



“FTIR, Uv- Vis And GCMS Analysis Of Potential Bioactive Compounds From *Tinospora Cordifolia* And Its Antibacterial Activity”

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Abstract:

Medicinal plants are assuming greater importance in the primary health care of individuals and communities in many developing countries. Endophytes are the microorganisms inhabiting the living tissues of plants. Endophytic fungi from medicinal plants have created a huge potential in generating novel drugs. The secondary metabolites and their semi-synthetic derivatives play an important role in anticancer drug therapy. The present study is investigated for the isolation of endophytes from the inner bark of twigs and leaves of *Tinospora cordifolia*, collected from the Shankaraghatta regions, Shivamogga district of Karnataka and examined as a potential source of anticancer drug lead compound. Bioactive components of *Tinospora cordifolia* have been evaluated using GCMS, UV-VIS and FTIR and its antibacterial activity. GC-MS analysis revealed the chemical profile of extract of different compound, distinct peak, retention time (RT), molecular formula, molecular weight (MW) and chemical structure. The GC-MS analysis of methanol extracts detected the presence of 30 phytochemical compounds. The UV-VIS profile showed the presence of peaks at 190-500nm revealing the presence of secondary metabolites in *T. cordifolia*. The results of FTIR analysis confirmed the presence of phenol, alkanes, alkenes, alcohol, aromatic, aliphatic amines and amine compound. The results show that important bioactive compounds present in plant extract and these constituents may be responsible for pharmacological activities.

Keywords: GC MS, UV-VIS, FTIR, *Tinospora cordifolia*

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INTRODUCTION

Nature is the richest source of several natural therapeutic compounds. Menispermaceae, one of the largest plant families with huge and varied secondary metabolites, used in the management of several ailments. The medicinal value of plants can be correlated to different phytochemicals, as they offer a wide diversity of pharmacological activities. Due to these pharmacological properties, a great attention has been derived towards the medicinal plants.

T. cordifolia is a traditional medicinal plant belonging to Menispermaceae family and it is a common climbing shrub that grows on other trees. It's native to India. Its root, stems, and leaves are used in Ayurvedic medicine. It contains many different chemicals that might affect the body. Some of these chemicals might have antioxidant and anti-inflammatory effects. Others might affect the immune system. People use these plants for hay fever, athletic performance, diabetes, high cholesterol, upset stomach, and many other conditions.

Various bioactive compounds of medicinal plants exhibit stimulating pharmacological actions like antibacterial, antifungal, anticancer, anti-inflammatory and antioxidant properties [1,2,3]. The potential of these bioactive compounds should be analyzed for their candidature in the treatment of various ailments [2,4]. Plant-based medicines are often prepared from crude plant extracts comprising of complex mixture of different phytochemicals [4]. These phytochemicals have unique and complex structures, and are used in treating prolonged as well as contagious diseases [4,5]. An enormous pool of bioactive secondary metabolites exists in various plant species, but merely a small proportion of them have been examined and sustained to be significant source of bioactive agents. In the search for new compounds, and also for quality control, development of suitable screening methods is very important.

The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information on chemical and pharmacological activities, which helps to

select the biologically active plants [6]. In recent years, Fourier-transform infrared (FTIR) and gas chromatography-mass spectrometry (GCMS) has commonly been employed for detection of functional groups and identification of various bioactive therapeutic compounds that are present in medicinal plants [7,8]. GCMS is one of the best, fast and accurate techniques to detect various compounds, including alcohols, alkaloids, nitro compounds, long chain hydrocarbons, organic acids, steroids, esters and amino acids [9], and requires a small volume of plant extracts. Hence, in the present study, GCMS, FTIR and UV technique were used for the detection and identification of phytochemical compounds present in this medicinal plant. Here we discuss the *in vitro* antibacterial activity of endophytic fungal extracts against gram positive and gram- negative bacteria.

MATERIALS AND METHODS

Plant material, *T. cordifolia* was collected from Shankaraghatta region, Shivamogga district, Karnataka, India.

Plant collection, sterilization and inoculation of implants.

Freshly collected plant material, *T. cordifolia* leaves and twigs is washed thoroughly under running tap water followed by sterile distilled water to remove the adhered debris. Twigs and leaves were surface sterilized under aseptic condition in sequential steps by immersing in mercuric chloride (1mg/1ml) for 1min and 70% ethanol for another min followed by washing finally with distilled water. Twigs and leaves of *T. cordifolia* were aseptically cut into small pieces (0.5x0.5cm²) and placed 5-6 pieces on each of the solidified sterile Potato Dextrose Agar (PDA) media. The inoculated plant implants were incubated till the growth of distinguishable fungal endophytes.

Identification of endophytic fungi

For the identification of endophytic fungal isolates, slides were prepared from cultures and were stained with lactophenol cotton blue reagent and examined with a bright-field and phase contrast microscope. Identification was

based on morphological characteristics such as growth pattern, hyphae, the colour of the colony and medium, surface texture, margin character, aerial mycelium, mechanism of spore production and conidia characteristics using standard identification manuals^[10]. The identified fungi were sub cultured in PDA slants for further use and stored in refrigerated conditions.

Mass production of identified fungi

Identified fungal species were cultured on PDB broth for large scale cultivation. The inoculated flasks were incubated at room temperature ($26\pm 2^{\circ}\text{C}$) for 8-15 days and allowed to grow the fungal mats.

UV and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper by using a high-pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-400 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in ranging from $400\text{-}4000\text{ cm}^{-1}$ and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

GCMS Analysis

GCMS analysis of endophytic fungal extract were performed using a Perkin - Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite - 1, fused silica capillary column (30 mm x 0.25 mm 10 x 1 μm DF, composed of 100% Di methyl poly siloxene). For GCMS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2 μl was employed (split ratio of 10:1); injector temperature

2500 $^{\circ}\text{C}$; ion-source temperature 2800 $^{\circ}\text{C}$. The oven temperature programmed from 1100 $^{\circ}\text{C}$ (isothermal for 2 min) with an increase of 100 $^{\circ}\text{C}/\text{min}$ to 2000 $^{\circ}\text{C}$, the 0 $^{\circ}\text{C}/\text{min}$ to 2800 $^{\circ}\text{C}$, ending with a 9 min isothermal at 2800 $^{\circ}\text{C}$, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo mass.

Interpretation on mass spectrum GCMS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Antimicrobial activity of crude extracts of endophytic fungi from *T. cordifolia*.

Preparation of bacterial inoculum.

The effect of different endophytic fungal extracts were treated against bacterial strains such as *Escherichia coli*, *Pseudomonas syringae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and *Knoellia sinensis*. These bacterial species were again sub-cultured in sterile nutrient agar plates as well as inoculated in the nutrient broth for further antibacterial studies as pure inoculum.

Muller Hinton agar medium (MHA).

The Hi-media containing MHA was prepared around 19g in 500ml of distilled water. Stirred well. Using autoclave equipment, the medium is autoclaved at 15lbs pressure at 121 $^{\circ}\text{C}$ for 15min. The MHA media was mixed well and poured onto 100mm petri plates (15-20ml/plate) and allowed for solidification.

Disc diffusion assay.

The antibacterial activity of the endophytic fungal extracts was evaluated by disc diffusion method. Filter papers were used to punch and












form uniform discs of size 6mm in diameter. Sample solutions of desired concentrations was applied over the disc with the help of the micropipette in an aseptic condition and then air dried. MHA agar plates were prepared and the test organisms were swabbed on it. The discs were then carefully placed onto the agar medium using sterile forceps. The antibiotic streptomycin was used as a standard. The plates were further incubated at 37°C for 48 hours. The zone of inhibition was calculated.

RESULTS AND DISCUSSION.

Identification of Endophytic fungi:

Different endophytes were isolated and identified from *T. cordifolia* (Table 1).

Table 1: Identification of fungal endophytes from different parts of *T. cordifolia*.

Fungal Endophytes	PDA Plates	Microscopic view (40X)
<i>Fusarium sp</i>		
<i>Aspergillus sp</i>		
<i>Rhizopus sp</i>		
<i>Cladosporium sp</i>		
<i>Curvularia sp</i>		
<i>Colletotrichum sp</i>		
<i>Acremonium sp</i>		
<i>Mucor sp</i>		

Mass production of identified fungi

Identified fungal species were cultured on PDB broth for large scale cultivation (Fig 1).



Fig 1: Different endophytes were grown on PDB media.

UV-Visible Spectrometry: The UV-VIS profile of plant extract was taken at the 190 to 500nm wavelength due to the sharpness of the peaks and proper baseline. The UV-visible spectra were performed to identify the compounds containing σ -bonds, π -bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed the peaks of all the four seasons. Endophytic fungal extracts, only six of the major endophyte fungal extracts are taken for the UV spectrometric analysis (Fig. 2-6). The precise position and relative intensities of these maxima give valuable information on the nature of the secondary metabolites. Occurrence of peaks of endophytes reveals the presence of secondary metabolites in the *T. cordifolia*. On comparison of the twigs and leaves spectra shows that the extracts have similar compounds reported.

1. Sample 1- *Cladosporium sp*.

Peaks between 190 and 500nm with the absorption 4.285 and 4.606 respectively (Fig. 2).

2. Sample II- *Cylindrocladium sp*.

Peaks between 190 and 500nm with the absorption 3.63 and 3.72 respectively (Fig. 3).

3. Sample III- *Aspergillus sp*.

Peaks between 190 and 500nm with the absorption 3.2 and 3.82 respectively (Fig. 4).

4. Sample IV- *Mucor sp*,

Peaks between 200 and 500nm with the absorption -1.23 respectively (Fig. 5).

5. Sample V- *Curvularia* sp.

Peaks between 200 and 500nm with the absorption -0.84 respectively (Fig. 6).

6. Sample VI- *Collatotrichum* sp.

Peaks between 200 and 500nm with the absorption -2.3 respectively (Fig. 7).

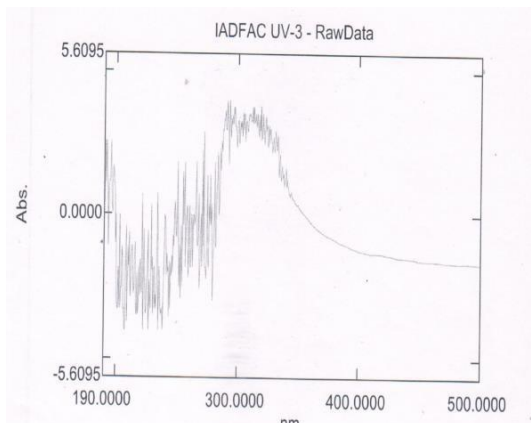


Fig. 2: Sample 1- *Cladosporium* sp.

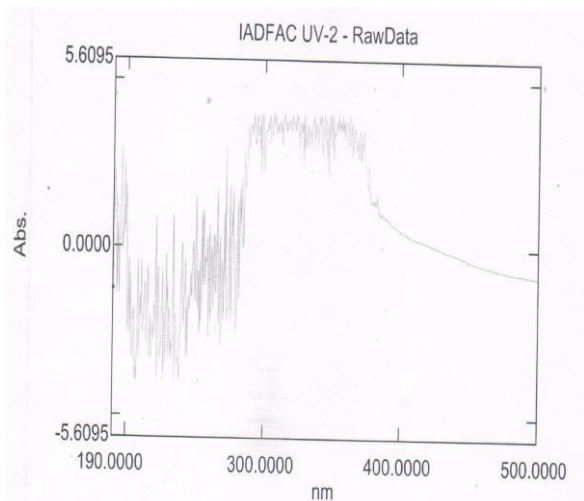


Fig 3: Sample II- *Cylindrocladium* sp.

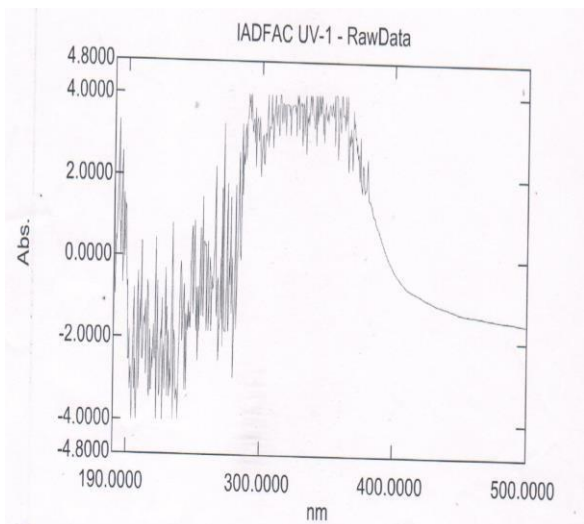


Fig. 4: Sample III- *Aspergillus* sp.

Fig 5: Sample IV- *Mucor* sp,

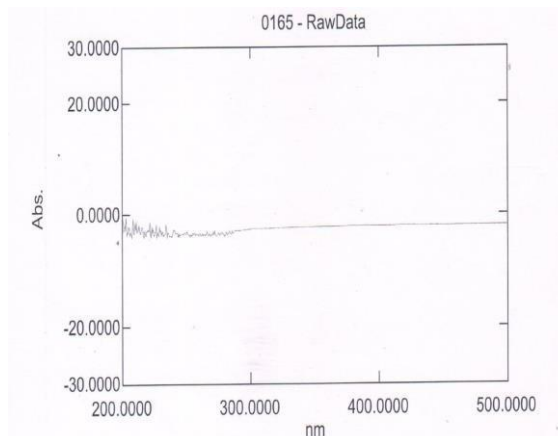


Fig. 6: Sample V- *Curvularia* sp.

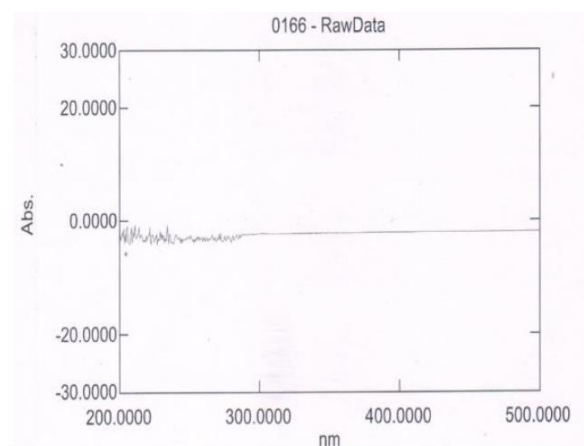
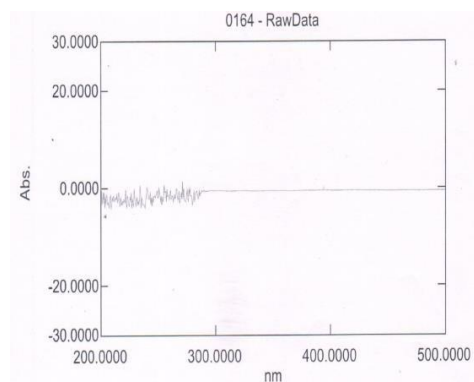


Fig. 7: Sample VI- *Collatotrichum* sp.



FTIR Spectroscopic analysis

Infrared spectroscopy involves the absorption of electromagnetic radiation in the infrared region of the spectrum which results in changes in the vibrational energy of molecules. Since, usually all molecules have vibrations in the form of stretching, bonding etc., the absorbed energy will be utilized in changing the energy levels associated with them.

Functional groups identification: The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peak's values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peak's

ratio. The results of FTIR analysis confirmed the presence of phenol, alkanes, alkenes, alcohol, aromatic, aliphatic amines and amine compound. Endophytic fungal extracts, only six the major endophytic fungal extracts are taken for the FTIR techniques (Fig. 8-12 and Table 2-7).

Fig 8: Sample 1- *Cladosporium* sp.

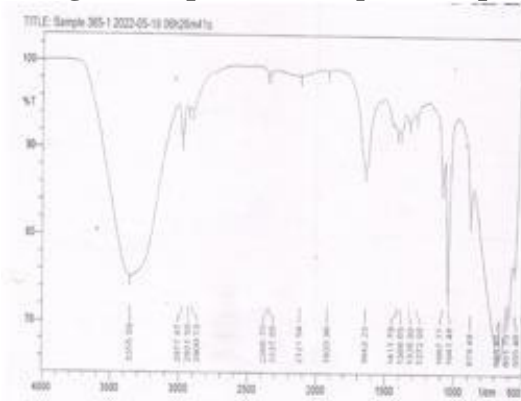


Table 2: Sample 1- *Cladosporium* sp.

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2283	O-H Stretch, H-Bonded	Alcohols, Phenols
2.	1643	C-H Stretch	Alkanes
3.	1087	-C=C- Stretch	Alkenes
4.	1041	C-C Stretch (In-Ring)	Aromatics
5.	848	C-N Stretch	Aliphatic Amines
6.	609	N-H Wag	1°, 2° Amine

Fig 9: Sample II- *Cylindrocladium* sp.

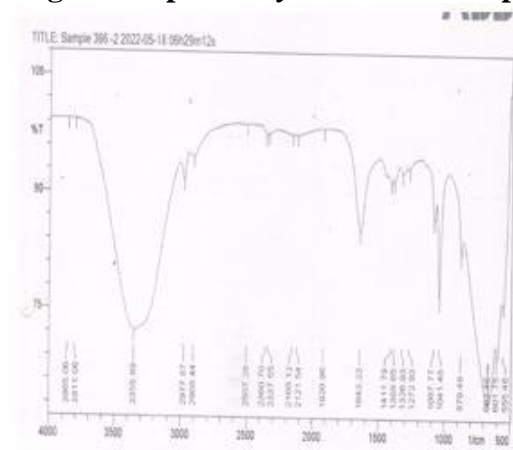


Table 3: Sample II- *Cylindrocladium* sp.

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2337	O-H Stretch, H-Bonded	Alcohols, Phenols
2.	1496	C-H Stretch	Alkanes
3.	1180	-C=C- Stretch	Alkenes
4.	1064	C-C Stretch (In-Ring)	Aromatics
5.	856	C-N Stretch	Aliphatic Amines
6.	609	N-H Wag	1°, 2° Amine

Fig 10: Sample III- *Aspergillus* sp.

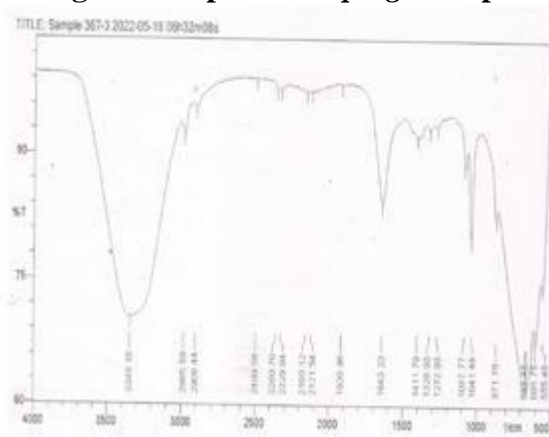


Table 4: Sample III- *Aspergillus* sp.

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2923	O-H Stretch, H-Bonded	Alcohols, Phenols
2.	1944	C-H Stretch	Alkanes
3.	1288	-C=C- Stretch	Alkenes
4.	1064	C-C Stretch (In-Ring)	Aromatics
5.	979	C-N Stretch	Aliphatic Amines
6.	609	N-H Wag	1°, 2° Amine

Fig 11: Sample IV- *Mucor* sp

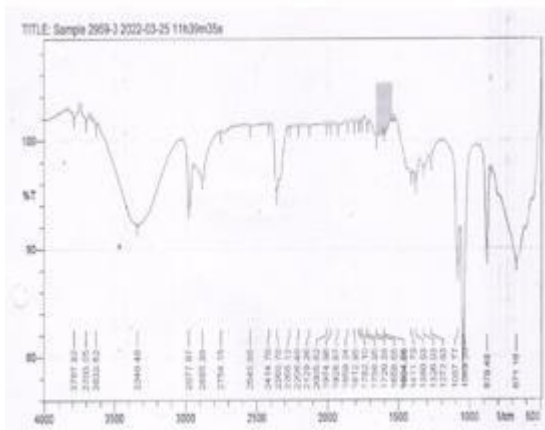


Table 5: Sample IV- *Mucor* sp

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2013	O–H Stretch, H–Bonded	Alcohols, Phenols
2.	1951	C–H Stretch	Alkanes
3.	1774	–C=C– Stretch	Alkenes
4.	1535	C–C Stretch (In–Ring)	Aromatics
5.	1288	C–N Stretch	Aliphatic Amines
6.	1064	N–H Wag	1°, 2° Amine

Fig 12: Sample V- *Curvularia* sp.

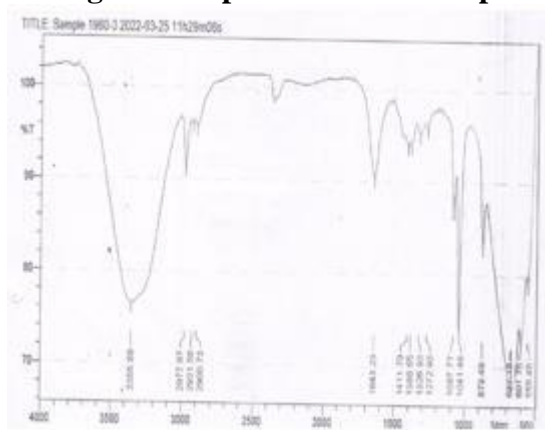


Table 6: Sample V- *Curvularia* sp.

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2939	O–H Stretch, H–Bonded	Alcohols, Phenols
2.	1797	C–H Stretch	Alkanes
3.	1350	–C=C– Stretch	Alkenes
4.	1188	C–C Stretch (In–Ring)	Aromatics
5.	972	C–N Stretch	Aliphatic Amines
6.	609	N–H Wag	1°, 2° Amine

Fig 13: Sample VI- *Collatotrichum* sp

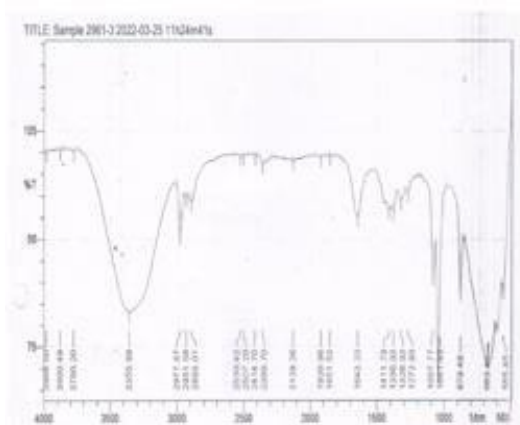


Table 7: Sample VI- *Collatotrichum* sp

SL.NO.	Frequency (Cm ⁻¹)	Bond	Functional Groups
1.	2916	O–H Stretch, H–Bonded	Alcohols, Phenols
2.	2283	C–H Stretch	Alkanes
3.	1527	–C=C– Stretch	Alkenes
4.	1188	C–C Stretch (In–Ring)	Aromatics
5.	972	C–N Stretch	Aliphatic Amines
6.	609	N–H Wag	1°, 2° Amine

Gas Chromatography Mass spectroscopy of the purified methanol endophytic extract of fungal species:

The partially purified crude extract was subjected to GCMS analysis which showed retention time, area %, molecular formula and molecular weights of the several compounds were identified and tabulated (Fig. 13-18 and

Table 8-13). The gas chromatography results of fungal crude extract reveal that major active compounds which are present inside the endophytes.

Fig. 13: Sample 1- *Cladosporium* sp.

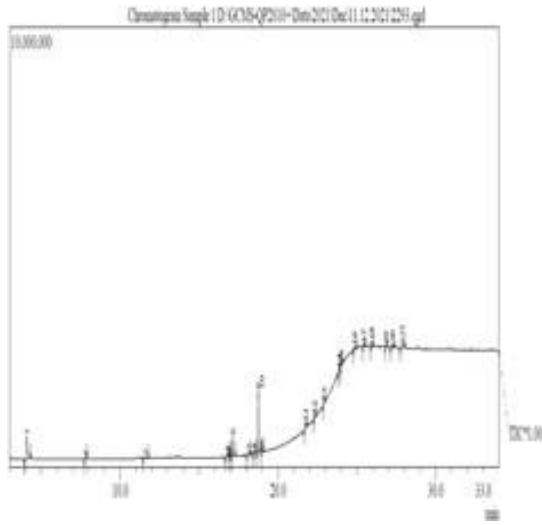


Fig. 16: Sample IV- *Mucor* sp,

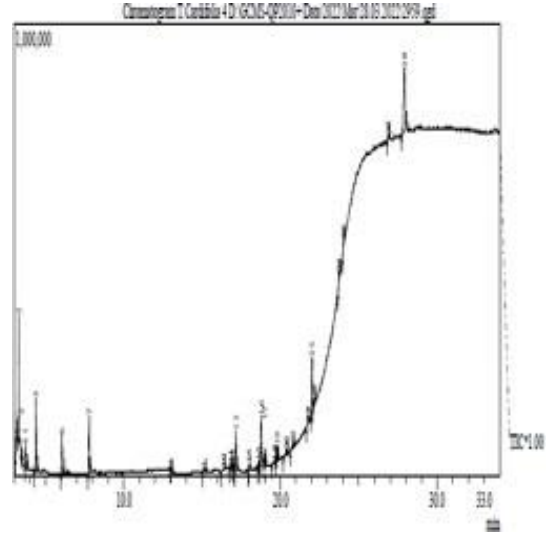


Fig. 14: Sample II- *Cylindrocladium* sp.

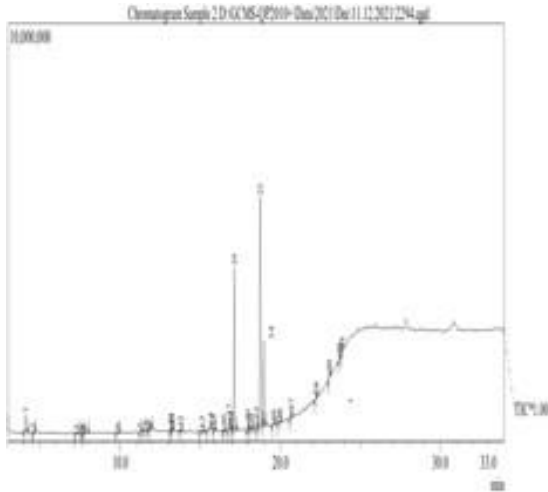


Fig. 17: Sample V- *Curvularia* sp.

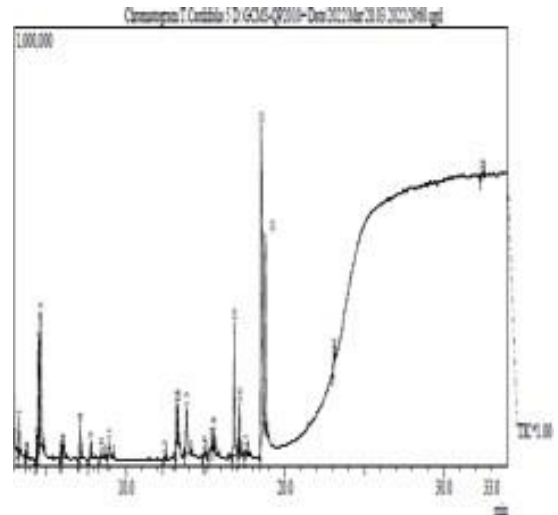


Fig. 15: Sample III- *Aspergillus* sp.

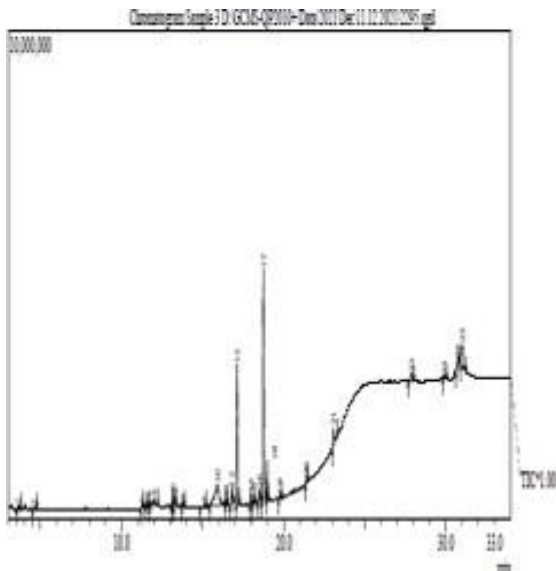


Fig. 18: Sample VI- *Collatotrichum* sp

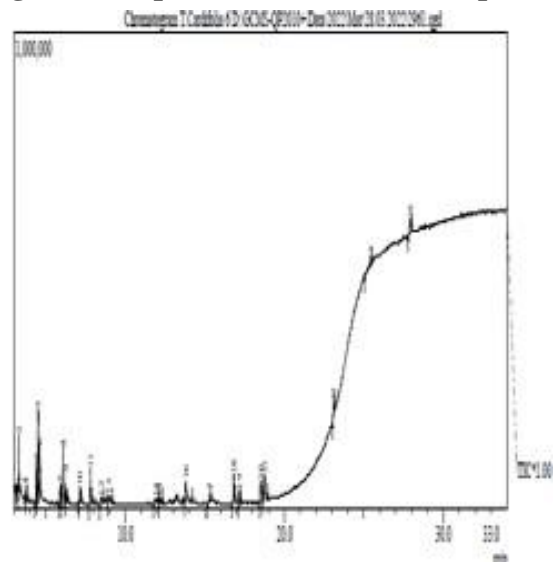


Table 8: Bioactive compounds for all the six endophytic fungal extracts

Sl. No	RT	COMPOUNDS	MOLECULAR FORMULA	MOL WEIGHT	CAS#	ENTRY #
1	4.086	1,2-Cyclopentanedione	C ₅ H ₆ O ₂	98	3008-40-0	1558
2	7.8	Azulene	C ₁₀ H ₈	128	275-51-4	5054
3	11.587	Heptadecanoic acid, trimethylsilyl ester	C ₂₀ H ₄₂ O ₂ Si	342	55517-58-3	25322
4	16.805	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	242	1002-84-2	20371
5	16.953	Ethyl 9-hexadecenoate	C ₁₈ H ₃₄ O ₂	282	54546-22-4	22873
6	17.135	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284	628-97-7	91920
7	18.171	9-Octadecenoic acid (Z)-, methyl ester	C ₁₉ H ₃₆ O ₂	296	112-62-9	98788
8	18.502	9,12-Octadecadienoic acid	C ₁₈ H ₃₂ O ₂	280	60-33-3	22757
9	18.754	14-Methyl-8-hexadecyn-1-ol	C ₁₇ H ₃₂ O	252	64566-18-3	72583
10	18.979	Octadecanoic acid, ethyl ester	C ₂₀ H ₄₀ O ₂	312	111-61-5	24293
11	21.735	Benzenemethanamine, N,N,.alpha.-trimethyl-, (S)-	C ₁₀ H ₁₅ N	149	17279-31-1	14431
12	22.323	5,10-Dihydroxy-2-methoxy-7-methyl-1,4-anthracenedione	C ₁₆ H ₁₂ O ₅	284	74815-58-0	91617
13	22.917	Cyclononasiloxane, octadecamethyl	C ₁₈ H ₅₄ O ₉ Si ₉	666	556-71-8	27563
14	24.015	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C ₃₀ H ₅₀	410	111-02-4	146890
15	25.439	Dehydroergosterol 3,5-dinitrobenzoate	C ₃₅ H ₄₄ N ₂ O ₆	588	6059-43-4	160692
16	25.93	Cyclodecasiloxane, eicosamethyl	C ₂₀ H ₆₀ O ₁₀ Si ₁₀	740	18772-36-6	162623
17	26.841	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	C ₁₃ H ₂₂ OSi ₂	250	0-00-0	71033
18	27.876	Ergosterol	C ₂₈ H ₄₄ O	396	57-87-4	143813
19	4.593	Glycerin	C ₃ H ₈ O ₃	92	56-81-5	1169
20	7.247	2(3H)-Furanone, dihydro-4-hydroxy	C ₄ H ₆ O ₃	102	5469-16-9	2175
21	7.631	1-[p-Tolylsulfonyl]-2-methylaziridine	C ₁₀ H ₁₃ NO ₂ S	211	25856-77-3	47526
22	7.793	Naphthalene	C ₁₀ H ₈	128	91-20-3	5057
23	9.837	2-Acetamido-2-deoxy-d-mannolactone	C ₈ H ₁₁ NO ₆	217	0-00-0	50837
24	11.284	Butane, 2,3-bis(trimethylsiloxy)-	C ₁₀ H ₂₆ O ₂ Si ₂	234	53274-85-4	19688
25	11.582	2-Oxiraneethanol, 2-t-butylidimethylsilyloxymethyl-acetate	C ₁₃ H ₂₆ O ₄ Si	274	0-00-0	85586
26	11.908	DL-Arabinitol	C ₅ H ₁₂ O ₅	152	6018-27-5	15409
27	13.169	Ethyl. alpha. -d-glucopyranoside	C ₈ H ₁₆ O ₆	208	0-00-0	45406
28	13.256	beta.-D-Glucopyranoside, methyl	C ₇ H ₁₄ O ₆	194	709-50-2	37120
29	13.767	Methyl 6-O-[1-methylpropyl]-.beta.-d-galactopyranoside	C ₁₁ H ₂₂ O ₆	250	0-00-0	70806
30	15.115	Tetradecanoic acid, ethyl ester	C ₁₆ H ₃₂ O ₂	256	124-06-1	21343

Screening for antimicrobial activity of endophytic fungi isolated from an endangered plant *T. cordifolia* by disc diffusion method:

Antibacterial activity of endophytic extract of *T. cordifolia* has been assessed by

measuring the diameters of zones of growth inhibition on some strain of bacteria and the results are presented as shown in Table 9. Six endophytic fungi of *T. cordifolia* were screened for antimicrobial activity of which, majority of isolates showed antimicrobial

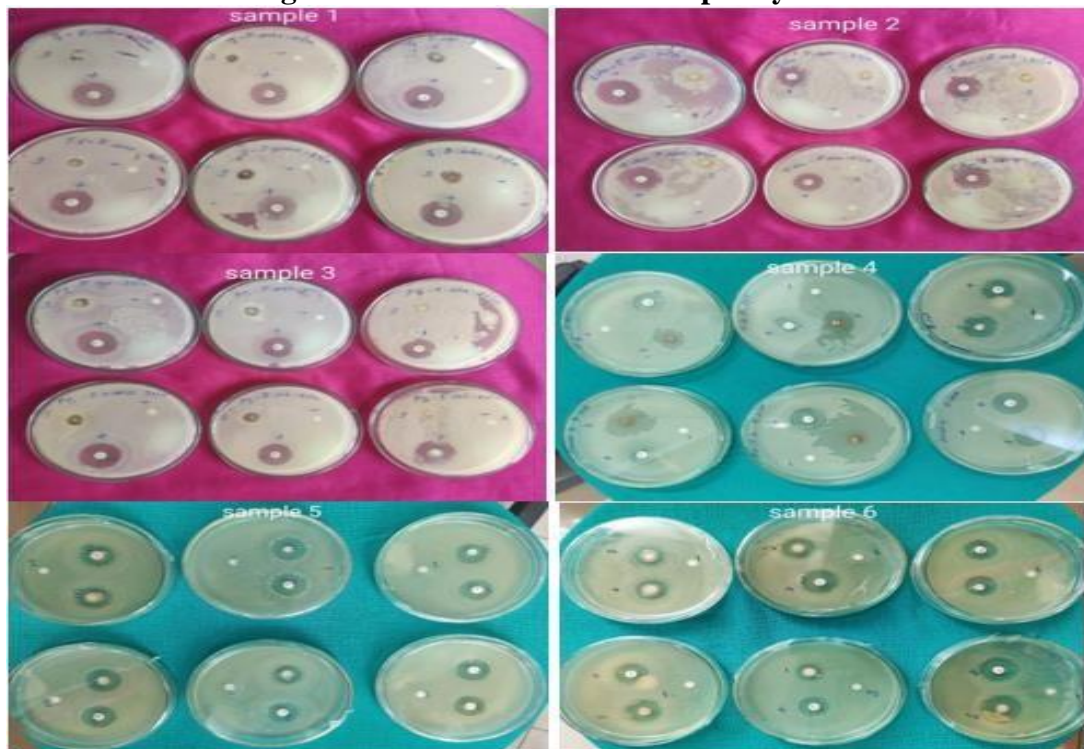
activity with high zone of inhibition, whereas some of the isolates showed with less zone of inhibition against the test pathogens (Fig 19). Inhibition growth of the highest zone has been

shown by endophytic extract against gram-negative bacteria. Streptomycin has been used as a standard.

Table 9: Antimicrobial activity showing zone of inhibition

Test Samples	<i>E. coli</i> (mm)	<i>S. aureus</i> (mm)	<i>P. syringae</i> (mm)	<i>B. subtilis</i> (mm)	<i>P. aeruginosa</i> (mm)	<i>K. sine</i> (mm)
Sample 1	12	12	24	15	10	9
	24	22	21	21	21	20
Sample 2	25	25	25	25	20	20
	23	25	27	22	19	20
Sample 3	11	10	12	10	12	11
	24	23	22	20	20	20
Sample 4	23	22	21	24	19	30
	22	22	20	19	20	20
Sample 5	21	22	23	21	20	21
	21	21	23	20	19	21
Sample 6	22	20	18	22	22	21
	22	19	19	23	22	22

Fig. 19: Antimicrobial activity of endophytic fungi determined by disc diffusion method against Standard antibiotic Streptomycin.



CONCLUSION

The fungal endophytes can be utilized as the potential bio resources for the production of

secondary metabolites other than host plant. Significant molecules having unique pharmacological purpose. These endophytes

are rich sources of bioactive secondary metabolites which hold tremendous potential for industrial and commercial use. In the present study, the endophytic fungi were isolated and identified from the medicinal plant *T. cordifolia*. The plant was also identified taxonomically. A total of 8 different endophytes were isolated and identified from their natural habitat. Among which six has been used for different analytical techniques. UV-visible spectra were performed to identify the compounds containing σ -bonds, π -bonds, and lone pair of electrons, chromophores and aromatic rings. The profile showed different peaks at with varying the absorption at different level for all the four seasons. The precise position and relative intensities of these maxima give valuable information on the nature of the secondary metabolites. Occurrence of peaks between 190-500 nm reveals the presence of secondary metabolites in the *T. cordifolia*. The FTIR analysis confirmed the presence of phenols, alkanes, alkenes, alcohol, aromatic, aliphatic amines and amine compounds. GCMS analysis which revealed the presence of major 30 bioactive compounds. All the six endophytic extracts were screened for antibacterial activity, most of the endophytes showed zone of inhibition against gram positive and gram-negative bacteria. So, it is recommended as a plant of phytopharmaceutical importance. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

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