp. isolated from *Mentha piperita* showed 8mm zone against *Pseudomonas aeruginosa* isolated from infected freshwater fishes which is lower than the present study [32].

Table 3: Zone of inhibition measured in millimeter against pathogenic bacterial strains.

Zone of inhibition (ZOI) in millimeter						
Bacterial strains	***Bacillus cereus***	***Escherichia coli***	***Staphylococcus aureus***	***Shigella flexneri***	***Salmonella typhi***	***Pseudomoas aeruginosa***
Zone of inhibition of positive controls	Chloram phenicol	Amoxicillin	Clindamycin	Azithromycin	Ciproflaxin	Aziocillin
	30mm	24mm	28mm	33mm	25mm	30mm
Zone of inhibition (ZOI) of isolated endophytic fungal ethyl acetate extract and endophytic bacterium						
Verticillium sp.	16mm	11mm	20mm	26mm	13mm	19mm
Chrysosporium sp.	-	-	14mm	15mm	-	-
Bipolaris sp.	23mm	17mm	20mm	32mm	20mm	23mm
Arthrinium sp.	17mm	12mm	19mm	23mm	-	12mm
Fusarium sp.	13mm	-	22mm	24mm	-	10mm
Alternaria sp.	19mm	11mm	22mm	25mm	13mm	21mm
Ulocladium sp.	23mm	-	20mm	28mm	10mm	16mm
Geomyces sp.	28mm	17mm	24mm	30mm	20mm	28mm
Bacillus acidiceler	-	24mm	23mm	-	23mm	22mm
Paenibacillus apiaries	-	20mm	23mm	-	21mm	21mm

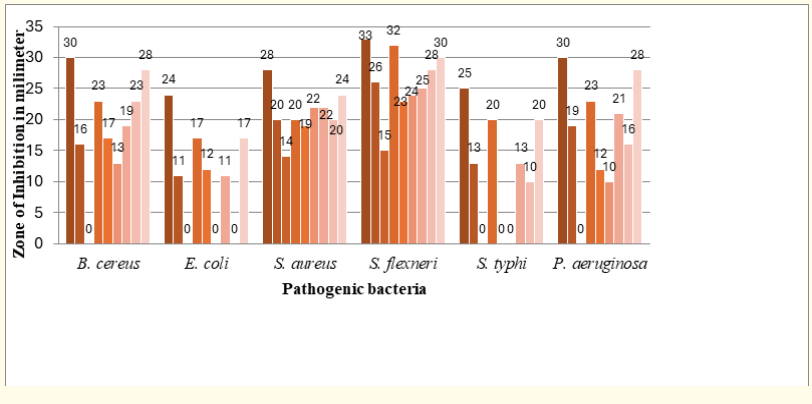

Figure 5: Activity of Endophytic fungal ethyl acetate extract against different pathogenic bacteria on the basis of ZOI formation.

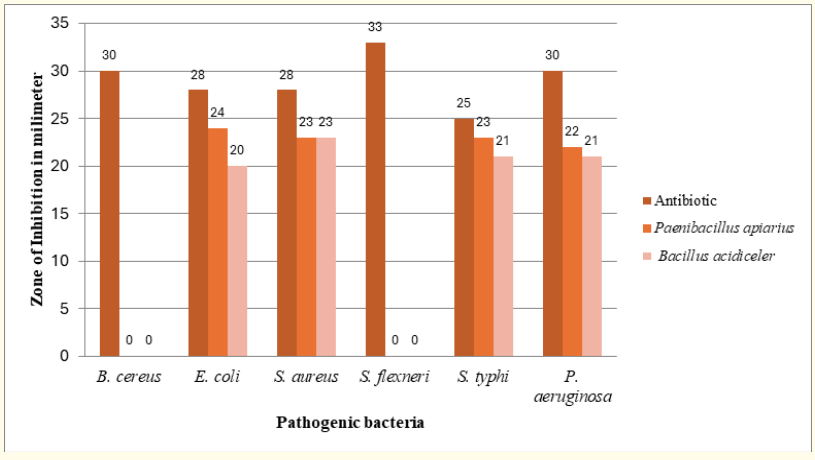

Figure 6: Activity of Endophytic bacterium against different pathogenic bacteria on the basis of ZOI formation.

Table 4: Zone of inhibition of isolated Endophytic fungal ethyl acetate extract and Bacterial extracts measured in millimeter against pathogenic bacterial strains.

Zone of inhibition (ZOI)						
Bacterial strains	***B. cereus***	***E. coli***	***S. aureus***	***S. flexineri***	***S. typhi***	***P. aeruginosa***
Zone of inhibition (ZOI)of positive controls	Chloramphenicol	Amoxicillin	Clindamycin	Azithromycin	Ciproflaxin	Aziocillin
	27mm	24mm	19mm	16mm	25mm	22mm
Zone of inhibition (ZOI) of endophytic fungal ethyl acetate extract and bacterial extract						
Alternaria sp.	15mm	14mm	14mm	12mm	12mm	15mm
Alternaria sp.	14mm	12mm	10mm	14mm	9mm	10mm
Ulocladium sp.	20mm	11mm	13mm	29mm	16mm	17mm
Bacillus sp.	11mm	-	-	11mm	10mm	10mm
Micrococcus sp.	-	10mm	14mm	12mm	10mm	12mm
Serratia sp.	-	-	15mm	10mm	10mm	10mm

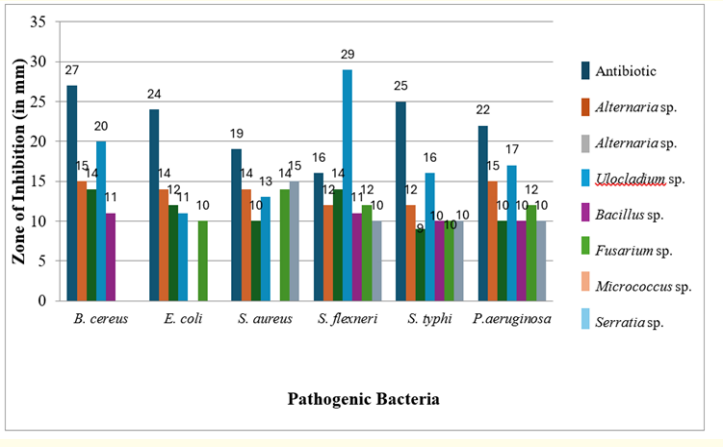

Figure 7: Activity of endophytic fungal ethyl acetate extract and endophytic bacterial extracts against different pathogenic bacteria on the basis of ZOI formation.

Determination of minimum inhibitory concentration (MIC) by Resazurin dye

All the endophytic fungal ethyl acetate extract and bacterial extract were checked for their MIC by Resazurin dye method. Enany and coworkers reported that *Ulocladium* extract was not effective against pathogenic bacteria which was isolated from *Mentha piperita* [32], but in this study *Ulocladium* sp. was found more effective against all pathogenic bacterial strains (Table 5, 6 Figure 8).

Conc. of extract Mg/100µl	*S. aureus*		*E. coli*		*B. cereus*		*S. typhi*		*S. flexneri*		*P. aeruginosa*	
10	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-
2.5	-	-	-	-	-	-	-	-	-	-	+	-
1.25	+	+	+	-	-	-	+	-	+	-	+	-
0.625	+	+	+	+	+	-	+	+	+	-	+	+
0.312	+	+	+	+	+	+	+	+	+	+	+	+
0.156	+	+	+	+	+	+	+	+	+	+	+	+
0.078	+	+	+	+	+	+	+	+	+	+	+	+
0.039	+	+	+	+	+	+	+	+	+	+	+	+
0.0195	+	+	+	+	+	+	+	+	+	+	+	+
MIC (mg/ 100µL	2.5	2.5	2.5	1.25	1.25	0.625	2.5	1.25	1.25	0.625	5	1.25

- = no bacterial growth, + = bacterial growth

Paenibacillus apiarius *Bacillus acidicereler*

Table 5: MIC of ethyl acetate extract of Endophytic bacterial Isolates against Bacterial pathogens.

Conc. of extract mg/100µl	*B. cereus*						*E. coli*						*P. aeruginosa*						*S. aureus*						*S. flexneri*						*S. typhi*					
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.5	+	-	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	-	-	-	+	+	+
1.25	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
0.625	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.312	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.156	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.078	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.039	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
0.0195	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MIC(mg/ 100µl)	5	2.5	2.5	2.5	2.5	5	2.5	2.5	5	5	5	5	2.5	2.5	2.5	5	5	5	5	2.5	2.5	5	5	5	2.5	2.5	1.2	5	5	5	2.5	2.5	2.5	5	5	5

- = no bacterial growth, + = bacterial growth

Alternaria sp. *(A. bidentata)* *Alternaria* sp. *(W. somnifera)* *Ulocladium* sp. *Bacillus* sp. *Micrococcus* sp. *Serratia* sp.

Table 6: MIC of ethyl acetate extract of endophytic Fungal isolate against Bacterial pathogens.

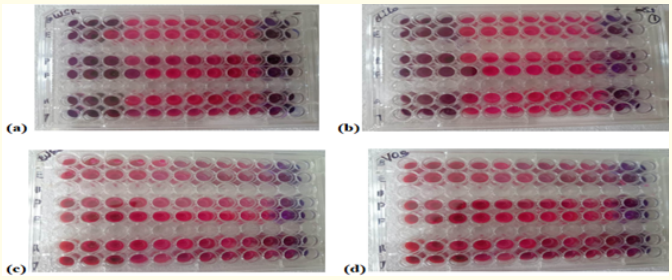

Figure 8: Determination of MIC of different endophytic fungal ethyl acetate extract against various pathogenic bacterial strains (a) *Alternaria* sp. (b) *Ulocladium* sp. (c) *Bacillus* sp. (d) *Serratia* sp.

Conclusion

The current research findings on endophytic isolates indicate that they can be used to produce bioactive compounds for pharmacological purposes without affecting human beings. Seasonal variations play a key role in plant's antimicrobial and antioxidant properties. Phytoconstituents are the constituent molecules which helps plants in exhibiting bioactive potential. Phenolic compounds and saponin levels appear to be higher in winter and autumn season. The availability and concentration of phytochemicals in plants are affected by seasonal fluctuations. These changes in phytochemicals have been widely attributed to variations in environmental variables or abiotic factors such as composition of soil, responses against a variety of season specific pathogens and changes in temperature and rainfall. Biotic factors such as development of the plant and plant genotype also change rapidly and these too may affect the composition of the plant parts. These variations seem to influence the harvest time which ensure optimum quality and yields of medicinal plants. From the Current research work, it can be concluded that endophytic microbes can be a good source for herbal formulation. These organisms can be harmless and can be used to produce bioactive compounds for pharmaceutical use. The obtained results indicates that Endophytic organisms can be a good source for obtaining biofertilizers, biocontrol agents and antioxidants agents. Therefore, the plants from Uttarakhand exhibited good antimicrobial potential in comparison to the plants from Himachal Pradesh due to the environmental variations such as composition of soil, temperature of that particular area, rainfall, microflora of plant's soil, plant's genotype etc.

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