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Research Article

Extraction, characterization and biological applications of Secondary Metabolites of Endophytic Fungi of *Codium variegatum*

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Abstract

Many chemical substances produced by some fungi find application in various spheres and therefore draw scientific interest. Among such fungi, the ones living in plant tissue attract particular attention. *Codiaeum variegatum* was selected as a host plant because of unique bioactive compounds produced by its fungal colonizers. In order to discover them, it was decided not to skip stages and conduct the study thoroughly. As a preliminary stage, fungi collection had to be conducted followed by cultivation in conditions where both temperature and light remain stable. For isolation of secondary metabolites of the studied fungal isolates, alcohol was used first followed by fractional separation using planar and column chromatography where each fraction had to be clearly identified. Further, the composition of obtained fractions was analyzed. The final stage consisted of bioassay. In order to find out about the composition of metabolites, techniques such as UV-Vis, FTIR and GC-MS were used. In order to determine the biological activities of discovered secondary metabolites, tests for free radical neutralization, inhibition of α-amylase and antibacterial activity were performed. Among the tested compounds, Tris (2, 4-di-tertbutylphenyl) phosphate was found which was known before for antioxidant properties. Under infrared spectroscopy examination, characteristic bands corresponding to OH groups, double bonds of carbon and phenyl rings could be observed. As amounts increased, so did radical cleanup power; enzyme interference grew stronger, bacterial growth slowed in clear patterns. From start to finish, findings point toward tiny fungi tucked inside *C. variegatum* leaves holding steady promise not loud claims for future drug-linked discoveries.

Keywords: Endophytic fungi, *C. variegatum*, Secondary metabolites, Anti-diabetic, Antibacterial activities.

Introduction

Hidden among plant cells are small allies known as endophytic fungi. These organisms take up residence without causing harm [1]. Researchers lately keep returning to these life forms when searching for useful chemicals tucked inside their structures. Alkaloids, terpenoids, flavonoids, phenolics, and polyketides emerge through their natural processes not loud, yet powerful in effect. Certain ones stop harmful microorganisms; some ease cellular stress or interfere with abnormal tissue growth, working steadily behind the scenes. From quiet presence comes measurable impact. Some compounds let crops handle tough conditions, also pointing to possible medical breakthroughs. It turns out these mushrooms mirror the chemical makeup of their host plants almost exactly [2].

The match stays strong because nature keeps delivering helpful molecules without pulling up roots every season. Growing them in labs means scientists slowly boost the amount of powerful ingredients made. Steady environments keep the organisms active, allowing wider uses in health aids, wellness items, even topical treatments. Pulling fewer wild plants means less pressure on forests and their living inhabitants. Not many people know that hidden inside Garden Croton. Its scientific name is *Codiaeum variegatum*. They are tiny fungi yet to be fully studied. Bright leaves make it popular in warm places, where it grows easily outdoors. Earlier work looked at some chemicals in the plant, still

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what lives inside its stems and leaves stays mostly unknown. Hidden microbes might hold keys to fresh kinds of molecules [3] never seen before. New health threats like unstoppable infections, damage from internal rusting processes, and blood sugar problems push science to find better answers. These inner fungi may offer unusual chemistry, possibly leading to medicines with different ways of working. Instead of taking more from nature, looking deep into plant life opens doors quietly. Studying these organisms adds to knowledge about how green beings team up with microscopic partners. Clues emerge not just for healing tools [4], but also how nature builds complex substances step by step. Surprises wait within silent partnerships shaped over long stretches of time.

Discovery happens when attention shifts beneath surfaces most overlook. Each small organism carries quiet promise without loud claims. Understanding unfolds slowly through patient observation rather than force. What lies unseen often shapes outcomes in ways not first imagined. This work fills a missing piece by pulling out fungal partners from *C. variegatum*, one leaf at a time. Through careful steps, those hidden microbes are teased apart, then coaxed into revealing their chemical tools. In order to conduct this research, each fungal compound has been purified using the latest methods of chromatography and spectrometry [5]. Composition of any discovered compound can be determined thanks to modern approaches which do not require any guesswork [6]. The last stage includes checking abilities of each compound to inhibit oxidation, affect response of the organism to blood sugar and block bacteria. Why is this unique approach worth mentioning? Firstly, it combines the process of discovery, determination of chemical nature of substances and evaluation of biological activity [7]. Secondly, it was implemented based on a decorative plant which is rarely examined thoroughly. Thus, it gives access to the pool of potentially useful resources which are yet to be explored in the field of medicinal biology. Each result makes it evident that common ornamental plants have many hidden features. The present study aimed to isolate and identify endophytic fungi from *C. variegatum* and to characterize their bioactive compounds using advanced analytical techniques. It further sought to evaluate the antioxidant, antidiabetic, and antibacterial potential of these compounds in order to explore their possible applications in medicinal biology.

Materials and methods

Collecting leaf sample

From homes, rice water got saved and left out at normal air warmth until needed. Right away, lively grown leaves of *Codiaeum variegatum* were picked fresh for catching hidden fungi inside as shown in **Fig. 1**. No leaf with spots, tears, or sickness made it through; those flaws mess up the tiny life living within. Every bit arrived from places never touched by bug sprays, keeping outside chemicals far off the scene. Clean hand coverings and sterilized cutting tools were used in order to prevent contamination with additional microorganisms. Each sample cut from the plant was placed immediately in sterile plastic wraps. Time of transportation to the laboratory had to be reduced in order to maintain original conditions which might help preserve fungal communities intact. Preparation of each sample started not later than a day after picking. Care should have been taken while transporting them in order to provide adequate shading to prevent stress to the plant and possible change in its biological characteristics.

Fig. 1: Image of *Codium variegatum* leaves

Surface Sterilization of Plant Material

Freshly picked *Codiaeum variegatum* leaves were washed under running tap water for several minutes in order to remove dust, grime and other contaminants that might stick to their surface. That rinse cuts

down microbes living on the surface before deeper steps begin [8]. To clear off organisms growing outward without harming those tucked inside, a wipe-down with 70% ethanol lasted exactly one minute which breaks open outer layers of unwanted cells. Afterward, several rounds of washing followed, using only pure sterile water, repeated between three and five times so none of the alcohol stayed behind. Getting rid of extra ethanol matters. Without removal, it might slow down fungus growth later. To check if sterilizing worked, the researchers put rinse water on special plates like PDA ones and kept them warm for two to three days. No germs showed up and compostion of PDA medium is shown in **Table 1**. That meant surfaces were clean. These PDA plates feed fungi well. They get their power from mashed potatoes and sugar [9]. The sugar fuels cell activity. Potatoes add vitamins and minerals needed for life. A mild acid level helps mushrooms thrive but blocks bacteria. Once cleaned, leaves sat undisturbed in a safe space until ready for next steps.

Table 1: Composition of Potato Dextrose Agar Medium

Component	Quantity (per 1 L)	Function
Potato infusion	200 g (from potatoes)	Provides essential nutrients, vitamins, and growth factors
Dextrose (Glucose)	20 g	Serves as a carbon and energy source for fungal growth
Agar	15–20 g	Solidifying agent to provide a surface for growth
Distilled Water	1000 mL	Solvent for media preparation
pH	~5.6	Slightly acidic, favors fungal growth over bacteria

Endophytic Fungi Isolated

After cleaning the surface, pieces of *C. variegatum* leaves got sliced into tiny bits around half a centimeter wide with a sterilized blade as shown in **Fig. 2**. While working, every effort stayed focused on keeping things germ-free so nothing unwanted could sneak in. Once ready, those clean leaf parts landed gently onto PDA dishes, making sure their freshly cut sides touched the gel below. Because of that positioning, hidden fungi living inside the plant cells slowly crept out into view [10]. These prepared dishes then moved into a warm chamber held steadily at 28°C, staying there between three and seven days. Periodically it was examined by means of microscopy as soon as fuzzy formations appeared along leaf edges. Secondary metabolite production takes place within the tissue; therefore, these fungi are endophytic. Once colonies become isolated, sterile loop was used to transplant them to sterile PDA dishes in order to have clean culture. Colonies had to be repeatedly transferred to new plates until they looked morphologically homogeneous; thus, only one species could be isolated each time. Every sample was named by means of assigning it a tag such as Isolate B1 and stored.

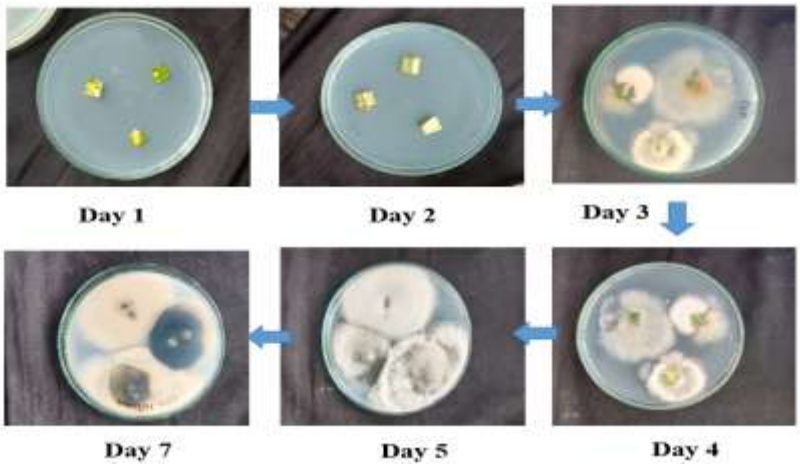


Fig. 2: Above is shown the gradual development of endophytic fungi isolated from *Codiaeum variegatum* on Potato Dextrose Agar (PDA) plates during the course of 7 days incubated at 28°C

Mass Cultivation of Endophytic Fungi

In order to induce secondary metabolites production on a larger scale, fungal colonies extracted from previous stages were transferred to cultivation on a larger scale was shown in **Fig. 3**. Strong fungal colony was placed with care into sterilized Potato Dextrose broth with the help of sterile tool [11]. Seed culture was allowed to grow at 28°C within one-two week period. At the beginning of cultivation rapid development was observed followed by a slowing-down when fungal cells reached maximum density.` During this period, some metabolites start to flow to the medium; others, on the contrary, remain in living biomass. Gradually, cloudy medium starts forming accompanied with fungal mycelium strands. Length of incubation process had to be determined in order to achieve maximum metabolic output; thus, processes of decomposition had to be prevented due to depletion of nutrients. Once it was completed, a mixture of spent broth medium and fungal biomass was prepared for isolation of metabolites.

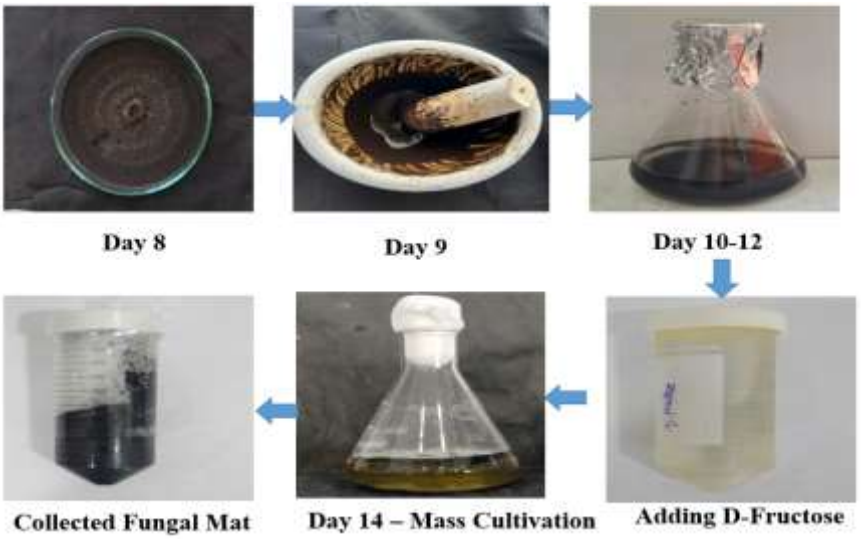


Fig. 3: Further development of Endophytic Fungi isolated from *Codiaeum variegatum* on Potato Dextrose Agar (PDA) dishes during the period of 8-14 days of incubation at 28°C

Extracting of Secondary Metabolites from Endophytic Fungi

Once the required time passed, fungal culture was subjected to series of procedures aimed at separation of secondary metabolites was shown in **Fig. 4**. Filtering was done with Whatman No.1 paper and led to the separation of mycelium from the liquid phase. Thanks to this division, researchers had an opportunity to work both with intracellular and extracellular metabolites. Clear liquid containing secondary metabolites secreted by the fungus was extracted with alcohol [12] which was effective for it was capable of capturing many kinds of active ingredients of uneven polarity such as phenols and pigments produced by plants. The mixture was left for some time being stirred occasionally in order to ensure that all useful compounds had transferred into alcohol. Then upper aqueous phase, saturated with isolated compounds, was separated carefully and evaporated using spinning heating under reduced air pressure [13]. Low-temperature distillation process helps avoid degradation and preserve chemical compounds intact. Brownish liquid formed after the procedure consists of numerous pigments. Extract obtained this way was stored in a frozen form in order to facilitate its further isolation and purification.

Purification of Secondary Metabolites from Endophytic Fungi

Extracted from the fungal culture in a crude form, the product contains many chemical components differing from each other in chemical structure. These differences determine their behavior with different solvents; therefore, separation can be carried out using filtering techniques based on the level of polarity.

Analysis of Secondary Metabolites from Endophytic Fungi

The fractionated extracts from the column were analyzed further using various spectroscopic techniques to identify atom connections and molecular bonds. The instrument tracking how the light is absorbed

from samples provided clues to its structure while another device measured the vibration in molecules in conjunction with a system registering vapor trails relative to molecular weight.

Fig. 4: Crude extract containing secondary metabolites which is obtained by means of ethanol extraction and subsequent solvent evaporation

Analysis of Secondary Metabolites from Endophytic Fungi by Thin Layer Chromatography

The first evaluation of the crude extract was conducted using the thin-layer chromatography approach. The stationary phase utilized silica gel plates which kept everything in place during the test. Small amounts of the sample were directly applied onto the surface using the micropipette. Into a closed vessel with a developing agent, the plate was carefully inserted, and liquid started to climb up by itself. The polarity and affinity to the plates determined the speed at which compounds would move. The solvent was evaporated in the air at the end of the process [14]. Ultraviolet light served as a means to detect spots on the plate, and each had a different appearance under the influence of shortwave and long-wave lights. Positions revealed which substances managed to pass further. The numbers were obtained based on the ratio of distances and provided clues without sophisticated equipment around.

Analysis of Secondary Metabolites from Endophytic Fungi by Column Chromatography

Using TLC results, a purified form of the crude extract was produced using column purification. The column consisted of a glass tube with silica grains as the stationary phase. The raw dose was carefully poured on top of that bed and allowed to descend through each section stronger in attraction than the previous. The substances began to separate as they had different responses to the grain and solvent interaction [15]. During elution, distinct colors emerged; the first one being yellow, then orange, followed by rust-red hues moving further down. Each represented a different type of compound traveling independently. Drops from the bottom were collected in eight fractions labeled from F1 to F8 - as if sorting puzzle pieces by color. Similar samples collected into fractions remained separated at all times awaiting further testing.

Analysis of Secondary Metabolites from Endophytic Fungi by UV-Visible Spectroscopy

A small amount of laboratory-grade alcohol dissolved the fungal matter without difficulties. As it binds so effectively with the extract, the solution became even and transparent. The clarity of samples is essential when it comes to light absorption measurement [16]. Boiling water was subsequently cooled and used for dilutions and thorough washing of all small tools. Quartz capillaries of exactly a centimeter diameter were used for analysis as sunlight resistance is much higher compared to other materials. The machine monitoring visible and invisible radiation registered each intake shade. With uninterrupted transition, wavelengths shifted from 200 to 800 nanometers. Measurements were taken when the operation had completed, and accuracy was important in the case of minute concentrations.

Analysis of Secondary Metabolites from Endophytic Fungi by Fourier Transform Infrared Spectroscopy

The potassium bromide used for spectroscopy enabled production of pellets for the FTIR test due to excellent light transmittance properties. Raw material of endophyte fungus culture underwent further examination. If required, the laboratory ethanol assisted with the preparation of the sample before placing into a spectrograph, while clean water took care of rinsing [17]. All glass instruments and holders needed deep washing followed by complete drying prior to testing to ensure reliable and repeatable results.

Analysis of Secondary Metabolites from Endophytic Fungi by Gas Chromatography–Mass Spectrometry

A small quantity of dry material of the endophytic fungus was dissolved in laboratory ethanol. When the solution became clear enough, it was filtered using a syringe with a micropipette equipped with 0.22-micron membrane filter. There are cases when substances are difficult to analyze; in such situations, it was necessary to add silyl reagent to improve detection of the compound. This allows to keep the molecular structure intact under the effect of heat in a mass spectrometer [18]. The filtered solution was poured into a quartz vial tightly sealed and ready for testing with the GC–MS apparatus. The gas chromatograph with mass selective detector split molecules within a special column due to increasing temperature. Each detected signal compared to known molecular spectra in the database of NIST based on mass fragmentation data.

Biological Activities of Secondary Metabolites from Endophytic Fungi

From various plant secondary metabolites, there were tests for biological functions. It is not any lab technique – tried and proven approaches helped to investigate anti-oxidant, anti-inflammatory, and antibacterial abilities of the substances.

Antioxidant Activity of Secondary Metabolites from Endophytic Fungi by DPPH Assay

For the evaluation of the antioxidant function, there was an experiment involving the formation of DPPH radicals. This chemical is highly stable on its own and has a violet color, which turns yellow when reacting with an antioxidant. The initial stage was preparation of a solution consisting of methanol with DPPH concentration of 0.4 mM. Two milliliters of this mixture had varying concentrations of fungal extract from 20 up to 100 micrograms per milliliter [19]. In low lighting conditions, the mixture sat for twenty minutes. The machine measured absorbance at 517 nanometers, which helped to calculate the percentage of inhibited radicals (see Equation 1).

$$Antioxidant\ activity\ \% = {}^{(Abs\ control - Abs\ sample)}/_{Abs\ control} \times 100 \qquad (1)$$

Anti-diabetic Activity of Secondary Metabolites from Endophytic Fungi

Fungus may have anti-diabetic effects as it inhibits α-amylase. It is responsible for carbohydrates metabolism in the stomach and serves as a target in such tests. For the experiment, researchers mixed the enzyme with a buffer of pH equal to 6.8. Various concentrations of fungal extract were added and left under heating of exactly 37 degrees for five minutes. Starch was added [7] further to start the reaction and was subsequently heated. Denaturating of α-amylase enzymes was conducted with DNS solution which was briefly boiled under boiling water. Further, the light reading was taken at 540 nanometers wavelength.

Antibacterial Activity of Secondary Metabolites from Endophytic Fungi

The antibacterial activity of the fungal extract was tested through the agar well diffusion technique on selected bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Bacterial cultures were cultivated overnight and uniformly spread on nutrient agar plates with the help of a sterile swab [10]. Wells of 6 mm diameter were formed in the agar using a sterile cork borer. Different amounts of the fungal extract (25 µL, 50 µL, 75 µL, and 100 µL) were dissolved in the respective wells. Gentamycin was employed as the positive control. After incubation of the inoculated plates at 37°C for 24 h, the zones of inhibition were calculated in millimeters. All experiments were performed in a sterilized environment.

Results and Discussion

Isolation of Endophytic Fungi

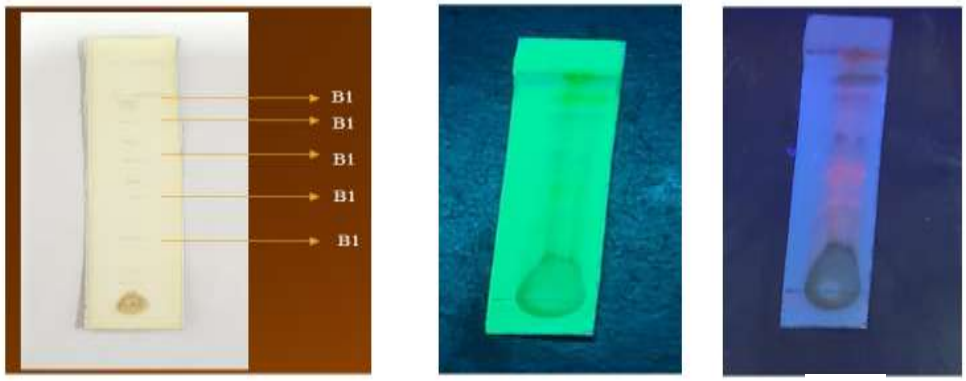
Following sterilization, growth of fungus was observed from the leaves of *C. variegatum.* The hyphal growth emerging out of the tissue confirmed the presence of endophytic fungi. The colonies that were

formed had distinct appearances like the color and morphology of their surfaces. When transferred to fresh media, each colony grew on its own, later tagged clearly and kept ready for upcoming tests.

Analysis of Secondary Metabolites from Endophytic Fungi

Thin Layer Chromatography Analysis of Secondary Metabolites from Endophytic Fungi

A thin layer chromatography test (TLC) helped check what substances were in the raw mix taken from the fungus grown in culture as shown in **Fig. 5((a) and (b))**. Under ultraviolet light,first at 254 nm, then at 365 nm where the plate showed many separate marks, each one a sign of a different chemical present. These marks settled at various levels along the silica surface, since each traveled a unique distance during analysis. Because some chemicals attract more to the gel while others prefer the moving solvent, where they land depends heavily on how polar they are.



(a) (b) (c)

Fig. 5: Thin Layer Chromatography analysis of crude fungal extract which shows the separation of multiple compounds (a) under normal light (b) under UV illumination of 254 nm and (c) under UV illumination of 365 nm

Column Chromatography of Secondary Metabolites from Endophytic Fungi

A purification step used column chromatography on the raw extract taken from the endophytic fungus, relying on how each substance interacts with polar environments. Moving down the tube, separate colored zones became visible inside the silica-packed [11] glass column as shown in **Fig. 6**. When the solvent flowed through, chemicals traveled unevenly where some stuck more to the solid material, others preferred the liquid carrier. Because of these differing behaviors, the original mixture split into individual parts, gathered one after another and marked from F1 to F8.

Fig. 6: Column chromatography separation of fungal extract which shows the distinct colored bands corresponding to different fractions (F1–F8)

GC-MS Analysis of Secondary Metabolites from Endophytic Fungi

A machine called GC-MS Analyzer helped spot the chemicals inside the fungus mix. The graph and table are shown in **Fig. 7 and Table 2**. Into this device went the sample, with molecules splitting apart depending on how easily they turned to vapor and stuck in the tube. Once out came each separate

substance, it got zapped into charged bits, seen clearly by the detector, forming a one-of-a-kind pattern of signals. They were compared to the known databases of endophytic fungi in order to recognize what compounds were present in the extract. Many bioactive compounds were identified from the analysis. The highest spiked compound observed in the graph was Tris (2, 4-di-tert-butylphenyl) phosphate, indicating its abundance in the extract. This particular substance is known for its antioxidant properties. Modified versions like Octylsilanetriol (3TMS) were also detected, confirming the proper modification of compounds in the process.

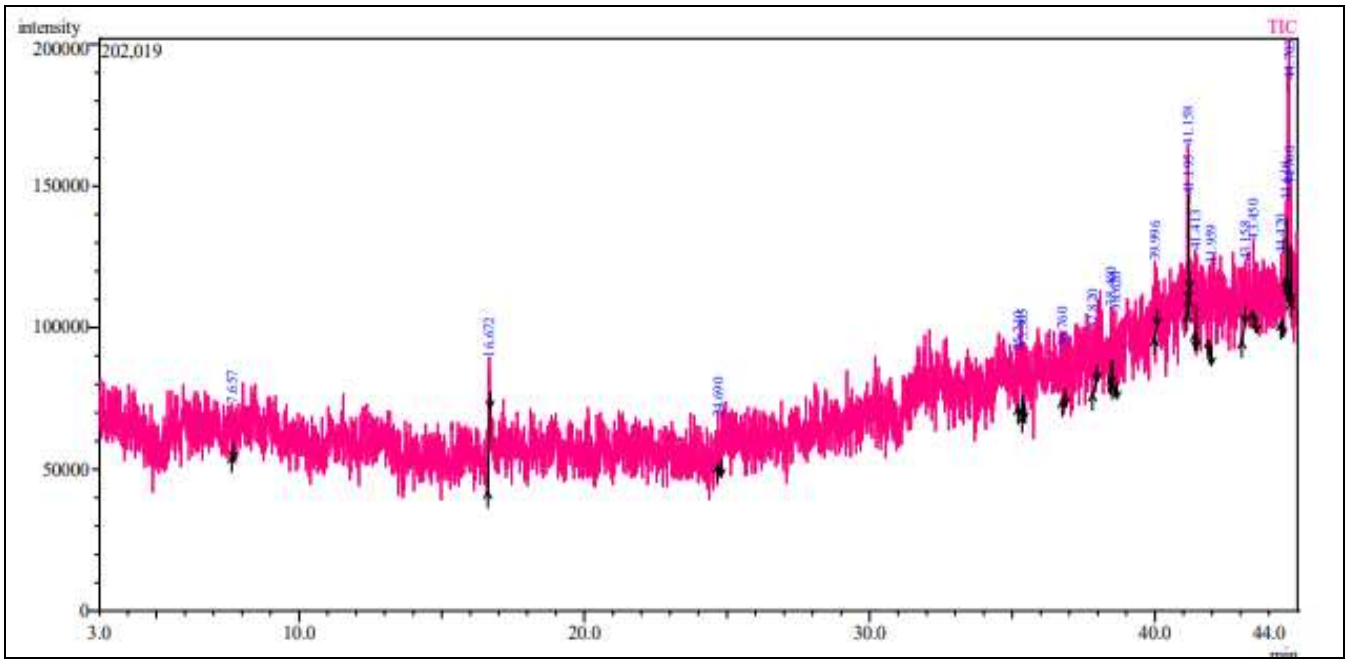


Fig. 7: GC–MS chromatogram of endophytic fungal extract that indicates multiple peaks of different bioactive compounds.

Table 2: GC-MS identified compounds of endophytic fungal extract along with retention time and peak area percentage

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	7.657	36784	1.96	18963	3.08	6-O-Tosyl-1,3:2,4:5,7-trimethylene-.beta.-sedoheptitol
2	16.672	77120	4.11	24636	4.00	DIETHYL PHTHALATE
3	24.690	63509	3.39	16948	2.75	
4	35.210	145730	7.77	19873	3.23	3,3,5,5,7,7,9,9,11,11,13,13-Dodecamethyl-1,15-bis(1,3
5	35.365	50166	2.67	24219	3.94	7.alpha.,11.alpha.-Dihydroxytomatidine, O,O,O,N-tetr
6	36.760	69836	3.72	18792	3.05	
7	37.820	97822	5.21	22477	3.65	14-Methyl-pentadecane-1,2-diol, isopropylidene deriva
8	38.460	65955	3.52	25553	4.15	3-Trifluoromethylbenzoic acid, octadecyl ester
9	38.620	145179	7.74	29302	4.76	Digitoxin
10	39.996	55903	2.98	26528	4.31	Melezitose
11	41.158	123716	6.60	55721	9.06	2-Nonadecanone 2,4-dinitrophenylhydrazine
12	41.195	49351	2.63	34399	5.59	BUTYL TRIS(TRIMETHYLSILYL) ORTHOSILICA
13	41.413	49839	2.66	31931	5.19	Androstan-17-one, 3,11-bis[(trimethylsilyl)oxy]-, O-(p
14	41.959	127730	6.81	33450	5.44	Octylsilanetriol, 3TMS
15	43.158	95246	5.08	22164	3.60	1,2-Dimethoxy-4-(1,3-dimethoxy-1-propenyl)benzene
16	43.450	95203	5.08	26116	4.24	2-(4b,8,8,10a-Tetramethyl-2-methylenetetradecahydro
17	44.420	56170	2.99	24488	3.98	Silicic acid, diethyl bis(trimethylsilyl) ester
18	44.610	46250	2.47	28183	4.58	
19	44.703	386694	20.61	90532	14.71	Tris(2,4-di-tert-butylphenyl) phosphate
20	44.760	37598	2.00	41083	6.68	TRISTRIMETHYLSILYL ETHER DERIVATIVE OF
		1875801	100.00	615358	100.00	

UV-Visible Analysis of Secondary Metabolites from Endophytic Fungi

From the analysis of the UV–Visible scan of the fungal extract, it can be seen that its activity ranges from 200 to 800 nm as shown in **Fig. 8**. Spikes are visible from both UV as well as visible regions, depicting the molecules having certain absorption spectra. A marked peak exists in the region 240-260 nm that refers to ring structures of the molecules, which is a common structure of bioactive compounds. Ring-like forms are usually found in natural phytopharmaceuticals [12] produced by microorganisms. Another rise exists between 300 and 380 nm indicating the existence of conjugated electron systems having energy transmission in distributed bonding arrangements. Such type of behavior exists in flavonoids which are not easily oxidized. Faint peaks above 400 nm especially in the blue region indicate colored components.The presence of the material gives them colors.

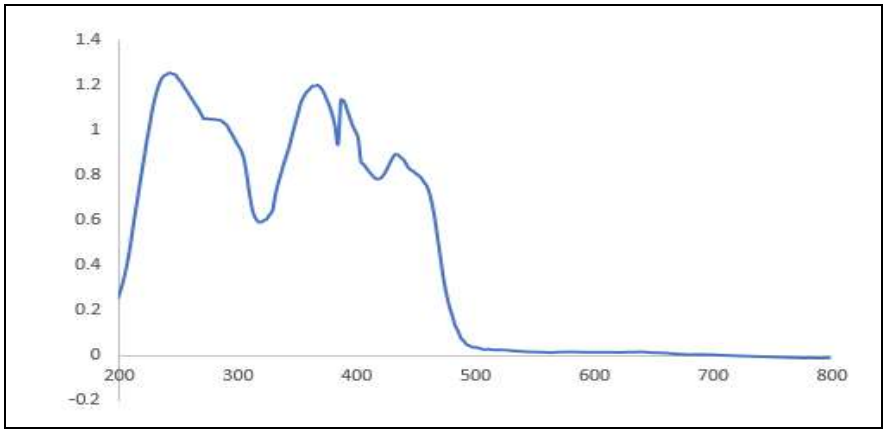


Fig. 8: UV–Visible absorption spectrum of endophytic fungal extract in the range of 200-800 nm that depicts the characteristic peaks of absorbance

FTIR Analysis of Secondary Metabolites from Endophytic Fungi

Analyzing the FTIR results of endophytic fungi extract, it is evident that a number of chemical features exist in it as shown in **Fig. 9**. The broad band seen between 3200-3400 cm^{-1} depicts vibration of the O-H bonds, suggesting the presence of hydroxyl functional groups, associated with phenolics with antioxidative property. The band observed near 2900 cm^{-1} represents vibrations related to stretching of C-H bonds, referring to alkane type compounds. The prominent peak in the region 1600-1700 cm^{-1} corresponds to C=O bond pull and indicates carbonyls, present as ketone, aldehyde or amide. Such type of functional groups are commonly present in bioactive substances. Further peaks seen from 1400 to 1500 cm^{-1} suggest the presence of C=C movements that confirm the presence of aromatic compounds. Towards the lower side, the absorption peaks observed in the region 1000-1200 cm^{-1} correspond to vibration of C-O bonds, suggesting alcohol or ether or ester type compounds may be present in the extract.

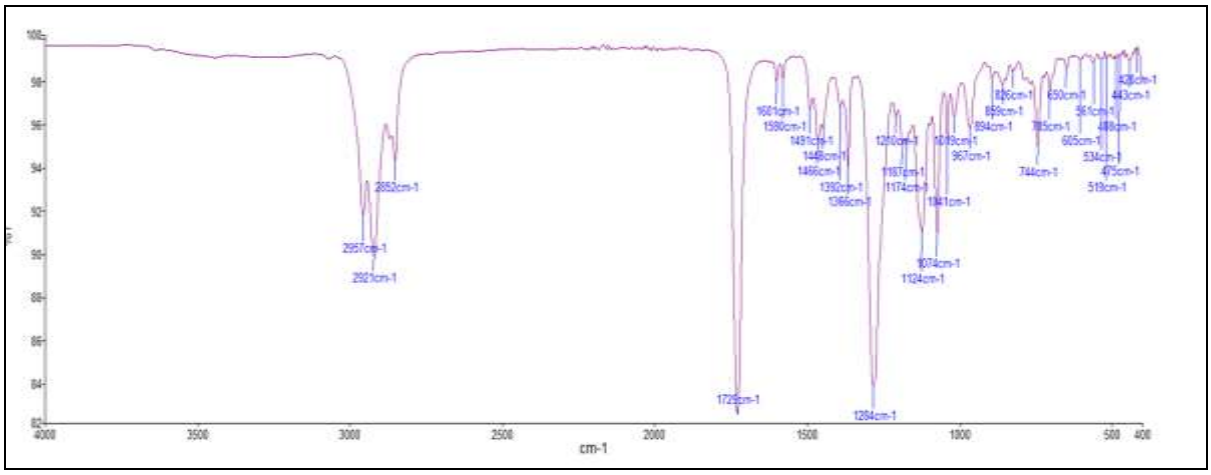


Fig. 9: FTIR spectrum of endophytic fungal extract that depicts the absorption peaks for various functional groups

Biological Activities of Secondary Metabolites from Endophytic Fungi

Antioxidant Activity of Secondary Metabolites from Endophytic Fungi

The free radical scavenging ability of the endophytic fungal extract was evaluated using the DPPH [13] test. **Fig. 10** correlates well with the data represented in **Table 3**. It can be seen that the higher the amount of fungal extract the greater its scavenging activity. From 20 micrograms per milliliter concentration, it could inhibit 28 percent of radicals, which was quite satisfactory. Increasing it to 40, the percentage increased to almost 36. From 60 the figure crossed 44 while at 80 the percentage went above 57 indicating almost 60% of inhibition. In case of the highest dosage of 100 micrograms, over three quarters (76.11%) radicals were destroyed. Clearly, the more the concentration of extract the higher the effect. It tends to capture free radicals, particularly in abundant quantity.

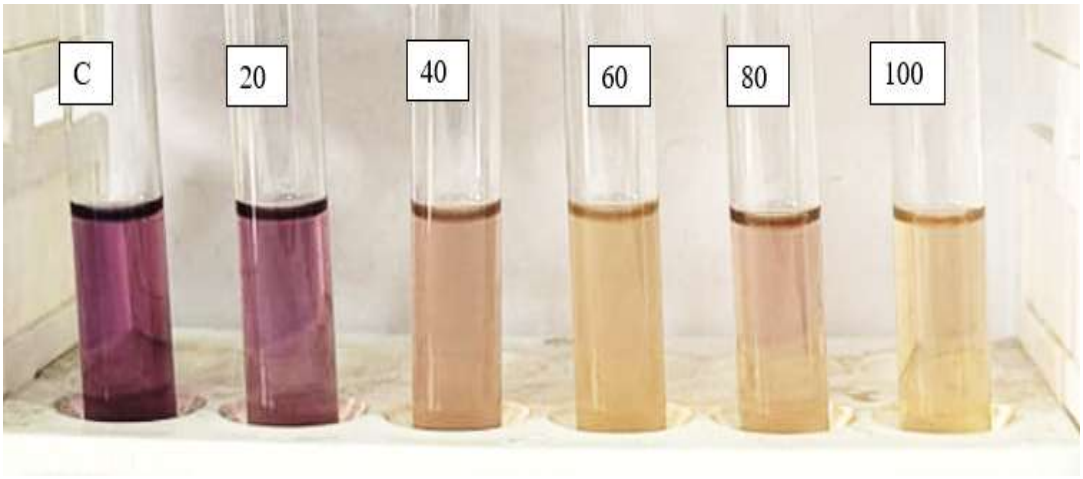


Fig. 10: Radical Scavenging Activity of DPPH against Endophytic Fungal Extract of Various Concentrations

Table 3: Radical Scavenging Activity of DPPH against Fungal Extract of Various Concentrations

Concentration (µg/mL)	Absorbance	Inhibition (%)
Control	0.98	0
20	0.71	28.13
40	0.63	35.82
60	0.54	44.83
80	0.42	56.57
100	0.23	76.11

Anti-diabetic Activity of Secondary Metabolites from Endophytic Fungi

The antidiabetic activity of the endophytic fungal extract was tested using the alpha-amylase [14] inhibition assay. As depicted in **Fig. 11** and **Table 4**, there was a marked increase in inhibitory activity of fungal extract depending upon its concentration. In the minimum concentration of 20 µg/mL, the extract produced 23.17% inhibition, indicating moderate alpha-amylase inhibitory action.This inhibition increased progressively with higher concentrations: 44.92% at 40 µg/mL, 68.29% at 60 µg/mL, and 76.83% at 80 µg/mL. A maximum of 84.15% inhibition was observed at the maximum tested concentration of 100 µg/mL.These results indicate that the fungal metabolites effectively interfere with

the enzymatic breakdown of starch into glucose. The observed dose-dependent response highlights the extract's efficacy in modulating αamylase activity.

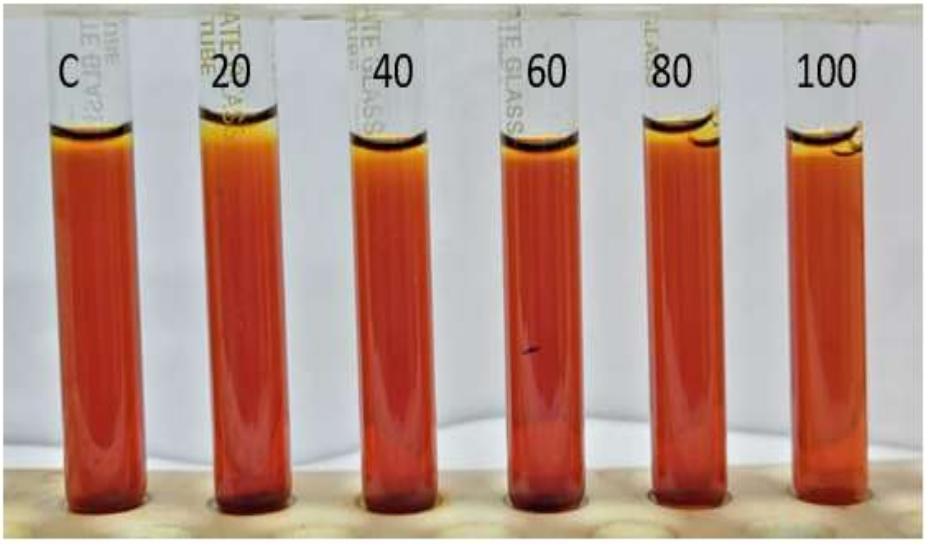


Fig 11: α-amylase inhibitory activity of endophytic fungal extract at different concentrations

Table 4: α-amylase inhibitory activity of fungal extract at varying concentrations

S.No	Concentration(µg/mL)	Absorbance	% of antidiabetic activity
1	Control	0.98	-
2	20	0.75	23.17
3	40	0.54	44.91
4	60	0.31	68.29
5	80	0.22	76.82
6	100	0.15	84.14

Antibacterial Activity of Secondary Metabolites from Endophytic Fungi

From inside plant-dwelling fungi, an extract was tested on harmful bacteria [15] using a standard lab technique with agar-filled plates. As seen in **Fig. 12** and laid out clearly in **Table 5**, higher amounts of the substance led to stronger blocking effects on every germ strain examined. Clear rings formed where the solution sat, spreading wider when more liquid ranging from 25 microliters up to 100 was applied. Even at its weakest dose, some impact appeared, but just on one type: *Staphylococcus aureus* made a small circle, seven millimeters across. Other germs like *Bacillus subtilis, Pseudomonas aeruginosa*, along with *Escherichia coli* stayed untouched then, hinting they need stronger doses before any reaction kicks in. Once quantity climbed, each bug began responding with visible halts in growth; *S. aureus* kept widening its limit steadily until it reached thirteen millimeters, while *gentamycin*, used as benchmark, hit seventeen. From 8 mm up to 11 mm, *B. subtilis* had growth blocked. In contrast, *P. aeruginosa* displayed clear areas stretching 7 mm through 11 mm. When it came to Gram-negative types, *E. coli* reacted more strongly zones grew from 8 mm to 12 mm as amounts increased. Across results, the fungus-based solution held back both kinds of bacterial groups, whether thick-walled or thin-walled.

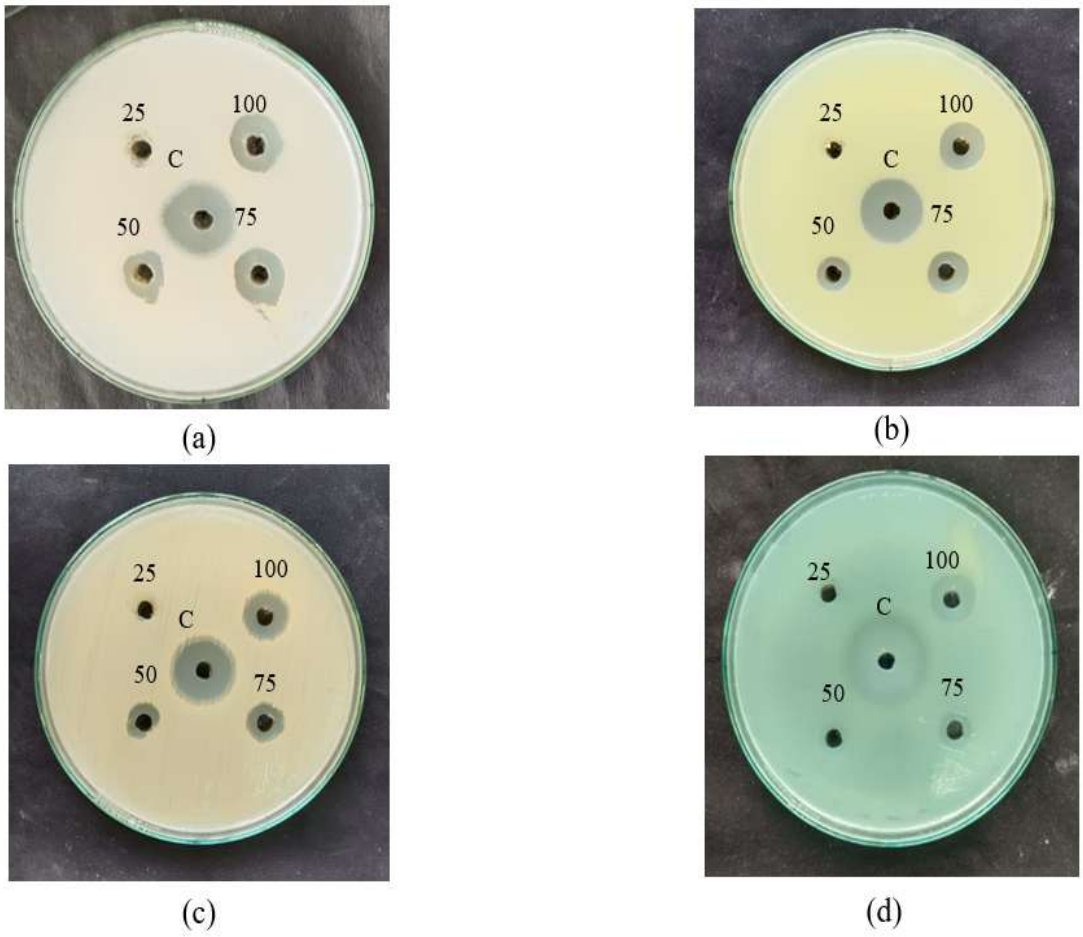


Fig. 12: Antibacterial activity of endophytic fungal extract showing zones of inhibition against test organisms *a) Staphylococcus aureus b) Bacillus subtilis c) Pseudomonas aeruginosa d) Escherichia coli*

Table 5: Quantitative analysis of antibacterial activity of fungal extract based on zone of inhibition against pathogenic bacteria

S. No.	Name of the microorganism	Zone of inhibition (mm in diameter)				
		Positive control (gentamycin)	25 µl	50 µl	75 µl	100 µl
1	*Staphylococcus aureus*	17	07	09	11	13
2	*Bacillus subtilis*	19	-	08	10	11
3	*Pseudomonas aeruginosa*	18	-	07	09	11
4	*Escherichia coli*	17	-	08	10	12

Conclusion

This work showed how scientists pulled out, cleaned up, identified, and tested natural substances made by tiny fungi living inside *C. variegatum*. Because many kinds of fungus were found here, it proves the plant hosts plenty of hidden microbial life. Using methods like thin layer and column separation, researchers spotted several chemicals, each acting differently under analysis. Tools such as UV light readings, infrared scans, and gas-based detection helped map their makeup revealing parts like OH groups, carbon double bonds, and ring-like structures. One standout molecule, Tris (2, 4-di-tert-butylphenyl) phosphate, stood out due to its complex design and likely function. When checked in lab

tests, these extracts fought off oxidation, slowed enzymes tied to blood sugar issues, also blocked certain bacteria. So far, findings suggest fungi from this ornamental shrub may offer useful molecules, possibly aiding medicine development down the line.

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Conflict of interest

The authors declare that there is no conflict of interest. This research was conducted solely for academic purposes, with no financial, commercial, or personal interests influencing the outcomes.

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