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ISOLATION, CHARACTERIZATION, ANTIBACTERIAL ACTIVITY, AND SCREENING USING UV-FTIR TECHNIQUES OF *CUVULARIA SP.* FROM *T. CORDIFOLIA*

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Abstract:

Medicinal plants are frequently utilized in therapy because of their natural therapeutic properties and the existence of bioactive chemicals that can treat a variety of ailments. Medicinal plants are employed in phytotherapy, which is the utilization of plant extracts and active substances to promote health and aid the body's natural healing processes. Secondary metabolites found in herbal plants, such as flavonoids, flavones, anthocyanins, lignans, and coumarins, have been proven to show anticancer activities. The current study demonstrated that secondary metabolites exhibit a range of bioactive compounds. The endophyte extract from Cuvularia sp was tested against several bacteria, including E. coli, S. aureus, B. subtilis, K. pneumoniae, P. aeruginosa, and P. syringae, and it demonstrated a zone of inhibition against these bacteria. Endophytic extract characterisation is utilized for several analytical methods, including FTIR, which finds functional groups including, O–H (alcohols, Phenols), C-H (Alkanes), C=C (Alkenes), C-C (Aromatic rings), C-N (Aliphatic Amines), N-H (1°, 2° Amines). The present study evaluates Curvularia sp samples for UV-Vis spectrophotometric analysis, identifies significant bioactive compounds, including carotenoids, flavonoids, photosensitive proteins, chromophores, phenolic groups, nucleic acids, etc, which are all helpful for the preparation of pharmaceutical drugs, and also shows the antioxidant, anticancer activity.

***KEYWORDS**: Anti-cancer activity, Cuvularia sp, FTIR, UV-Vis, Photosensitive,*

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INTRODUCTION:

Herbal treatments provide people with potent medications that can be used to treat a wide range of illnesses and dangerous conditions. *T. cordifolia*, often known as Guduchi,
is a naturally occurring herbal plant that is a member of the Menispermaceae family of moonseeds. Guduchi is a huge, widely dispersed, glabrous, perennial deciduous vine that grows widely in Sri Lanka, India, and Myanmar. It has a succulent stem and papery bark. The leaves are simple, heart-shaped, and dark green. The stems seem to have warty tubercles closely spaced over their surface. Succulent, the bark has deep clefts, spots, and enormous lenticels that resemble rosettes. On auxiliary and terminal racemes, the tiny, greenish-yellow flowers are unisexual. While fruits mature in the winter (November), flowers bloom from March to June. Fruits have an orange-red hue, and a wide range of phytochemicals, or bioactive compounds, are found in medicinal plants. Sugars, alkaloids, tannins, flavonoids, and other primary and secondary plant metabolites make up phytochemicals. *T. cordifolia* is known by several names, including Amara, Amritvalli, Chinmarrhuha, Chinnodebha, and Vatsadani. It can be used for numerous illnesses, including diabetes, gout, skin conditions, and jaundice.

MATERIALS AND METHODS:

1. Collection of plant material:

Plant material, *Tinospora cordifolia*, was collected from the Shankaraghatta region, Shivamogga district, Karnataka, India. The plant material was cleaned with distilled water and mercuric chloride after being cleaned with running tap water to get rid of any undesired detritus. Aseptic procedures were used to study plant materials, and sterile scissors were used to remove interior tissues.

2. Inoculation of implants

The plant *T. cordifolia*, parts, i.e., leaves and twigs, were cut into small pieces (0.5-0.5cm^2) were placed 5- 6 pieces on a petri dish (9cm) containing solidified Potato Dextrose Agar (PDA) media and incubated at 28
℃ to 30 ℃ for 7-14 days. Purity of the culture was determined by colony morphology that is seen on the petri dish.

3. Morphological identification of the endophytic fungi.

The endophytic fungal isolates were identified morphologically by characters including colour, texture, and spores using a stereo microscope and a clean glass slide stained with lactophenol cotton blue. The reproductive structures, that is, both macro and micro morphological features, were noted (1). According to standard manuals, the endophytic fungi were recognised by looking at their growth pattern, hyphae, colour of the colony and medium, surface texture, margin character, aerial mycelium, spore production method, and conidia characteristics (2). The endophytic fungi were examined under a 40x magnification objective lens.

4. Mass production of the identified endophytic fungi.

Further, for large-scale production sample was required to analyse other tests, identified fungal species were cultivated on Potato Dextrose Broth (PDB) medium, period of 8 to 15 days. The sterile inoculation flasks were kept at room temperature (26±2ºC) to facilitate the growth of fungal mats. Additionally, these fungus mats are utilised for further phytochemical tests and in vitro tests. The leftover fungus broth is centrifuged for 10 minutes at 3000 rpm.

5. Preliminary Phytochemical tests of Secondary metabolites.

Qualitative testing was used to identify secondary metabolites in methanolic extracts of *Curvularia spp* of
T. cordifolia. Cardiac glycosides, amino acids, terpenoids, triterpenoids, alkaloids, flavonoids, tannins, carbohydrates, and steroids were all recorded using standard protocols (3).

1. Steroids: To take 1 ml of fungal crude extract from the endophyte. Add one or two drops of strong sulfuric acid. Concentrated sulfuric acid layer exhibits yellow with green fluorescence, while the upper layer turns red. Consequently, it suggests that steroids are present (4).
2. Terpenoids: To take 1 ml of fungal crude extract from the endophyte. Add 3 millilitres of concentrated sulfuric acid to create a layer. The presence of terpenoids is indicated by the appearance of a reddish-brown hue (5).
3. Tannins: 1% $FeCl_3$ solution is combined with fungal crude extract from endophytes, a green or brownish green blue colour is produced (6).
4. Saponins: After mixing 1 millilitre of the endophyte fungal crude extract with 20 millilitres of distilled water, stir for 15 minutes. If foam develops, saponins are present (7).
5. Alkanoids: To take 1 ml of Wagner's solution is added to 3 ml of the endophyte fungal crude extract. A reddish-brown precipitate appears in the presence of alkaloids.
6. Flavonoids: One millilitre of concentrated sulfuric acid and two millilitres of fungal extract are well mixed together in the test tube with one millilitre of diluted ammonia. Flavonoids are

present when a yellow hue forms (8).

7. Triterpenoids: Five drops of concentrated sulfuric acid are combined with two millilitres of the endophytes' crude fungal extract. The presence of triterpenoids is indicated by the appearance of a greenish blue colour(8).
8. Carbohydrates: Benedict's reagent is combined with 2 millilitres of fungal crude extract from the endophytes, and the mixture is allowed to boil in a water bath (8).
9. Cardiac glycosides: Sodium nitroprusside was added to 5 millilitres of the crude extract along with sodium hydroxide and pyridine. When pink to crimson coloration forms, cardiac glycosides are present.
10. Amino acids: adding 0.25% ninhydrin reagent to the fungal extract, it was brought to a boil for a short while. Amino acid content is shown by the formation of blue.

Antibacterial activity of *Curvularia sp*: The antibacterial activity was done by using the disc diffusion method; the fungal extract of *Curvularia spp* was tested against Gram-negative bacteria, including *Pseudomonas syringae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, as well as Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Knoellia sinensis* (9). The endophytic extracts were dipped onto the sterile discs. For fifteen minutes, the MHA (Muller Hinton Agar) media, forceps, and petri plates were autoclaved at 121°C. The media was then poured onto Petri plates and left to harden for an hour in the Laminar Air Flow Chamber. In addition to utilizing cotton swabs to disperse bacterial cultures, approximately 10µl of bacterial cultures were put onto solidified media. The discs that were submerged in Rhizopus sps endophytic extracts and put on MHA media were therefore utilized as a negative control, whereas the discs that were submerged in double-distilled water and Streptomycin, which demonstrated antibiotic activity, were used as a positive control. Every plate underwent a 24-hour incubation period at 37°C. Measurements were made to verify the antibacterial action of the zone of inhibition surrounding the discs.

UV and FTIR spectrometry of *Curvularia sp*:
The extracts were subjected to proximate analysis utilizing both visible and ultraviolet light. Before being examined with a UV and FTIR spectrophotometer, the extracts were centrifuged for 10 minutes at 3000 rpm to filter them through Whatman No. 1 filter paper using a high-pressure vacuum pump. The sample should be diluted to a 1:10 ratio using the same solvent. When the extracts were scanned in the 200–400 nm wavelength range using a Perkin Elmer Spectrophotometer, the characteristic peaks were discovered. The unique peaks and their functional groups in the 400–4000 cm–1 range were identified via FTIR analysis using the Perkin Elmer Spectrophotometer apparatus. The values of the UV and FTIR peaks were recorded. Two analyses were performed for each analysis to confirm the spectrum.

RESULTS AND DISCUSSION:

1. Identification and Mass production of *Curvularia sp.*

The endophytic fungi isolated from *T. cordifolia* were identified as *Curvularia sp*. by analyzing their morphological features under a phase contrast and brightfield microscope (Fig. 1). Furthermore, PDB was utilized to mass-culture the isolated endophytic fungi, and the extracts were employed for further investigation.

2. Preliminary tests of Secondary metabolites:

Preliminary tests were carried out to confirm the presence of secondary metabolites in *Curvularia sp* methanolic extracts. Alkaloids, flavonoids, tannins, terpenoids, steroids, carbohydrates, and amino acids were detected by the assays (Fig. 2 and Table 1). Qualitative analysis of secondary metabolites is one method of discovering more about specific bioactive compounds that an organism produces (10). We can understand the possible therapeutic effects of secondary metabolites, such as flavonoids, terpenes, tannins, alkaloids, etc., in the treatment of various illnesses by identifying their existence. It paves the way for further research to identify the precise bioactive compounds present in them and their significant biological impacts (11).

3. Antibacterial activity of *Cuvularia sp* extract:

The methanolic extract of *Cuvularia sp*. was tested against six different bacterial species, including Gram-positive bacteria *S. aureus*, *B. subtilis*, and *K. sinensis* [3. (d) (e) (f)] and Gram-negative bacteria *P. syringae*, *E. coli*, and *P. aeruginosa* [Fig. 3(a) (b) (c)]. *Cuvularia* species inhibited every bacterium, although the most potent inhibitors were *P. syringae* (10 mm) and *P. aeruginosa* (15 mm), followed by *S. aureus* (15 mm), *E. coli* (13 mm), *B. subtilis* (14 mm), and *K. sinensis* (10 mm). The findings indicate that endophytic extracts from *Cuvularia sp*. may be chosen for further research and the identification of potential bioactive components because they can create antibiotic compounds (12).

4. UV Spectrometry of *Curvularia sp*:

The UV-Vis spectrophotometric study of the *Curvularia sp* sample revealed important bioactive

substances, such as pigments or photosensitive proteins at 557.5 nm, flavonoids at 567 nm, and carotenoids or porphyrins at 596.5 nm. Chromophores such as phenolic groups or chlorophyll derivatives are indicated by a peak at 422.5 nm, whereas nucleic acids or aromatic groups are indicated by peaks at 306 nm and 249.5 nm. The samples' rich pigment, antioxidant, and aromatic component makeup is highlighted by these results, indicating their possible biological relevance.

5. FTIR Spectrometry of *Curvularia sp*:

The presence of bioactive chemicals is indicated by the wide variety of functional groups found in *Curvularia sp*. FTIR spectral analysis, which is shown in Table 3. O-H stretching vibrations are represented by a noticeable absorption at 3355.89 cm^{-1}, which suggests the existence of phenolic and alcoholic groups, both of which have antibacterial and antioxidant qualities. Peaks at 2121.54 cm^{-1} and 2977.87 cm^{-1} indicate C–H stretching, which points to the existence of alkanes, which are usually connected to hydrocarbon or fatty acid structures. C=C stretching vibrations, which are characteristic of alkenes and unsaturated compounds, are represented by the peak at 1643.23 cm^{-1}. The C–C in-ring stretching at 1087.77 cm^{-1} clearly shows aromatic rings. In addition, the N–H wag at 601.75 cm^{-1} indicates the existence of primary and secondary amine groups, while a peak at 879.48 cm^{-1} shows C–N stretching, indicating the presence of aliphatic amines. According to these functional groups, *Curvularia sp*. might generate a range of secondary metabolites that could have industrial or medicinal uses.

Table 1: Phytochemical analysis of secondary metabolites from endophytic fungal species.

Sl.No	Secondary Metabolites tests Samples	*Cuvularia sp*
01	Alkaloids	+
02	Flavonoids	+
03	Carbohydrates	+
04	Saponins	+
05	Triterpenoids	-
06	Tannins	+
07	Terpenoids	+
08	Steroids	-
09	Cardiac glycosides	+
10	Aminoacids	+

(+ Indicates Present),(- Indicates Absence)

Table 2: UV–Visible spectral peaks and interpretation of *Curvularia sp:*

SL. No.	Peaks Wv(nm)	Absorbance	Interpretation
1	596.5	0.0932	Carotenoids or porphyrins
2	567	0.0996	Flavonoids
3	557.5	0.1014	Pigments or photosensitive proteins
4	422.5	0.2616	Chromophore, associated with aromatic compounds such as phenolic group, chlorophyll derivatives, complex organic compounds
5	306	0.2616	Nucleic acid
6	249.5	0.0092	Nucleic acids or aromatic groups

Table 3: FTIR spectral data and functional group analysis of *Curvularia sp.*

SL.NO.	Frequency (Cm^{-1})	Bond	Functional Groups
1.	3355.89	O–H Stretch, H–Bonded	Alcohols, Phenols
2.	2977.87	C–H Stretch	Alkanes
3.	2121.54	C–H Stretch	Alkanes
4.	1643.23	–C=C– Stretch	Alkenes
4.	1087.77	C–C Stretch (In–Ring)	Aromatics
5.	879.48	C–N Stretch	Aliphatic Amines
6.	601.75	N–H Wag	1˚, 2˚ Amine

Figure 1: Fungal endophyte *Cuvularia sp.*

A). Photographic image of PDA showing the growth. B). Microscopic image at 40x.

Figure 2: Qualitative analysis of fungal extract to confirm the presence of Secondary metabolites.

Figure 3: Antibacterial activity of *Cuvularia sp.* against a) *E. coli*, b) *P. syringae*, c) *P. aeruginosa*, d) *S. aureus*, e) *B. subtilis*, f) *K. sinensis*

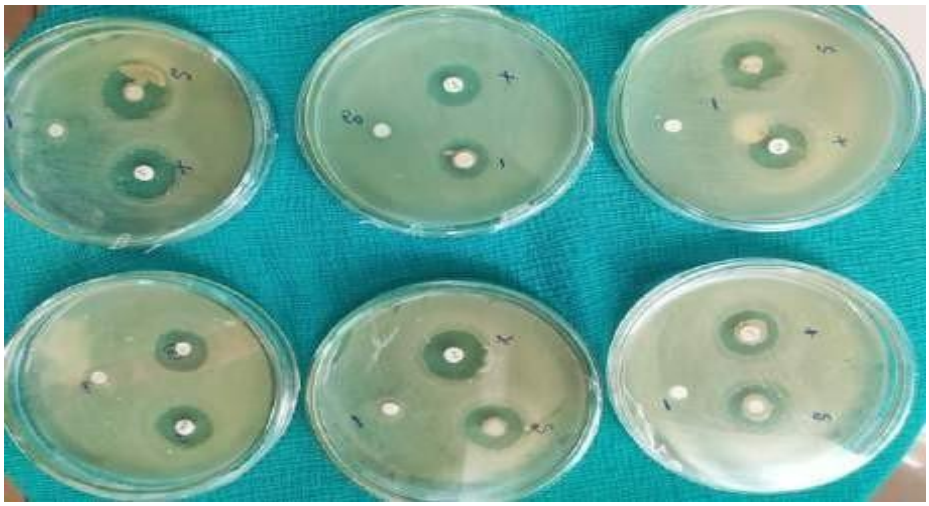


Figure 4: UV Spectrometry of *Curvularia sp*:

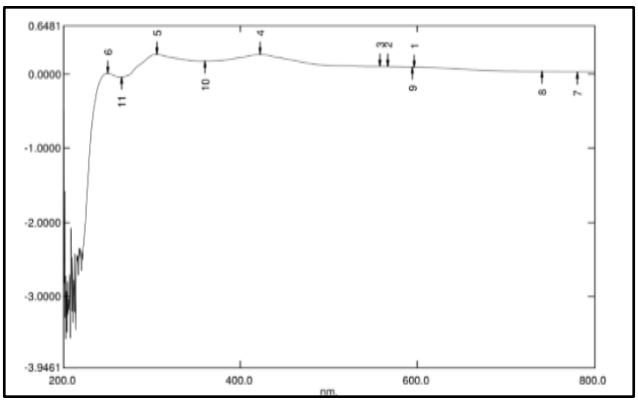
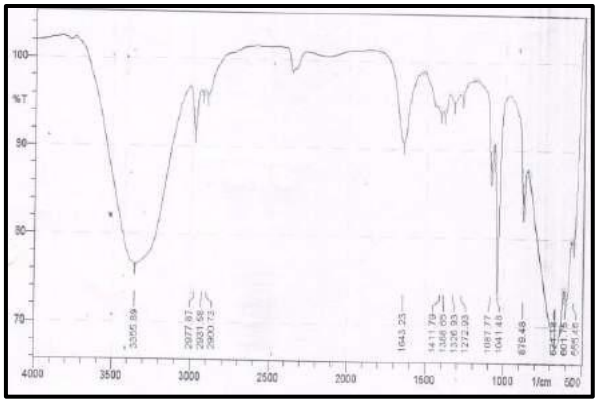


Figure 5: FTIR Spectrometry of *Curvularia sp*:



CONCLUSION:

Medicinal plants have been employed in traditional medicine and our daily lives since the dawn of humanity. Secondary metabolites, which exhibit the bioactive compounds produced by living organisms, might disclose natural symbiotic relationships. When tested against the S. asoca endophytic extract, the bacteria *E. coli*, *S. aureus*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*, and *P. syringae* showed the zone of inhibition. The present study evaluates *Curvularia sp* samples for UV-Vis spectrophotometric analysis, identifies significant bioactive compounds, including carotenoids or porphyrins at 596.5 nm, flavonoids at 567 nm, and pigments or photosensitive proteins at 557.5 nm. A peak at 422.5 nm indicates chromophores, such as phenolic groups or chlorophyll derivatives, while peaks at 306 nm and 249.5 nm indicate nucleic acids or aromatic groups. The FTIR functional groups present in this extract are O–H (alcohols, Phenols), C-H (Alkanes), C=C (Alkenes), C-C (Aromatic rings), C-N (Aliphatic Amines), N-H (1˚, 2° Amines). These drugs showed antioxidant, anticancer activity.

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