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NATURAL BIOACTIVE COMPOUNDS SYNTHESIS IN ENDOPHYTIC MICRO-FUNGI FROM VARIETY OF THERAPEUTIC PLANTS IN SIVAKASI, TAMIL NADU

[1]Dr.Sundaram Muthukrishnan and [2]Mr.Rajaguru Gangatharan
[1]Assistant Professor and [2]M.Sc., Biotechnology
[1]Department of Biotechnology,
[1]Ayya Nadar Janaki Ammal College, (Autonomous), Sivakasi, Tamil Nadu, India.

Abstract: Medicinal plants include endophytic fungus that can create a range of bioactive chemicals with different pharmaco-potentials. The endophytic fungi *Fusarium solani* and *Curuvularia hominis* were isolated from different medicinal plants. GC-MS examination of endophytic fungus' intra- and extracellular products showed the presence of a variety of bioactive compounds, including n-Hexadecanoic acid, Tridecanoic acid, cis-Vaccenic acid, 6-Octadecenoic acid, Chloromethyl 11-chlorododecanoate, Benzene, tert-Butyl(5-isopropyl-2-methylphenoxy) dimethylsilane, 9,12-Octadecadienoic acid, Z,Z-4,15-Octadecadien-1-ol Acetate, 9-Octadecenoic acid, cis-13-Octadecenoic acid, cis-Vaccenic acid, Octadecanoic acid, Glycerol 1-palmitate, Triazin, Eicosane, Oleic Acid, Acetamide, 2,2,2-trifluoro-N, pennogenin diacetate, 1,2,3-propanetr,doecanoic acid 1,2,3-propanetr,acetamide,2,2,2-trifluoro-N-,colchiceine,N-desacetyl-n-T-FA,Acetamide,2,2,2-trifluoro-n-(5),5-(4-chlorophenyl)-6-ethyl,5alpha-cyano-3-methoxymethylen, anti-microbial, anti-fungal, anti-HIV, antitumor, anthelmintic, solubilizing agent, anti-cancer, antiviral, fungicidal, insecticidal, bactericidal, herbicidal, antimalarial and antimicrobial agents. The phytochemical composition analysis of endophytic extracts also revealed the presence of flavonoids, phenols, saponins, carbohydrates, glycosides, alkaloids and proteins. The intra and extracellular endophytic extracts exhibited strong antibacterial and antioxidant activity, which was screened with the agar well diffusion method and DPPH, H_2O_2 activity respectively. Therefore, the isolated endophytic fungal species can be utilized to generate a variety of bioactive chemicals from natural sources that utilized in the creation of innovative therapeutics and lessen the environmental impact of manufactured chemical drugs.

Keywords: Bioactive compounds, **Entophytic fungi, antibacterial, GC-MS, FTIR, Phytochemical study, Anticancer, antiviral activity.**

I. INTRODUCTION

A significant and cutting-edge source of naturally occurring bioactive chemicals, plant endophytic fungus has potential uses in the fields of agriculture, health, and food production. endophytic fungus for the production of bioactive substances obtained from plants, including diosgenin, vinblastine, camptothecine, hypericin, and paclitaxel. The relationships that exist between endophytic fungus and the plants that serve as their hosts, as well as some effective methods for boosting the production of these bioactive substances and their possible uses. It is advantageous for us to comprehend and utilize plant endophytic fungus more fully (Zhao *et al*., 2010). Novel bioactive compounds are in high demand to combat challenges of microbial resistance. In recent years, secondary metabolites from endophytic bacteria have drawn attention from researchers due to their novel structures and significant biological activities (Ngidi *et al*., 2021).

Endophytes are known to produce metabolites such as alkaloids, terpenoids, steroids, quinones, isocoumarin derivatives, flavanoids, phenols, phenolic acids, and peptide. Recently endophytes are viewed as outstanding source of secondary metabolites bioactive natural products. Endophytes are able to produce secondary metabolites these may be an antibiotic, therapeutic agent, agrochemical and an enzyme. Endophytes are rich in bioactive compound which have an important in the fields of medicine, agriculture

and industry. There are a large number of bioactive compounds that have been isolated from endophytic fungi and these bioactive natural products have demonstrated a broad range of biological activities. The production of metabolites is mainly depending on the environmental factor and some nature of host plant also because most of the endophytes have a mutual relationship with the plant to favour them. Endophytes are mostly unexplored but they have enormous number of sources as antioxidant, insecticidal, antitumor, antidiabetic, immunosuppressive and biocontrol compounds (Hussain *et al*., 2014).

The liquid-liquid extraction method has been utilized to extract bioactive substances and secondary metabolites from endophytic fungus. The process of extracting samples into immiscible organic and aqueous solutions by partitioning them is known as liquid extraction. Following the appropriate shaking and separation procedures, inorganic solvents were employed for the methodological extraction of the material. Because this approach takes longer, larger amounts of solvents are needed for the liquid-liquid extraction process. Two widely used techniques to identify the bioactive chemicals in fungal samples are FTIR and GC-MS (Kim *et al*., 2012)

The present study concentrated on isolating endophytic micro-fungi and using FTIR and GC-MS analysis to test for bioactive chemicals in them. To further identify the presence of different phytoconstituents, biochemical assays for protein, carbohydrates, flavonoids, tannins, and alkaloids were used. To ascertain the intra- and extracellular fungal extracts' bioactive potential, tests for antibacterial properties were conducted.

II. MATERIALS AND METHODS

Sample collection

The medicinal herbs were gathered from their original location in Sivakasi (9.4533° N, 77.8024° E). Within twenty-four hours of collection, ten plant samples with medicinal values— *Acalypha indica, Portulaca grandiflora, epipremnum aureum, Jatropha gossypifolia, Senna auriculata, Leucas aspera, and Clitoria ternatea*—were collected and stored in separate sterile ziplock bags. Fresh plant leaves and stems were removed for the isolation procedure in order to prevent contamination. The aforementioned medicinal plants have been used for wound healing, abdominal pain, diarrhea, dysentery, parasitic infestation, fever, malaria, respiratory issues, and anticancer properties. They have also been used for their prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive, and cytotoxic activities (Jagannath *et al*., 2021).

Surface sterilization

The samples were cleaned under running water to get rid of any dust and debris. The samples were then divided into 1-cm-long pieces and cleaned twice or three times in sterile distilled water before being placed within a laminar airflow chamber. The samples were cleaned with distilled water up to twice after first being rinsed with a 5% sodium hypochlorite solution. Following a 70% ethanol treatment, the samples were cleaned with sterile distilled water. Following that, 1% mercuric chloride was applied to the samples, and they were then cleaned with sterile distilled water. Ultimately, the samples were rinsed twice, first for two minutes in 79% ethanol and then for a maximum of two minutes in sterile distilled water (Alkahtani, *et al*., 2020)

Sample inoculation

Malt Extract Agar (MEA), Sabouraud Dextrose Agar (SDA), or Potato Dextrose Agar (PDA) medium were used to prepare the inoculation media. These media were ready and autoclaved for 20 minutes at 121 degrees Celsius to disinfect them. Antibiotics like tetracycline and amoxicillin were added to the medium at a palm-bearable temperature during the transfer to a sterile Petri plate in order to lessen the contamination of both gram-positive and gram-negative bacteria. Using clean forceps, surface-sterilized samples were infected following solidification. For fifteen days, the infected samples were incubated at 28 °C (Olusola *et al*., 2020)

Lactophenol cotton blue mounting

The staining solution was lactophenol and lactophenol cotton blue stain (Hi Media laboratories private limited). The ready slides were sealed with DPX mountant. The Compendium of Soil Fungi (Domsch, 1980) and "The Genera of Hyphomycetes from the Soil" (Barron, 1972) were used to identify the fungus species.

Diversity analysis

The diversity investigation was conducted using the fungal colony that surfaced from the inoculated plant samples. The colony was stained and evaluated under a microscope. Paraffin oil was used to stain dark fungal strains, such as black or grey strains, while

lacto phenol cotton blue dye was used to color the white strains. Applying a few drops of dye to the tiny slides, the fungal strain was extracted using a sterile needle. Subsequently, the fungus strain on the slides was teased to disperse the spores and conidia, and it was subsequently magnified (Olusola, 2020).

Production of fermentation medium

The fungal strain was added to the medium used for secondary metabolite synthesis. In addition to 20% of mineral sources like yeast extract, dipotassium hydrogen ortho phosphate, magnesium sulfate with hepta hydrate, sodium chloride, and ferrous sulfate, the fermentation medium was made using 50% potato extract and 5% carbon source like dextrose. In an autoclave to preserve sterility, the production medium above this concentration was created in a 200 ml Erlenmeyer flask holding a 500 ml capacity. Antibiotics like tetracycline and amoxicillin were added to the production medium at a concentration of 200 mg/L after it reached room temperature. After adding the spore-containing fungal strain to the fermentation medium, it was cultured for 15 days at 28°C (Sharma *et al*., 2016**)**.

Extraction of metabolites

In order to separate the bioactive chemicals produced by the endophytic fungi present in the production medium, the primary step involved the extraction of secondary metabolites. The intra-and extracellular metabolites were extracted from the production medium using the liquid-liquid extraction technique. The liquid broth of the production medium was collected for the extracellular metabolites following a 20-day incubation period. Initially, filter paper and a glass funnel were used to filter the production material. No mycelium or fungal culture may be present in the filtrate. This filtrate was combined with the same volume of ethyl acetate and left to incubate at room temperature for four hours. Next, using a separating funnel, the filtrate and ethyl acetate mixture were divided. Following collection of the organic layer, **(**Sharma, *et al*., **2016)** Using a mortar and pestle and methanol as a solvent, the mycelium from the production medium was removed in order to disturb the intracellular metabolites. A piece of grass cloth was used to filter the ground mycelium extract. After the extract was lyophilized, devices like FTIR and GCMS were used to evaluate it (Hajjaj *et al*., 1998)

`The extraction of secondary metabolite was the major process to screen out the bioactive compounds produced by the endophytic fungi present in the production medium. The liquid-liquid extraction method was used to extract the metabolites (intra/extracellular) from the production medium. For the extracellular metabolites, the liquid broth of the production medium was collected after 20 days of incubation. Initially, the production medium was filtered with the usage of a glass funnel and filter paper. The filtrate must not contain any mycelium or fungal culture. This filtrate was mixed with an equal amount of ethyl acetate and allowed to incubate for four hours at room temperature. Then the filtrate and ethyl acetate mixture has been partitioning with a separating funnel. The organic layer was collected and used for the screening process of bioactive compounds (Sharma, *et al*., 2016). For the intracellular metabolites, the mycelium from the production medium was harvested and disrupted with mortar and pestle along with the solvent as methanol. The ground mycelium extract was filtered with grass cloth. The extract was lyophilized and then analyzed with the instruments such as FTIR and GCMS (Hajjaj *et al*., 1998).

FTIR Analysis

FTIR analysis was performed on the endophytic fungal extracts in order to identify the functional group that the metabolites contained. The FTIR employed a range of 400 to 4000 cm-1 for both transmittance and absorption. The sample was combined with the KBR powder and sent through a hydraulic pellet press to create a pellet suitable for examination. Next, the fungal samples' peak range was utilized to forecast the functional groups (Elango *et al*., 2020).

GCMS Analysis

GCMS has been used to identify the bioactive chemicals present in the fungal extract, both intracellular and intercellular. The liquid sample utilized for the GCMS test sample is free of contaminants and particles. The test samples were centrifuged for five minutes at 3000 rpm in order to accomplish this. The test sample was obtained from the clear supernatant. After dissolving the sample powder in methanol, the clear supernatant was obtained by centrifuging the mixture for intracellular extract (Elango *et al*., 2020).

Antibacterial assay

The test samples' antibacterial activity is evaluated using the agar well diffusion method. An extra 2% concentration of agar was added to the nutritional medium during preparation. Using an autoclave, the media was made sterile. Bacterial cultures of

Staphylococcus pneumoniae, *Shigella* sp., *Escherichia coli*, and *Streptococcus avarmitilis* were added to the nutrient agar-coated Petri plates. Then, on the cultivated agar plates, a 1 cm-diameter well was formed using the sterile well puncher. The Petri plate well was filled with test samples at varying concentrations of 10 µl and 20 µl. For the purpose of this agar well diffusion procedure, the solvent that was utilized to dissolve or extract the fungal metabolites served as the control. The solvents in control were ethyl(Fouda, *et al*., 2020).

Phytochemical analysis

For the phytochemical investigation, both the extracellular and intracellular extracts were utilized. In this study, 10 phytochemicals—including flavonoids, alkaloids, saponins, tannins, carbohydrates, terpenoids, steroids, phenols, glycosides, and proteins were examined (Roghini and Vijayalakshmi, 2018).

Flavonoids test

Alkaline test

Two to three drops of 2% diluted NaOH solution were added to the two milliliters of fungal extract. The test's yellow hue shift signifies the presence of flavonoids (Roghini and Vijayalakshmi, 2018).

Lead acetate test

A few drops of lead acetate were added to two milliliters of the extract. The test's yellow precipitate development indicates that the extract contains flavonoids (Roghini and Vijayalakshmi, 2018).

Ferric chloride test

The 2 ml of the fungal extract was taken and a few drops of the ferric chloride was added to it. The green color changes of the test indicate the presence of flavonoids (Roghini and Vijayalakshmi, 2018**).**

Sulphuric acid test

A few drops of ferric chloride were added to the 2 ml of fungal extract that was extracted. The test's changes in hue to green indicate the presence of flavonoids. (Roghini and Vijayalakshmi, 2018).

Saponins test

One ml of the fungus extract and five ml of distilled water were combined. The test was subjected to a 15–20 second violent shake. After then, take a ten-minute break from the test. The fungal extract contains saponins, as evidenced by the development of a stable form. (Roghini and Vijayalakshmi, 2018).

Tannins test

Ferric chloride test (Tannins test)

The 2 ml of the fungal extract was taken along with the 1 ml of 5% $FeCl_2$ was added to it. The color change indicates the presence of tannin in the sample. While the dark green-black color formation indicates the presence of condensed tannins, as well as the dark blue black color formation indicates the presence of hydrolyzed tannin in the sample **(**Roghini and Vijayalakshmi, 2018).

Gelatin test

The 2 ml of the fungal extract was taken along with 1% gelatin solution was added, and also 1ml of 10% NaCl has added it. The white color precipitate formation in the test indicates the presence of tannin in the sample **(**Roghini and Vijayalakshmi, 2018).

Lead acetate test

The 2 ml of the fungal extract was taken along with a few drops of lead acetate was added to it. The formation of white color precipitate in the test indicates the presence of tannin in the sample **(**Roghini and Vijayalakshmi, 2018).

Proteins test

Millon test

The 2 ml of the fungal extract was taken along with 2ml of the millon reagent added to it. The formation of white color precipitate in the test indicates the presence of proteins in the sample **(**Roghini and Vijayalakshmi, 2018).

Biuret test

The 2 ml of the fungal extract was taken. A drop of 2% $CuSO_4$, as well as 1ml of ethanol and sodium hydroxide pellet, was added to the extract. The test allows incubating for 2 to 5 minutes without shaking it. The formation of pink color indicates the presence of protein in the sample **(**Roghini and Vijayalakshmi, 2018).

Ninhydrin test

The 2 ml of the fungal extract was taken along with 2ml of the ninhydrin solution. The test was placed in a water bath for 5 minutes at 50°C. The formation of violet-blue or violet color in the test indicates the presence of protein in the solution **(**Roghini and Vijayalakshmi, 2018).

Aminoacid test

The 2 ml of the fungal extract was taken along with a few drops of lead acetate and diluted NaOH. The formation of a black color precipitate indicates the presence of protein in the sample **(**Roghini and Vijayalakshmi, 2018).

Carbohydrate test

Benedicts test

The 2 ml of the fungal extract was taken along with 2 ml of benedict reagent, followed by placed in a water bath for 2 minutes at 50°C. The formation of the orange and red colors indicates the presence of carbohydrates in the sample **(**Roghini and Vijayalakshmi, 2018).

Iodine test

The 2 ml of the fungal extract was taken along with the 2 ml of the iodine solution. The formation of dark blue or purple color indicates the presence of carbohydrates in the sample **(**Roghini and Vijayalakshmi, 2018)..

Fehlings test

Initially, the Fehling A and Fehling B solutions were diluted well and boiled for 1 minute. After boiling, the clear blue color Fehling solution was obtained. The 2 ml of the fungal extract was taken along with 1 ml of the prepared Fehling solution. The test was placed in a water bath for 5 minutes at 50°C. The formation of dark brick red color indicates the presence of carbohydrates in the sample **(**Roghini and Vijayalakshmi, 2018).

Terpenoids test

The 2 ml of the fungal extract was taken along with 2 ml of the chloroform was added to it. After the test becomes dry, the 2 ml of conc. H_2So_4 was added to the test. The test sample was placed water bath for 2 minutes at 50°C. The formation of a grey color indicates the presence of terpenoids in the sample **(**Roghini and Vijayalakshmi, 2018).

Steroids test

The 2 ml of the fungal extract was taken along with a few drops of chloroform and 1 ml of the con. H_2SO_4. The formation of the brown ring indicates the presence of steroids, and the appearance of the bluish brown ring indicates the presence of phyto-steroids **(**Roghini and Vijayalakshmi, 2018).

Glycosides test

The 2 ml of the fungal extract was taken along with a few drops of $FeCl_2$ as well as 1ml of glacial acetic acid and 1 ml of the con. H_2SO_4 was added to it. The formation of the brown ring indicates the presence of cardiac glycosides in the sample **(**Roghini and Vijayalakshmi, 2018).

Alkaloids test

Mayer test

The 2 ml of the fungal extract was taken along with a few drops of Mayer reagent and 1 ml of the con. H_2SO_4. The formation of yellow color in the test indicates the presence of alkaloids in the sample **(**Roghini and Vijayalakshmi, 2018).

.Phenols test

Ferric chloride test

The 2 ml of the fungal extract was taken along with 3–4 drops of 10% ferric chloride solution and 1 ml of potassium ferro cyanide. The formation of bluish-black color in the test indicates the presence of phenols in the sample**(**Roghini and Vijayalakshmi, 2018).

Litmus paper test

The red color changes in the litmus paper confirmed the presence of phenols in the sample when the litmus paper was dipped in the sample to be tested **(**Roghini and Vijayalakshmi, 2018).

Antioxidant analysis

The fungal species contained a property to prevent or slow down cell damage caused by free radicals or other unstable molecules. The quantitative presence of antioxidant properties in the endophytic fungal extract was estimated by DPPH, H_2O_2, and Nitric oxide scavenging analysis (Senguttuvan *et al*., 2014)

DPPH Radial scavenging activity

Initially, 100 µM of DPPH was weighed and mixed with 99% methanol. This DPPH mixture was incubated for a minimum of 3 hours in a dark room without any photo and aerobic reaction. After 3 hrs of incubation, the DPPH was ready to use for the antioxidant test. For DPPH scavenging activity, the clear test tubes or 96 well microtitre plate was used. The test samples were taken in different concentrations from 10 µl to 50 µl. Then the test samples were made up to 100 µl with DPPH solution. After the incubation, the violet color changes into yellow based on the concentration, indicating the physical presence of the antioxidant property. The test samples were examined under UV spectrophotometry with the absorption at 517 nm. The standard was prepared with ascorbic acid. As a test sample, the standard was also taken in different concentrations like 10 µl to 50 µl and makeup with DPPH up to 100 µl followed by incubation up to 30 minutes at 37°C. After incubation, the standard was examined under UV spectrophotometry with the absorption at 517 nm. The pure methanol was used as a blank, whereas the 3 ml of DPPH solution was used as a control DPPH scavenging activity (Senguttuvan *et al*., 2014). The percentage of DPPH was calculated with the following equation.

$$\text{DPPH (scavenging effect \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Whereas the $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ was the absorption of control and absorption of the sample at 517nm.

H_2O_2 Radial scavenging activity

The test samples were taken in different concentration of 10 µl to 50 µl and was made up to 100 µl with H_2O_2 solution (4mM H_2O_2 was dissolved in phosphate buffer). The test sample was examined under a UV spectrophotometer with the absorption at 230 nm. Pure methanol was used as the control. The standard (ascorbic acid) was also prepared like a test sample and incubated for up to 10 minutes at 37°C. After incubation, the standard was examined under UV spectrophotometry with absorption at 230 nm (Senguttuvan *et al*., 2014. The pure methanol was used as the blank, whereas the 3 ml of pure hydrogen peroxide solution was used as a control. The percentage of the H_2O_2 scavenging activity was calculated with the following equation.

$$H_2O_2 \text{ (scavenging effect \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Whereas the $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ was the absorption of control and absorption of sample at 230nm.

Whereas, the $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{sample}}$ was the absorption of control and absorption of sample at 546nm.

Fungal identification through its region sequencing:

Fungal sample was commercially sequenced at Barcode Biotechnologies, Goa, India. Briefly, genomic DNA was isolated using the standard protocol described in AllPrep Fungal DNA Kit (Qiagen). The ITS1 [5'-TCCGTAGGTGAACCTGCGG-3'] and ITS4 [5'-TCCTCCGCTTA TTGATATGC-3'] were used for ITS region amplification using the PCR conditions; 10 minutes at 95°C followed by 35 cycles of 30 seconds at 95°C, 40 seconds at 60°C, and 40 seconds at 72°C and final extension for 5 minutes at 72°C. PCR

amplicons were purified using QIAquick PCR Purification Kit (Qiagen). The amplicons were 2-way sequenced using Sanger's dideoxy sequencing process in ABI 3730xl DNA Analyzer.

III. RESULT

Endophytic microfungal isolates

The matured *Fusarium solani* was whiteish orange color colony with dark color spores in PDA medium. The microscopic examination of *Fusarium solan*i was Blue color elongated spore with ten segments along with conidia. The matured colony of *Curuvulria homonis* was blackish brown colony with brown color spores. The microscopic examination of *Curuvularia hominis* was brown color oval shape spores with segements along with curved conidia isolated from ten medicinal plant samples (Fig.1,2,3)

Fig.1 Structure of *Curuvalaria hominis* under 40X of microscope

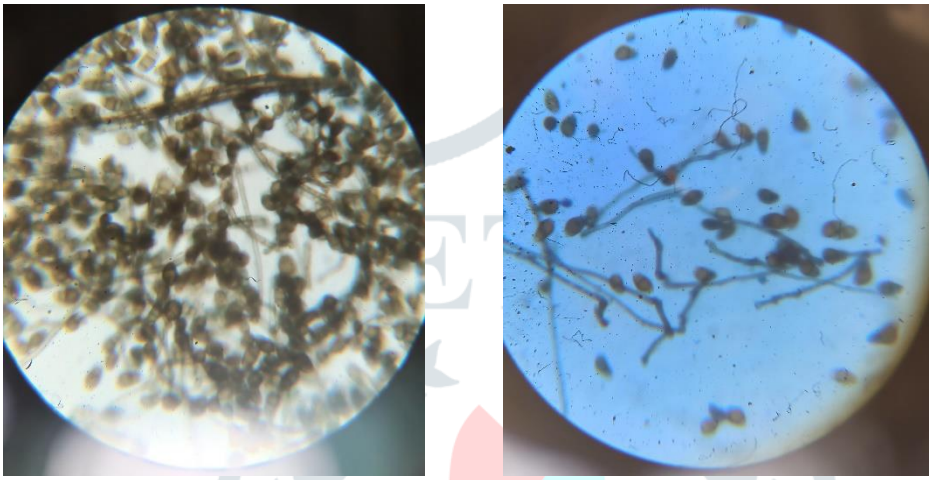


Fig.2. Structure of *Fusarium solani* under 40X of microscope

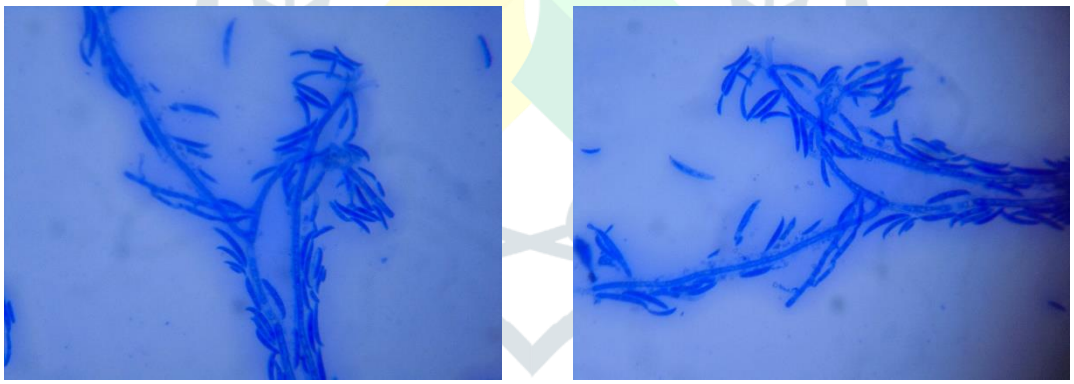


Fig.3. Pure culture of endophytic micro-fungi

Fusrium solani ***Curvularia hominis***

Fig 4. Extraction of secondary metabolites from the endophytic production medium

FTIR ANALYSIS

FTIR analysis of *Curuvularia hominis* ethyl acetate extract

The fungal ethyl acetate extract as *Curuvularia hominis* in FTIR analysis observed the essential functional group peak intensity range of 501.49 cm-1 (Strong, C-I stretching, halo compound), 594.08 cm-1 (Strong, C-I stretching, halo compound), 732.95 cm-1 (Strong, C-I stretching, halo compound), 848.68 cm-1 (Strong, C-CI Stretcing, halo compound), 1103.28 cm-1 (Strong, C-F Stretcing, Fluoro compound), Which are tabulated (Table:1 and Fig:5)

FTIR analysis of *Fusarium solani* ethyl acetate extract

The fungal ethyl acetate extract as *Fusarium solani* in FTIR analysis observed the essential functional group peak intensity range of 509.21 cm-1 (strong, C-I stretching, halo compound), 594.08 cm-1 (strong, C-Cl stretching, halo compound), 732.95 cm-1 (strong, C-CI stretching, halo compound), 786.96 cm-1 (Strong, C-CI stretching, halo compound), 879.54 cm-1 (week, C-H bending, 1,2,4 trisubstituted), which are tabulated (Table:2 and Fig:5)

ANTIBACTERIAL ACTIVITY OF MICROFUNGAL EXTRACT

Antibacterial activity of *Fusarium solani* ethyl acetate extract

The ethyl acetate extract of *Fusarium solani* was recorded the zone of inhibition against Escherichia coli had maximum level of zone of inhibition as 2.0mm (20 µL) and 1.2 mm (10 µL), *Shingella* zone of inhibition was recorded as 0.6 mm (20 µL) and 0.2mm

(10 µL), *Staphylococcus pneumoniae* is the highest zone of inhibition was recorded 4.2mm (20 µL) and 3.5 mm (10 µL) and then *Streptococcus avarmtillus* as 0.3mm (20 µL) and there is no inhibition at 10 µL concentration (Table.7).

Antibacterial activity of *Curuvalaria hominis* methanal extract

The methanal extract of *Curuvalaria* hominis was recorded the zone of inhibition against *Escherichia coli* had the highest level of zone of inhibition as 1.5mm (20 µL) and 0.8 mm (10 µL), *Shingella sp.*, had maximum level of zone of inhibition as 0.9 mm (20 µL) and 0.5mm (10 µL), *Streptococcus avarmtillus* zone of inhibition as 0.3mm (20 µL) and no inhibition at 10 µL concentration and then *Streptococcus avarmtillus* as 0.8mm (20 µL) and 0.4mm (10 µL) (Table.8).

PHYTOCHEMICAL ANALYSIS OF ENDOPHYTIC MICROFUNGAL EXTRACT

Phytochemical analysis of extra cellular extract of *Fusarium solani*

The phytochemical analysis of the ethyl acetate extract of *Fusarium solani* reveals the presence of variety of secondary metabolites in the sample such as flavonoids, saponins, glycosides, alkaloids and proteins in very high content as well as phenols was occurred in moderate level. Absence of Tannins, Steroids, terpenoids and carbohydrates was also noted by this phytochemical analysis (Table:9)

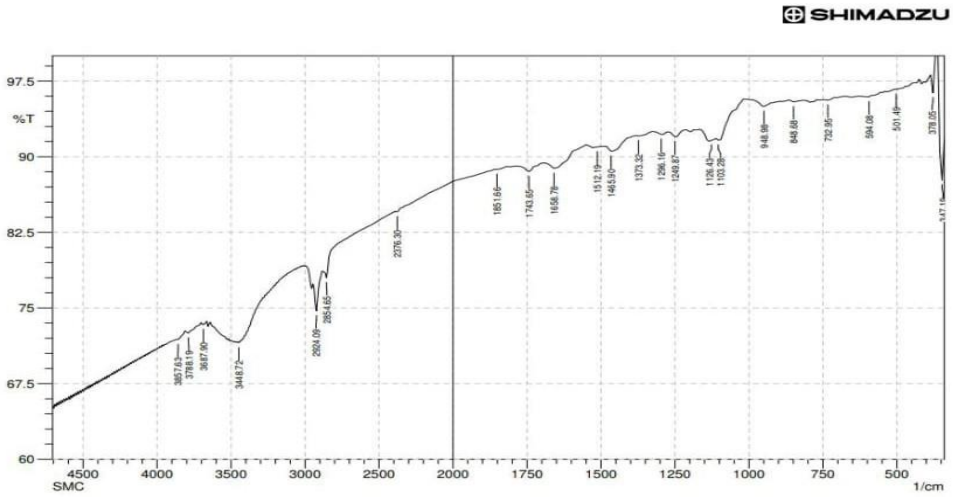
Phytochemical analysis of extra cellular extract of *Curuvularia hominis*

The phytochemical analysis of the ethyl acetate of *Curuvularia hominis* revels the presence of variety of secondary metabolites in the sample such as phenols, flavonoids, steroids, saponins, very high content as well as carbohydrates occurred in moderate level. Absence of phenols, glycosides, alkaloids was also noted by this phytochemical analysis (Table:9)

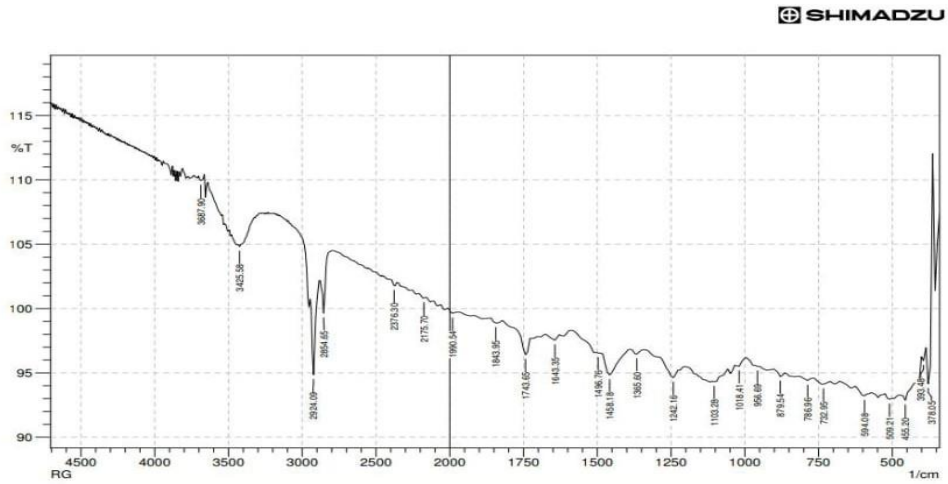
Phytochemical analysis of intra cellular extract of *Fusarium solani*

The phytochemical analysis of the methanol extract of *Fusarium solani* revels the presence of variety of secondary metabolites in the sample such as flavonoids, saponins, tannnins, very high content as well as alkaloids occurred in moderate level. Absence of phenols, protein, and carbohydrate was also noted by this phytochemical analysis (Table:9)

Fig.5.FTIR grapical peak representation of endophytic microfungal ethyl acetate extract extracellular metabolites

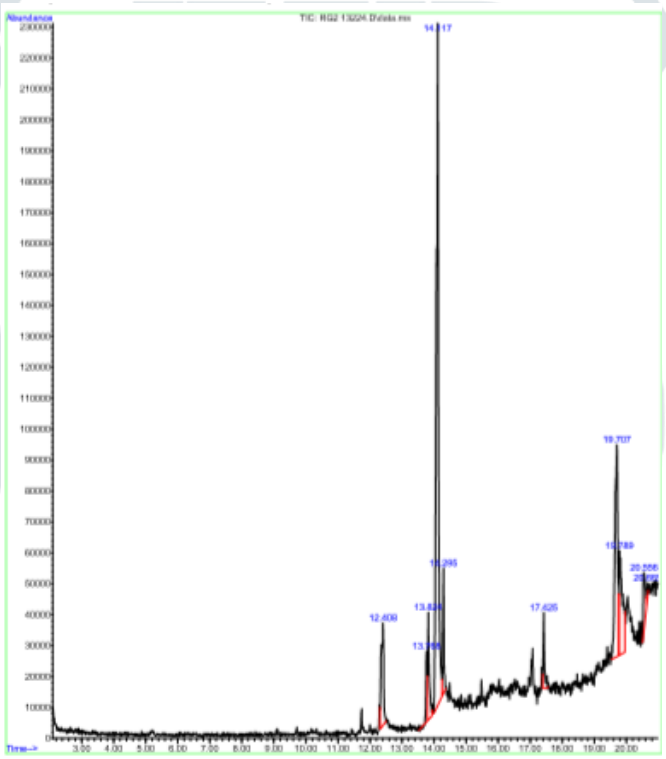


***Fusarium solani* ethyl acetate extract**

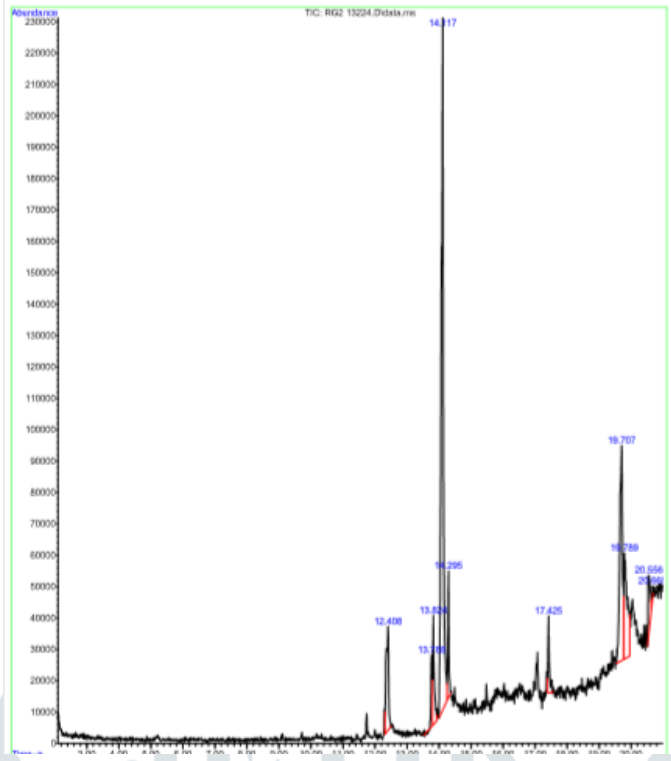


***Caruvularia hominius* ethyl acetate extract**

Fig.6.GC-MS analysis of grapical peak representaion of endophytic microfungal extracellular metabolites

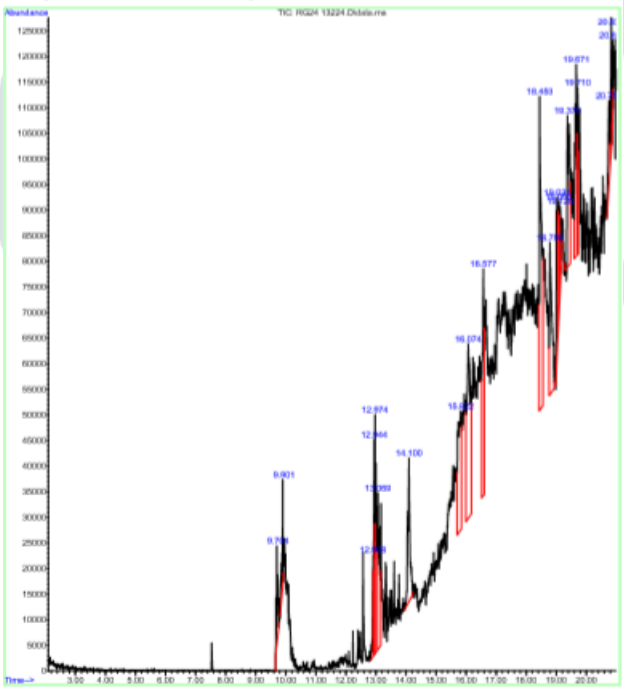


***Fusarium solani* ethyl acetate extract**

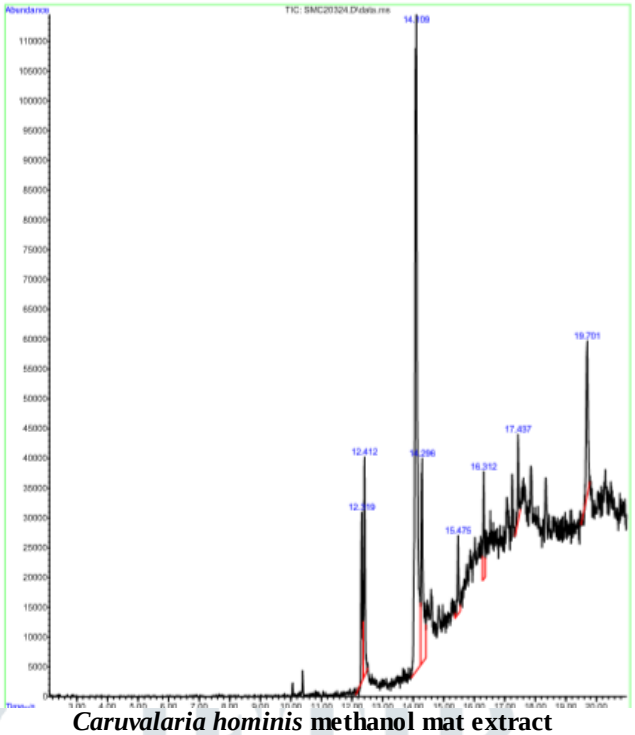


***Caruvularia hominius* ethyl acetate extract**

Fig.7.GC-MS analysis of grapical peak representaion of endophytic microfungal intracellular metabolites



***Fusarium solani* methanol mat extract**



***Caruvalaria hominis* methanol mat extract**

Phytochemical analysis of intra cellular extract of *Curuvularia hominis*

The phytochemical analysis of the methanol extract of *curuvularia hominis* revels the presence of variety of secondary metabolites in the sample such as phenols, alkaloids, steroids, saponins, flavonoids, very content as well as carbohydrate, and protein occurred in moderate level of presence in the sample was also noted by this phytochemical analysis (Table 9)

ANTIOXIDANT ACTIVITY OF ENDOPHYTIC MICROFUNGAL EXTRACT

DPPH Antioxidant scavenging activity of extracellular extract of *Fusarium solani*

The antioxidant scavenging activity of ethyl acetate extracellular extract of *Fusarium solani* was performed with DPPH. In DPPH scavenging activity the standard and test sample absorption was recorded with uv spectrophotometry. The high range of absorption was obtained at 10% concentration of test sample. The morderate range of scavengng activity was observed with DPPH solution as87% at 50% concentartion and 33.09% at 10% concentartion of scavenging activity when compare to other extracellular extract of endophytic fungi and standard (Table:10)

DPPH Antioxidant scavenging activity of extracellular extract of *Curuvularia hominis*

DPPH was used to test the antioxidant-scavenging ability of the *Curuvularia hominis* ethyl acetate extracellular extract. Using UV spectrophotometry, the standard and test sample absorption in the DPPH scavenging activity was observed. At 10% concentration of test material, the high range of absorption was achieved. DPPH solution showed a moderate range of scavenging activity, with 89% at 50% concentration and 26.98% at 10% concentration, when compared to other extracellular extracts of endophytic fungi and standard (Table:10)

H2O2 Antioxidant scavenging activity of intracellular extract of *Fusarium solani*

The antioxidant scavenging activity of methanol mycelium mat extract of *Fusarium solani* was performed with H2O2. In H2O2 scavenging activity the standard and test smaple absorption was recorded with uv spectrophotometry. The high range of absorption was obtained at 10% concentration of test sample. The maximum range of scavengng activity was observed with H2O2 solution as 89.78% at 50% concentration and 29.98% at 10% concentration of scavenging activity when compare to other intracellular extract of endophytic fungi and standard (Table:11).

H2O2 Antioxidant scavenging activity of intracellular extract of *Caruvalaria hominis*

The antioxidant scavenging activity of methanol mycelium mat extract of *Caruvalaria hominis* was performed with H2O2. In H2O2 scavenging activity the standard and test smaple absorption was recorded with uv spectrophotometry. The high range of absorption was obtained at 10% concentration of test sample. The maximum range of scavengng activity was observed with H2O2 solution as 78.65% at 50% concentartion and 23.12% at 10% concentartion of scavenging activity when compare to other intracellular extract of endophytic fungi and standard (Table:11).

GCMS ANALYSIS FOR BIOACTIVE COMPOUND SCREENING

GCMS analysis of *Fusarium solani* ethyl acetate extract

The fungal ethyl acetate extract of *Fusrium solani* in GC-MS analysis recorded the essential bioactive compound such as n-Hexadecanic acid,9,12-octadecadienoic acid, z,z-4,15-octadecadien-1-ol acetate,octadec-9-enoic acid,9-octadecenoic acid,n-propyl 11-octadecenoate,cis-vacceni acid,9-octadecenoic acid, ,octadecanoic acid,glycerol1-palmitate,,palmitoylcholoride,1-glyceroGCMS analysis of Bipolaris australiensis ethyl acetate extract palmitate,oleolchloride,cyclooctaneaacetia acid, which are tabulated (Table-4 and Fig -6).

GCMS analysis of *Fusarium solani* methanol mycelium extract

The fungal methanol extract of *Fusarium solani* in GCMS analysis recorded the essential bioactive compound such as osmium, hexacarbonyldi,osmium,octacarbonyldliod,2-(N_methl-N-nitroaino),21H,23H-prophine-2,7,12,17-tetra,9-octadecenoic acid,(Z)-oleic acid,dodecanoic acid,1,2,3-propanetr,dodecanoic acid 1,2,3,-propanter,benzamide4-bromo-N-(4-ch loroph..,5-acetyl-2-(3-bromo-benzoylamino,dodecanoic acid 1,2,3-propanetr,doecanoic acid 1,2,3-propanetr,acetamide,2,2,2-trifluoro-N-,colchiceine,N-desacetyl-n-T-FA,Acetamide,2,2,2-trifluoro-n-(5),5-(4-chlorophenyl)-6-ethyl. (Table-6 and Fig -7).

GCMS Analysis of *Curuvularia hominis* ethyl acetate extract

The fungal ethyl acetate extract of *curuvularia hominis* in GCMS analysis recorded the essential bioactive compound such as n-Hexadecanoic acid,tridecanoic acid, cis-vaccenic acid,6-octadecenoic acid (Table -3 and Fig -6).

GCMS Analysis of *curuvularia hominis* methanol mycelium extract

The fungal methanol extract of *Caruvalaria hominis* in GCMS analysis recorded the essential bioactive compounds such as n-hexadecanoic acid, Tridecanonic acid, cis-vaccenic acid, 6-octadecenoic acid, octadecenoic acid, Eicosane,octadecane, opyldecahydrod, 1-octadecanesulphonly chloride, methoxyacetic acid, benziothaiazol 3-amine tbdms, quinolone,1H-indole,benezene,(E)-2bromobytylox yachalcone,1-Benzazirene-1-carboxylic acid.which are tabulated (Table -5 and Fig -7).

DNA Sequencing

The Endophytic fungi *Fusarium solani* isolated from Phyllanthus niruri plant the fungal species was identified using ITS sequencing analysis. The sequenced ITS sequence submitted to the NCBI.

5'-3'

```
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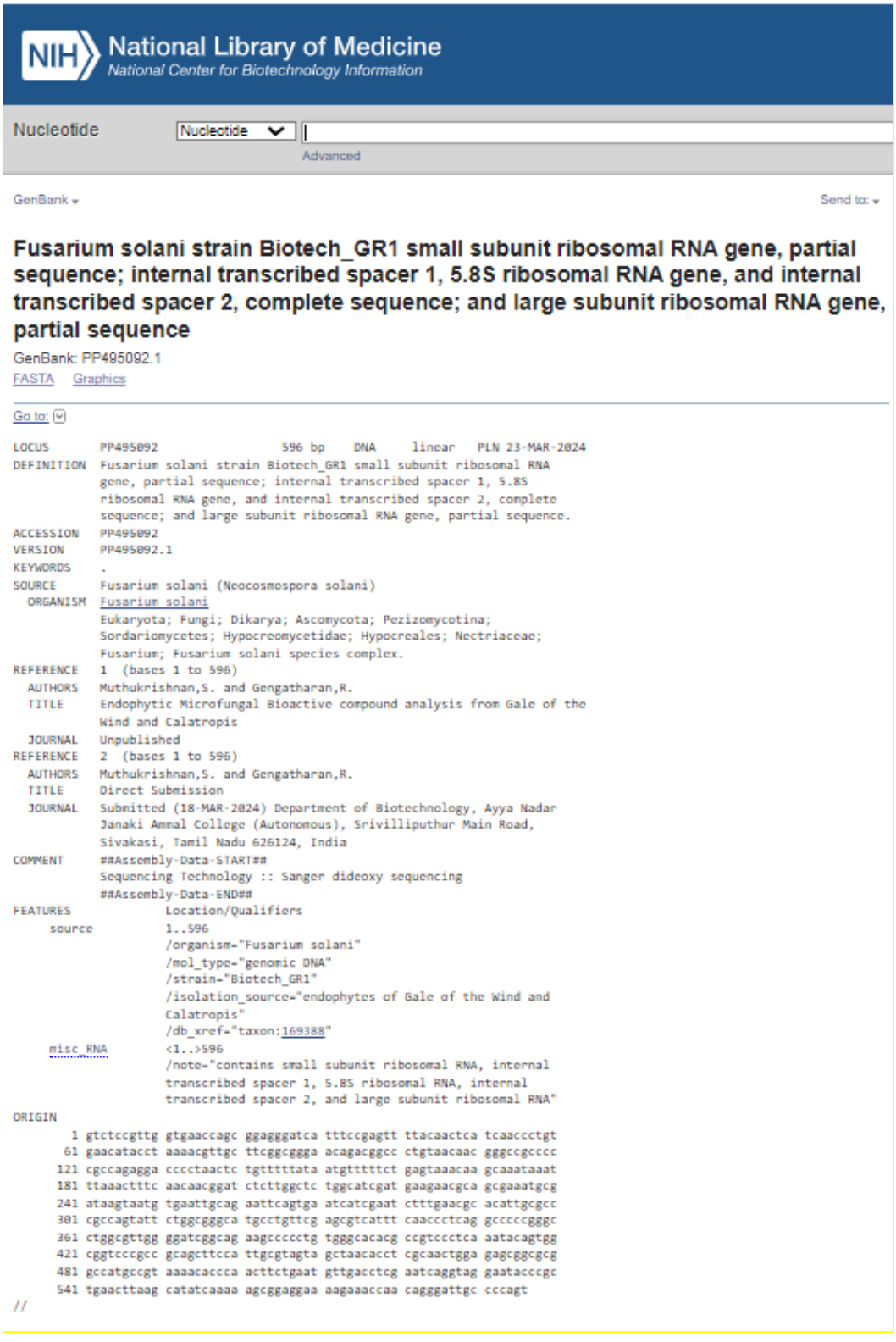

National Library of Medicine
National Center for Biotechnology Information

Nucleotide | Nucleotide | Advanced

GenBank | Send to:

Fusarium solani strain Biotech_GR1 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

GenBank: PP495092.1

FASTA Graphics

Go to:

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  AUTHORS   Muthukrishnan,S. and Gengatharan,R.
  TITLE     Endophytic Microfungal Bioactive compound analysis from Gale of the
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 596)
  AUTHORS   Muthukrishnan,S. and Gengatharan,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-MAR-2024) Department of Biotechnology, Ayya Nadar
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//
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Phylogenetic tree:

A diagrammatic representation or tree that illustrates the evolutionary relationships between several biological species of *Fusarium solani* fungi is called a phylogenetic tree. A phylogenetic tree was built using the *Fusarium solani* ITS rDNA sequence. The bootstrap values on neighbor-joining analysis of 500 resampled data sets are indicated by the number of nodes. Bar 0.0020 shows the divergence of the sequence. After the sequences were aligned with those in the GenBank database, phylogenetic tree analysis was performed on them.

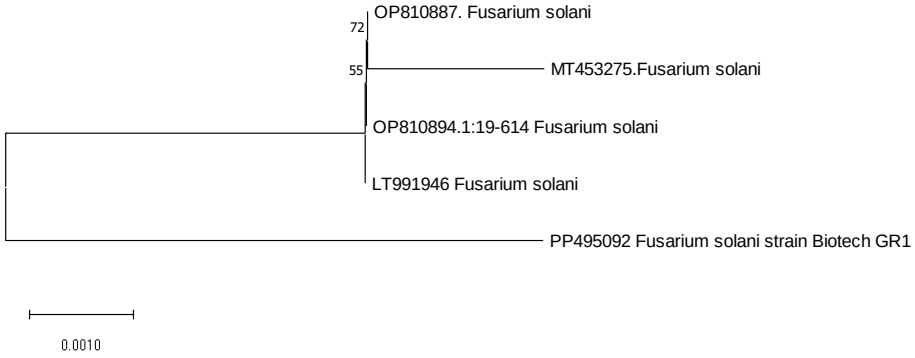


Table.1. FTIR analysis of ethyl acetate crude extract of *Fusarium solani*

.no	eak Value	roup	ompound
	09.21	-I stretching	alo compound
	94.08	-Cl stretching -Br stretching -I stretching	alo compound
	32.95	-Cl stretching -H bending	alo compound nonosubstituted
	86.96	-Cl stretching -H bending	alo compound ,2,3-trisubstituted
	79.54	-H bending	,2,4-trisubstituted
	56.69	-Ostratching	itro compound
	018.41	-F stretching	uoro compound
	103.28	-F stretching -N stretching -O stretching	uoro compound mine liphatic ether econdary alcohol
	242.16	-F stretching -O stretching -N stretching	uoro compound lkyl aryl ether mine
0.	365.6	-H bending -F stretching -H bending =O stretching	lcohol uoro compound henol lfonate lfonamide
1.	458.18	-H bending	lkane
2.	496.76	-H bending	lkane
3.	643.35	=N stretching =C stretching -H bending	nine / oxime lkene onjugated alkene mine

			yclic alkene
4.	743.65	-H bending =O stretching	romatic compound sters -lactone
5.	843.95	-H bending	romatic compound
6.	990.54	=C=S stretching =C=C stretching -H bending	othiocyanate llene romatic compound
7.	175.7	-C≡N stretching	hiocyanate
8.	376.3	-H bending	henol
9.	854.65	-H stretching -H stretching -H stretching	arboxylic acid lcohol mine salt lkane
0.	924.09	-H stretching -H stretching -H stretching	arboxylic acid lcohol mine salt lkane
1.	425.58	-H stretching	lcohol
2.	687.9	-H stretching	lcohol

Table.2. FTIR analysis of ethyl acetate crude extract of *Caruvalaria hominis.*

S.no	Peak value	Group	Compound
1.	347.19		
2.	378.05		
3.	501.49	C-I stretching	halo compound
4.	594.08	C-Cl stretching C-Br stretching C-I stretching	halo compound
5.	732.95	C-Cl stretching C-H bending	halo compound monosubstituted
6.	848.68	C-Cl stretching	halo compound
7.	948.98		
8.	1103.28	C-F stretching C-N stretching C-O stretching	fluoro compound amine aliphatic ether secondary alcohol
9.	1126.43	C-F stretching C-N stretching C-O stretching	fluoro compound amine tertiary alcohol aliphatic ether

10.	1249.87	C-F stretching C-O stretching C-N stretching	fluoro compound alkyl aryl ether amine
11.	1296.16	C-F stretching C-N stretching C-O stretching	fluoro compound aromatic amine aromatic ester
12.	1373.32	O-H bending C-F stretching	Alcohol fluoro compound phenol
13.	1465.9	C-H bending	Alkane
14.	1512.19	N-O stretching	nitro compound
15.	1658.78	C-H bending C=N stretching C=C stretching	aromatic compound imine / oxime alkene
16.	1743.65	C-H bending C=O stretching	aromatic compound esters δ-lactone
17.	1851.66	C-H bending	aromatic compound
18.	2376.3		
19.	2854.65	O-H stretching N-H stretching C-H stretching	carboxylic acid alcohol amine salt alkane
20.	2924.09	O-H stretching N-H stretching C-H stretching	carboxylic acid alcohol amine salt alkane
22.	3448.72	O-H stretching	Alcohol
23.	3687.9	O-H stretching	Alcohol
24.	3788.19		

Table.3. Screening and Characterization of Bioactive compounds from ethyl acetate extract of *Caruvalaria hominis*. in GC-MS analysis

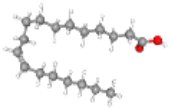
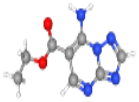
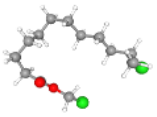
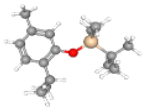
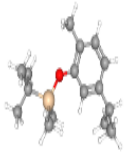
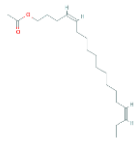
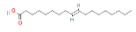
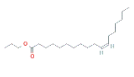
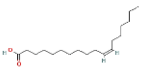
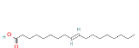
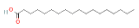
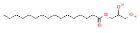
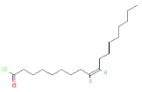
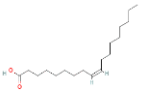
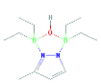
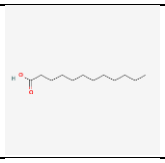
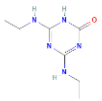
Peak value	Compound name	Molecular formula	Pubchem id	Molecular weight	Structure	Application
12.412	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	985	256.42 g/mol		Antiinflammatory,antioxidant,anticancer, Antinociceptive, cytotoxiccompound.
12.412	Tridecanoic acid	$C_{13}H_{26}O_2$	12530	214.34 g/mol		anthelminthic, anti-inflammatory and antimicrobial activities and anti-cancerous activity.
14.105	cis-Vaccenic acid	$C_{18}H_{34}O_2$	5282761	282.5 g/mol		Antibacterial reduce sicklecell, Reduction in the risk ofcaronary heartdisease (CHD)
14.105	6-Octadecenoic acid	$C_{18}H_{34}O_2$	5282754	282.5 g/mol		anticarcinogenic activity, suppressing in vitro growth of human melanoma, colorectal, and breast cancer cells, and exhibiting anti-atherogenic activity.
14.294	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 7-amino-, ethyl ester	$C_8H_9N_5O_2$	974047	207.19 g/mol		antimicrobial , anticancer, anti-inflammatory , anti-malarial , anti-diabetic , anti- HIV
14.294	Chloromethyl 11-chlorododecanoate	$C_{13}H_{24}Cl_2O_2$	543373	283.2 g/mol		antibacterial and antifungal properties
19.702	Benzene	$C_{16}H_{28}OSi$	610042	264.48 g/mol		Industrial manufacturing dyes,lubricant,drug, fuel
19.702	tert-Butyl(5-isopropyl-2-methylphenoxy)dimethylsilane	$C_{16}H_{28}OSi$	13581204	264.48 g/mol		Antitumor, antiinflammatory

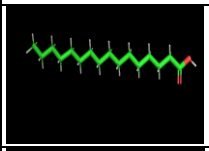
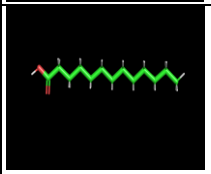
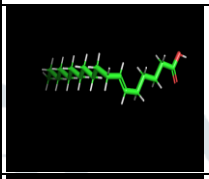
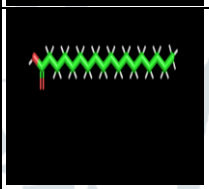
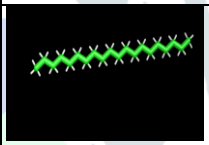
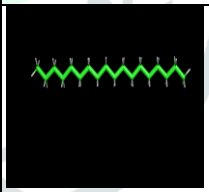
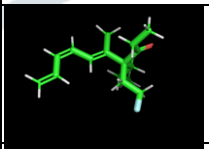
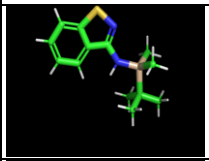
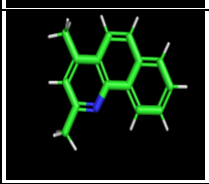
Table.4. Screening and Characterization of Bioactive compound from ethyl acetate extract of *Fusarium solani* in GC-MS analysis

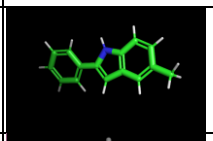
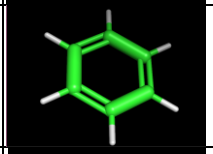
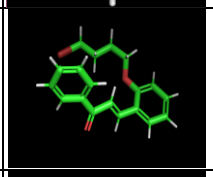
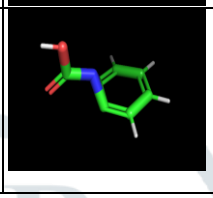
Retention Time(cm^{-1})	Compound Name	Molecular Formula	PubChem Id	Molecular Weight	2d Structure	Application
12.412	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	985	256.42 g/mol		Antiinflammatory, antioxidant, anticancer, Antinociceptive, cytotoxic compound.
13.764	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	3931	280.4 g/mol		hardening soaps, softening plastics and in making cosmetics, candles and plastics.
13.764	Z,Z-4,15-Octadecadien-1-ol Acetate	$C_{20}H_{36}O_2$	5363119	308.5 g/mol		Antiinflammatory
13.821	Octadec-9-enoic acid	$C_{18}H_{34}O2$	965	282.5 g/mol		hardening soaps, softening plastics and in making cosmetics, candles and plastics.
14.105	9-Octadecenoic acid	$C_{18}H_{34}O_2$	637517	282.5 g/mol		Anti -irritant, Used in cosmetics, Hydrating agent, Used for the treatment of inflammation of superficial tissues, Anti -aging agent, Anti -oxidant.
14.105	n-Propyl 11-octadecenoate	$C_{21}H_{40}O_2$	87131822	324.5 g/mol		
14.114	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	5312441	282.5 g/mol		Anticholestrol
14.114	cis-Vaccenic acid	$C_{18}H_{34}O_2$	5282761	242.40 g/mol		Reduce Unsaturated Fatty Acid Toxicity. Lipids
14.114	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	637517	282.5 g/mol		Anti -irritant, Used in cosmetics, Hydrating agent, Used for the

						treatment of inflammation of superficial tissues, Anti -aging agent, Anti -oxidant.
14.294	Octadecanoic acid	$C_{18}H_{36}O_2$	5281	284.5 g/mol		Antibacterial reduce sickle cell, Reduction in the risk of caronary heart disease (CHD)
17.423	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	14900	330.5 g/mol		protects the skin from external damaging factors, and preserves skin moisture.
17.423	Palmitoyl chloride	$C_{16}H_{31}ClO$	8206	274.9 g/mol		
17.423	1-Glycerol palmitate	$C_{19}H_{38}O_4$	14900	330.5 g/mol		identification and differentiation of enzymes that hydrolyze
19.711	Oleoyl chloride	$C_{18}H_{33}ClO$	5364783	300.9 g/mol		chemical modification of the jute fibbers to confer hydrophobicity and resistance to biofibers
19.711	Cyclooctaneacetic acid	$C_{10}H_{16}O_3$	536995	184.23 g/mol		preventing cis/trans-isomerization by replacing alkenes, replacing larger cyclic systems, increasing metabolic stability
19.711	9-Octadecenoic acid	$C_{18}H_{34}O_2$	445639	282.5 g/mol		Anti -irritant, Used in cosmetics, Hydrating agent, Used for the treatment of inflammation of superficial tissues, Anti -aging agent, Anti -oxidant.
19.787	3-Methylpyrazolobis(diethylboryl...		249918656			Antioxidant, antibacterial, antifungal

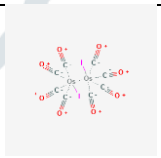
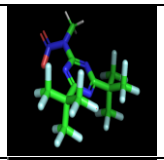
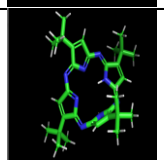
19.787	9-Octadecenoic acid (Z)-, 2-hydr...					
19.787	9-Borabicyclo[3.3.1]nonane,	$C_{13}H_{23}B$	5326191	190.13 g/mol		mild reagent for the reduction of carbonyl compounds, acid chlorides and alkenes
20.553	Dodecanoic acid,					
20.553	Dodecanoic acid,	$C_{12}H_{24}O_2$	3893	200.32 g/mol		plant metabolite, an antibacterial agent and an algal metabolite.
20.666	Triazin	$C_7H_{13}N_5O$	135408659	183.21 g/mol		anti-cancer, antiviral, fungicidal, insecticidal, bactericidal, herbicidal, antimalarial and antimicrobial agents.
20.666	5-Amino-3-metthiophene-2,4-d...					
20.666	Lauric anhydride	$C_{24}H_{46}O_3$	69527	382.6 g/mol		manufacturing of soaps and other cosmetics.

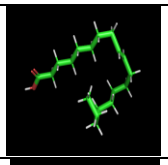
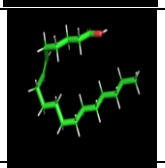
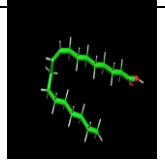
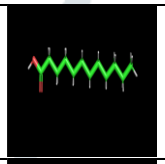
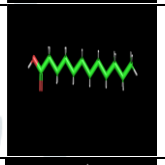
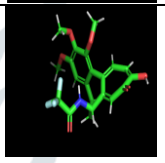
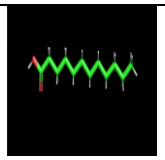
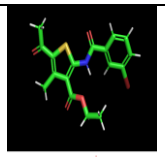
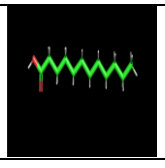
.05. Screening and Characterization of Bioactive compound from methanol mycelium extract of *Caruvalaria homonis* in GC-MS analysis

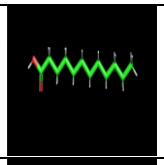
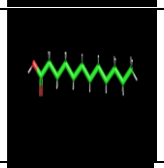
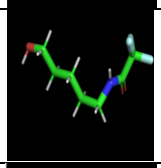
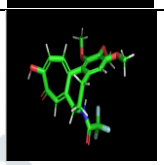
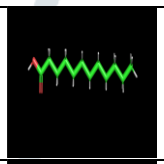
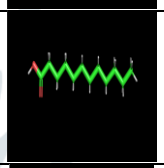
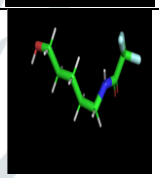
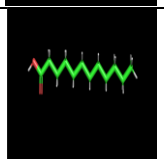
Peak value	Compound name	Molecular formula	Pubchem id	Molecular weight	Structure	Application
12.318	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	985	256.42 g/mol		antioxidant, antimicrobial, and anti-inflammatory activities
12.412	Tridecanoic acid	$C_{13}H_{26}O_2$	12530	214.34 g/mol		anthelminthic, anti-inflammatory and antimicrobial activities and anti-cancerous activity.
14.105	6-Octadecenoic acid	$C_{18}H_{34}O_2$	5282761	282.5 g/mol		hardening soaps, softening plastics and in making cosmetics, candles and plastics.
14.294	Octadecanoic acid	$C_{18}H_{36}O_2$	5281	284.5 g/mol		anticarcinogenic activity, suppressing in vitro growth of human melanoma, colorectal, and breast cancer cells, and exhibiting anti-atherogenic activity.
15.476	Eicosane	$C_{20}H_{42}$	8222	282.5 g/mol		wound healing anti-microbial and anti-inflammatory
15.476	Octadecane, 3-ethyl-5-(2-ethylbu...	$C_{18}H_{38}$	11635	254.5 g/mol		used as a solvent, a lubricant, transformer oil and an anti-corrosion agent. It plays an important role in pheromones as a chemical messenger
16.308	(5S,6aR,10aS)-5-Propyldecahydrod...	$C_{16}H_{25}FO$	17754612	252.37 g/mol		Antioxidant preservative
16.308	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	66281	353.0 g/mol		treatment of human and animal bacterial infections
16.308	Methoxyacetic acid, 2-pentadecyl...	$C_{18}H_{36}O_3$	542202	300.5 g/mol		gelling, viscosity and stabilizer reagents
17.433	1 1,2-Benzisothiazol-3-amine tbdms	$C_{13}H_{20}N_2SSi$	91733953	264.46 g/mol		helps to prevent the growth of bacteria, fungi, and algae in your household surfaces
17.433	Benzo[h]quinoline, 2,4-dimethyl-	$C_{15}H_{13}N$	610182	207.27 g/mol		Woundhealing,anti-bacterial,anti-oxidant,anti-malarial,antiviral,a

						nticonvulsant, anti HIV
17.433	1H-Indole, 5-methyl-2-phenyl-	$C_{15}H_{13}N$	83247	207.27 g/mol		Enrichmetabolism Anti-cancer Anti-viral
19.702	Benzene	C_6H_6	241	78.11 g/mol		Industrial manufacturing dyes, lubricant,drug, fuel
19.702	(E)-2-bromobutyloxychalcone	$C_{19}H_{19}BrO_2$	91733949	359.3 g/mol		antimicrobial activity and potential in reverting antimicrobial resistance in bacteria
19.702	1-Benzazirene-1-carboxylic acid,...	$C_7H_5NO_2$	22987358	135.12 g/mol		production of polymers, biopolymers, coatings, adhesives, and pharmaceutical drugs

reening and Characterization of Bioactive compound from methanol mycelium extract of *Fusarium solani* in GC-MS analysis

Retention Time(cm^{-1})	Compound Name	Molecular Formula	PubChem Id	Molecular Weight	3d Structure	Application
9.897	Osmium,hexacarbonyldi-.mu.-iodo...					treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases
9.897	Osmium, octacarbonyldiiododi-,	$C_8I_2O_8Os_2$	636326	858.3 g/mol		treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases
12.942	2-(N-Methyl-N-nitroamino)	$C_{12}H_3F_{18}N_5O_2$	542707	591.16 g/mol		
12.970	21H,23H-Porphine-2,7,12,17-tetra...	$C_{32}H_{42}N_8$	3479588	538.7 g/mol		antimicrobial, antitubercular, anticancer, antiviral, antimalarial, antidiabetic, antioxidant, analgesic, anti-inflammatory, anti-infection, and anti-infection

14.095	9-Octadecenoic acid,	$C_{18}H_{34}O_2$	637517	282.5 g/mol		potent antimalarial drug
14.095	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	5281125	282.5 g/mol		Preparation of lotions, Used as pharmaceutical solvent
14.095	Oleic Acid	$C_{18}H_{34}O_2$	445639	282.5 g/mol		Emulsifying agent, Anticancer effect, Reduce blood pressure, Reduction in the risk of caronary heart disease (CHD), Antioxidant and antipolymerization agent, Used in asthma inhaler
15.826	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		plant metabolite, an antibacterial agent and an algal metabolite.
16.072	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		used for treating viral infections including influenza
16.072	Colchiceine, N-desacetyl-N-TFA-	$C_{21}H_{20}F_3NO_6$	634905	439.4g/mol		to prevent or treat attacks of gout (also called gouty arthritis). This condition is caused by too much uric acid in the blood
16.573	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		A plant metabolite, an antibacterial agent and an algal metabolite.
16.573	Benzamide, 4-bromo-N-(4-chloroph...					
16.573	5-Acetyl-2-(3-bromo-benzoylamino...	$C_{17}H_{16}BrNO_4S$	603226	410.3g/mol		Wound healing, antibacterial, antioxidant, antimalarial, antiviral, anticonvulsant , anti HIV
18.454	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		plant metabolite, an antibacterial agent and an algal metabolite.

18.785	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		used for treating viral infections including influenza
19.031	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		plant metabolite, an antibacterial agent and an algal metabolite.
19.031	Acetamide, 2,2,2-trifluoro-N	$C_7H_{12}F_3NO_2$	4459265	199.17g/mol		used to reduce pressure in the eyes.
19.031	Colchiceine, N-desacetyl-N-TFA-	$C_{21}H_{20}F_3NO_6$	634905	439.4 g/mol		treatment of gout flares and Familial Mediterranean fever, and prevention of major cardiovascular events.
19.069	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		Plant metabolite an antibacterial agent and an algaal metabolite
19.126	Dodecanoic acid, 1-(hydroxymethy...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		used for treating viral infections including influenza
19.674	Acetamide, 2,2,2-trifluoro-N-(5,...	$C_7H_{12}F_3NO_2$	4459265	199.17 g/mol		to reduce pressure in the eyes. This pressure can be caused by or lead to an illness called glaucoma.
19.674	5-(4-Chlorophenyl)-6-ethylpyrimi...					used as a disinfectant , herbicide, and fungicide. Chlorophene is Used as a preservative in cosmetics.
19.674	5Alpha-cyano-3-methoxymethylenec...	$C_{30}H_{49}NO$	91745323	439.7 g/mol		
19.712	Pennogenin diacetate	$C_{31}H_{46}O_6$	541381	514.7 g/mol		antibiotic and antitumor activities, and are widely used as haemostatic agents.

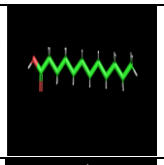
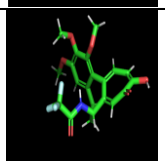
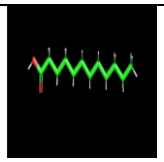
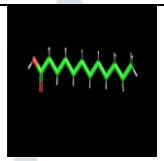
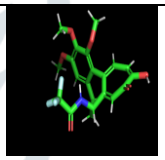
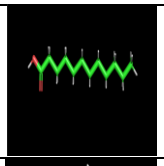
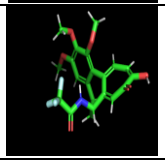
19.712	Dodecanoic acid, 1,2,3-propanetr...	$C_{12}H_{24}O_2$	3893	200.32 g/mol		used for treating viral infections including influenza
20.752	Androsta[17-16-b]furan-5'-imine...	$C_{29}H_{45}NO_2$	634906	439.7 g/mol		weight loss, to improve athletic performance, to reduce sexual problems
20.752	3-Methoxyandrosta[16,17-b]furan-...	$C_{29}H_{45}NO_2$	634897	439.7 g/mol		weight loss, to improve athletic performance, to reduce sexual problems
20.752	Cyclodisilazane-2,2,4,4-tetramin...					slightly to moderately toxic following acute oral, dermal, and inhalation exposure
20.828	3-Methoxyandrosta[16,17-b]furan-...	$C_{29}H_{45}NO_2$	634897	439.7g/mol		enhance athletic performance, increase energy, keep red blood cells healthy, enhance recovery and growth from exercise
20.828	Ethyl 3-hydroxy-2,6-di-p-chloros...					
20.828	1-(Trihexylsilyloxy)hexadecane	$C_{34}H_{72}Osi$	533033	525.0 g/mol		In the preparation of emulsions; also used as a substrate for the production of biosurfactants
20.865	5Alpha-cyano-3-methoxymethylenec...	$C_{30}H_{49}NO$	91745323	439.7 g/mol		
20.865	Colchiceine, N-desacetyl-N-TFA-	$C_{21}H_{20}F_3NO_6$	634905	439.4g/mol		to prevent or treat attacks of gout (also called gouty arthritis
20.865	Acetamide, 2,2,2-trifluoro-N-(5,...	$C_7H_{12}F_3NO_2$	4459265	199.17g/mol		to reduce pressure in the eyes

Table.07. Antibacterial activity of ethyl acetate extract of *Fusarium solani* and *caruvalaria hominis*

END0PHYTIC FUNGAI SPECIES	INTRACELLUR				Control
	Fusarium solani (mm)		*Curuvularia hominis* (mm)		Methanol (mm)
CONCENTRATION	10	20	10	20	15
Bacterial species					
E.coli	1.2	2.0	0.8	1.5	-
Staphylococcus pneumoniae	3.5	4.2	0.4	0.8	-
Steptococcus avarmtillus	0.0	0.3	0.3	0.7	-
Shigella sp.,	0.2	0.6	0.5	0.9	-

Table.08. Antibacterial activity of methanol mat extract of *Fusarium solani* and *caruvalaria hominis*

END0PHYTIC FUNGAI SPECIES	EXTRACELLUR				Control
	Fusarium solani (mm)		*Curuvularia hominis*(mm)		Ethyl acetate (mm)
CONCENTRATION	10	20	10	20	15
Bacterial species					
E.coli	0.6	1.5	1.0	1.4	-
Staphylococcus pneumoniae	0.7	1.0	1.2	1.5	-
Streptococcus avermitilis	0.5	1.0	1.4	1.6	-
Shigella sp.,	0.4	0.9	0.4	0.8	-

Table:09. phytochemical analysis of both ethyl acetate and methanol mycelium extract of *Fusarium solani* and *Caruvaluria hominis*.

S.NO		EXTRACELLULAR		INTRACELLULAR	
		Fusarium solani	*Caruvaluria hominis*	*Fusarium solani*	*Caruvaluria hominis*
1	FLAVANOID	+++	+++	+++	+++
2	SAPONIN	+++	+++	+++	+++
3	TANIN	– –	– –	+++	– –
4	CARBOHYDRATE	– –	– –	++	– –
5	PHENOL	++	+++	– –	– –
6	TERPENOID	– –	– –	– –	– –
7	STEROIDS		+++	– –	+++
8	GYCOSIDE	+++	– –	– –	– –
9	ALKALOIDS	+++	++	++	++
10	PROTEINS	+++	– –	– –	+++

(+++ Very high present ; ++ Highly present

+ present ; - absent)

Table.10. DPPH antioxidant scavenging activity of extracellular extract of *Fusarium solanai* and *caruvularia hominis*

Concentration	Standard		*FUSARIUM SOLANI*		*CARUVALURIA HOMINIS*	
Sample and standard	Absorption Value (OD)	% of scavenging	Absorption Value (OD)	% of scavenging	Absorption Value (OD)	% of scavenging
10	0.189	60.24	0.195	33.98	0.193	27.56
20	0.187	60.46	0.192	54.21	0.189	41.05
30	0.179	60.82	0.189	72.03	0.187	69.45
40	0.175	61.05	0.184	87.85	0.184	70.09
50	0.172	61.20	0.180	96.01	0.180	88.03

Table.11. H2O2 antioxidant scavenging activity of extracellular extract of *Fusarium solani* and *Caruvularia hominis*

Concentration of	**Standard**		***Fusarium solani***		***Caruvalaria hominis***	
Sample and standard	**Absorption Value (OD)**	**% of Scavenging**	**Absorption Value (OD)**	**% of scavenging**	**Absorption Value (OD)**	**% of scavenging**
10	0.180	88.95	0.126	29.87	0.137	22.43
20	0.177	89.12	0.118	46.03	0.111	33.59
30	0.171	89.96	0.109	53.76	0.096	45.67
40	0.169	90.25	0.096	79.12	0.088	78.84
50	0.165	91.00	0.088	89.05	0.056	89.32

IV. DISCUSSION

Endophytic fungi are commonplace creatures that live inside or between cells of plants, at least temporarily, and do not show outward signs of infection Endophytic fungi are derived from several biochemical processes and are classified into many structural classes, including quinones, phenols, coumarins, terpenoids, and steroids (Rodriguez *et al*., 2009 and Nisa *et al*., 2015) In the present study the two endo phytic fungi such as *Fusarium solani* and *Curuvularia hominis* have been recorded from the plant tissue and leaves of *Phyllanthus niruri* and *Calotropis gigantea*

The FTIR spectrum is the most preferred spectroscopic technique to examine the spectral composition of functional groups available in the sample by relating them to the respective absorption bands (Elango *et al*., 2020). In the present study also the fungal extract of methanol mat (intracellular) and ethyl acetate (extracellular) of *Fsarium solani* and *Curuvalaria hominis*. Functional group

were analysed by FTIR and recorded many functional group in extracellular extract of *Fusarium solani* C-I stretching, C-CI stretching, C-H bending , O-H stretching as a major functional group. In *curuvularia hominis* C-I stretching, C-F Stretcing, C-H bending, O-H stretching, as a major functional group.

Jagannathan *et al*., (2021) reported that twenty-five bioactive chemicals were found in the ethyl acetate extracts by GC-MS profile. Nine bioactive chemicals, including 2-Cyclopentenone, 2-(4-chloroanilino)-4-piperidino, Oxime-methoxy-Phenyl, Methanamine N-hydroxy-N-methyl, Strychane, Cyclotetrasiloxane, Octamethyl, and 1-Acetyl-20a-hydroxy-16-methylene, were found in Trichoderma reesei, an endophytic fungus separated from the flowers (Jagannath *et al*., 2021).Fungal extract was subjected to GC MS analysis to identify the bioactive compound of *Colletotrichum gloeosporioides* produced. The compounds were identified based on the NIST database by virtue of comparisons made of the actual mass spectral data acquired. The chromatogram predict the presence of 3-Dodecene, Benzene,1,3-bis(1,1-dimethylethyl), Dodecane,2,6,10- trimethyl-, 1-Undecanol, Phenol,2,4-bis(1,1- dimethylethyl)-, 1-Hexadecene, 1-Hexadecanol, Octahydro-2H-pyrido(1,2-α)pyrimidin-2-one, Pyrrolo[1,2- α]pyrazine-1,4-dione, hexahydro-3(2-methylpropyl)-, 1- Nonadecene, 9,12-Octadecadienoic acid, methyl ester(E,E)-, 9-Octadecanoic acid(Z)-methyl ester, 3- Eicosene,(E)-, Hexadecanoic acid, 2-hydroxyl-1- (hydroxymethyl)ethyl ester, 10-Heneicosene(c,t), Octadecanoic acid,3-hydroxypropyl ester, 1,2-Benzenedicarboxylic acid, diisooctylester. Hexadecanoic acid and octadecanoic acid methyl ester were mainly found in endophytic fungi obtained from medicinal plants (Mehdi, *et al*., 2021). The present study bioactive compounds were recorded in *Curuvularia hominis* extract such as n-Hexadecanoic acid,tridecanoic acid, cis-vaccenic acid,6-octadecenoic acid,(1,2,4)triazolo(1,5-a)pyrimidine-6-carboxylic acid,7-amino,ethyl ester,chloromethyl 11-chlorodecanoate, benzene, tret-butyl(5-isoprop-yl-2-methyiphenoxy)dimethylsilane. The bioactive compounds were recorded in only in *Curuvularia hominis* extract such as n-hexadecanoic acid, Tridecanonic acid, cis-vaccenic acid, 6-octadecenoic acid, octadecenoic acid, Eicosane,octadecane, opyldecahydrod, 1-octadecanesulphonly chloride, methoxyacetic acid, benziothaiazol 3-amine tbdms, quinolone,1H-indole,benezene.

Ukwatta, *et al*.,2020 reported that The agar well diffusion and agar disc methods are routinely utilized to examine the antibacterial efficacy of the extracted compounds.The agar well diffusion method is well-known to be used to test the antimicrobial. In present study *Fusarium solani* and *Curuvularia sp.* endophytic fungi against the bacterial species of *Escherichia coli*, *Shingella*, *Streptococcus pneumoneae* and *Staphylococcus pneumoniae*. Every individual extract was recorded different range of zone of inhibition for different species. The highest range of zone of inhibition observed in *Fusarium solani* in methanol mat extract and ethyl acetate as 1.0mm against *streptococcus avarmtillus* and *Staphylococcus pneumoniae aures*. In *caruvalaria sp.* the antibacterial zone of inhibition in ethyl acetate on the highest zone of inhibition is 1.6mm against *streptococcus avarmtillus*

According to Jannath *et al*., (2021) 24 endophytic fungus species were identified from the tissues of the medicinal plant Baliospermum montanum (Willd.) Muell, which is found all throughout India. 15% of the endophytic isolates tested positive for phenols, saponins, and terpenoids, while 70% of the isolates displayed alkaloids and flavonoids, according to the phytochemical study.

In this present work, phytochemical analysis was recorded such as the flavanoid, saponin, tannin, carbohydrate, phenol, terpe noid, steroids, glycoside, alkaloids and protein have tested with fungal extract of both ethyl acetate extract and methanol mat extract. *Fusarium solani* extract contain flavonoids, saponins, tannnins, alkaloids, phenols, glycosides. The *Curuvularia hominis* extract contain phenols, flavonoids, steroids, saponins, steroids, flavonoids was only recorded in all the extract of fungi. The significant Endophytic fungi *Fusarium solani* identified using ITS sequencing analysis and sequenced ITS sequence submitted to NCBI

V. CONCLUSION

Fusarium solani and *Curuvularia hominis*, two distinct endophytic fungus, were isolated from a variety of therapeutic plants. In order to ascertain the potential of bioactive compounds present in the extracts of endophytic fungi, a variety of antioxidant assays, including DPPH, nitric oxide, and H2O2 scavenging, were conducted on the ethyl acetate (extracellular) and methanol mat (intracellular) extracts of these fungi (Fusarium solani, Curuvularia hominis). GC-MS was also used to predict the bioactive compound such anticancer potential of bioactive metabolites, anti-microbial, anti-fungal, anti-HIV, antitumor, anthelmintic, solubilizing agent, antiviral, fungicidal, insecticidal, bactericidal, herbicidal, antimalarial and antimicrobial agents, Because of the presence of many bioactive

chemicals in the extracts that were detected by GC-MS, the endophytic fungal extracts demonstrated higher antibacterial activity and probable antioxidant activity against a variety of bacterial pathogens. Numerous components, including flavonoids, phenols, glycosides, carbohydrates, saponins, and proteins, were found in endophytic extracts according to a phytochemical composition analysis. The significant Endophytic fungi *Fusarium solani* identified using ITS sequencing analysis and sequenced ITS sequence submitted to NCBI

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