Phytochemistry and biodiversity of endophytic fungal metabolites isolated from medicinal plants

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ABSTRACT

It is critical to study endophytic fungi because they boost plant production which benefits both people and animals and meets nutritional needs. Four medicinal plants "*Datura sramonium, Echinacea purpura, Salvia rosmarinus and Thymus vilgrus*" were used to isolate twelve different fungal species. Preliminary light microscope identification showed that these species belonged to three genera *Asprigillus* species, *Alternaria* species, and *Fusarium* species. Antimicrobial activity was done for the intracellular (Mat) and extracellular extracts of all isolated fungi and showed that the extracellular extracts had higher activity than the intracellular (Mat) extracts. "*Candida albicans*" was the most sensitive fungal species, creating a disincentive zone (11±0.4 mm) with an (E2) fungal isolate, and "*Pseudomonas aurogonosa*" was the most sensitive bacterial species, producing a disincentive zone (26±0.5 mm) with an (E2) endophytic fungal isolate. Additionally, utilising (1, 1) diphenyl-2-picrylhydrazyl (DPPH) scavenging activity to assess the antioxidant activities for the twelve fungal isolates, it has been found that (T1) and (T3) isolated fungi have the highest antioxidant activities, with IC_{50} values of 36.8 and 36.8 µg/ml, successively. Alkaloids, flavonoids, anthocyanins, tannins, and anthraquinones were found in the extracellular and intracellular (Mat) fungal extracts, which were separated using thin layer chromatography (TLC), demonstrating that the extracellular cell extracts have a higher concentration of these phytochemical groups than the intracellular cells. Additionally, the polymerase chain reaction (PCR) was used to completely identify the two most active isolated fungi (R2 and T3), identifying both isolates as *Alternaria Alternata*.

Key words: Medicinal plants**;** Fungal endophyes**;** Antimicrobial**;** Antioxidant**;** TLC.

INTRODUCTION

Altersetin is the novel alkaloide compound comes from endophyte *Alternaria* species that have antibacterial activity against groups of bactria belong to gram positive bactria (Singh *et al*., 2023).

Endophytes, which comprise the microorganisms that inhabit plants and complete their whole life cycles there, include fungi and bacteria (Prima *et al*., 2022).

Just beneficial symbiosis and latent cytopathogenesis are the only two conceivable endophyte host plant interactions (Akram *et al*., 2023).

Furthermore, the endophytic fungus may produce bioactive metabolites for the host plant that aid in plant development and offer defence against abiotic pathogenic microbial assaults (Mulyani *et al*., 2023).

There are many different secondary compounds that endophytes can make, including steroids, tetralones, quinones, flavonoids, phenolic acids, alkaloids, terpenoids, xanthones and tetralones (Perumal *et al*., 2023).

Fungal endophytes are receiving increased attention as a result of the several advantages they might provide to the host plants. Examples include *Piriformospora indica*, which can increase the resistance of *Musa spp*. against *Fusarium oxysporum* by increasing the activities of its antioxidant enzymes, which aids the host plant in recovering from fungal infection (Cheng *et al*., 2020).

Endophytic fungi may operate as antagonists of plant pathogens, as indicated by (Vega *et al*., 2009; Vidal and Jaber, 2015; Vega, 2018; Jaber, 2018; Moraga, 2020), by causing the plant to develop systemic resistance and by enhancing plant development, which reduces the pathogens activity.

Although endophytic fungi are believed to be a source of novel active substances with biological activities that are used in innovations in the biotechnological industry, their true potential is still untapped and need additional research (Basappa *et al*., 2023).

MATERIALS AND METHODS

Materials

(Plant Samples): Medicinal plants that were mature and good health (displaying no visible

disease symptoms) were meticulously selected for sampling. Plant parts, for instance leaves, stems, and roots, were at random gathered from various Mepaco Company locations in Alsharqia, Egypt. The samples were then processed shortly after being collected in sterile bags and brought into lab. Fungal and bacterial strains used in antimicrobial test: "*Fusarium oxysporum DSMZ 62045, Trycophyton rubrum ATCC 28188, Candida albicans ATCC 10231, Aspergillus niger RCMB002033", "Escherichia-coli ATCC 7839, Pseudomonas aeruginosa ATCC 15442, Bacillus subtilis ATCC 35021, Staphylococcus aureus ATCC 12600"* strains were collected from RCBM (Regional center for mycology and biotechnology) at Al-Azhar University.

Methods

Isolation of endophytic fungi

Using a technique outlined by (Petrini, 1986), endophytic fungi were isolated from four medicinal plants "(*Datura stramonium, Echinacea purpura, Thymus vilgrus, and Salvia rosmarinus*". In this procedure, the plant components were cleaned in sterile water to eliminate dust before being chopped into tiny pieces using a sterile blade under aseptic circumstances. The plant parts were sterilised for 1 minute on their surface with 70% ethanol, then immersed for 1 minute in sodium hypochlorite solution (NaOCl) 0.25%, rinsed for 1 minute with sterile distilled water, and finally allowed to dry on filter paper. Four pieces of dried plant material were put into a "potato dextrose agar medium" (PDA) dish that contains chloramphenicol as an antibiotic.

Microscopic identification for twelve isolated fungi

The twelve purified isolated fungi were identified using the most well-known fungal identity keys, such as those published by (Booth, 1971; Ellis, 1971, 1976; Barron, 1977; Pitt, 1979; Barnett *et al*., 1990; Domsch *et al*., 1993; Kurtzman and Fell, 2000). Twelve selected isolated fungi were chosen, and an image analysis system using Soft-Imaging GmbH software (analysis Pro ver. 3.0) was utilised to perform the morphological characteristics. In general form, growth rate, colony diameter, colony texture, colony colour, and measures, the reverse pigmentation. Additionally taken are the species identifying traits,because they can provide the host plants with a variety of benefits. Fungi are being investigated more and more.

Antimicrobial activity

Twelve intracellular (Mat) and extracellular fungal extracts that defatting by n-Hexane then extracted by ethyl acetate then evaporate the ethyl acetate layer and weight 100 µg from each extract, dissolve the dry extracts in 1ml Dimethyl Sulfoxide (DMSO) (Sigma-Aldrich) then evaluate antimicrobial activities by using agar-well diffusion method. Test microorganisms were then added to the 5 mm-diameter wells that had been cork-bored into the infected plates under aseptic circumstances. DMSO was utilized suspend the tested extracts before; 100µl of each suspended sample was applied to each well. A solvent filled well served as the negative control. All plates were kept at 4°C for two hours prior to the start of the microbial growth to allow the chemicals to disperse. The agar dishes were progressively incubated for fungi and bacteria/yeast for three days at 28°C and for one day at 37°C respectively. The average diameter of disincentive zone for each test, with standard deviation determined from the triplicate samples, was the finding. (Collins *et al*., 1995).

Antioxidant activity

The (1,1) diphenyl-2-picryl hydrazyl (DPPH) scavenging activity technique, which was approved by (Melo *et al*., 2008), was used to test the antioxidant properties of twelve endophytic fungal extracts. The extracts had to be defatted with n-Hexane, extracted with ethyl acetate, evaporated, and then the residue had to be dissolved in methanol at 100 µg/ml. Following the concentration of six samples at 10, 20, 40, 80, 160, 320, 640, and 1280 µg/ml, extracts and ascorbic acid (as a standard) were dissolved in methanol to achieve the required concentrations using the dilution method. Weighed DPPH was added as a 0.004% (w/v) solution of methanol, 3ml of methanol and 0.004% of DPPH solution were put in each test tube after the necessary levels was reached. (Used as a blank). After 20 minutes, a UV spectrophotometer was used to measure the absorbance of each test container at a wavelength of 515 nm .The activity of antioxidant was calculated by the equation:

$$PI = [\{(AC- AT)/ AC\} \times 100]$$

Where AT denotes the sample's absorbance + DPPH at time 16 minutes and PI denotes percentage inhibition, the absorption of control at time zero and 16 minutes are referred to as AC and AT, successively (Yen and Duh, 1994).

Analysis of the intracellular (Mat) and extracellular extracts from the twelve isolated endophytic fungi for being present tannins,

flavonoids, alkaloids, anthocyanins and anthraquinones

Thin layer chromatography (TLC), as described by (Frost, 1966; Dedio *et al*., 1969), was used to analyse the phytochemical components of twelve fungal extracts using the CAMMAG LINOMAT 5 application method and TLC sheet (silica gel G-60 F254 aluminium sheet, TLC scanner unit Merck, Germany). The following developing solvents (TEF) were utilised to facilitate the isolation of fungal secondary metabolites: toluene, ethyl acetate, 90% (w/v), and formic acid (5:4:1, v/v/v).

Detection of alkaloids by spraying the TLC bands with Potassium bismuth iodide, also known as Dragendorff's solution, is made up of (27.2 g potassium iodide+ 20 ml nitric acid+ and 8 g of Bismuth (III) nitrate) dissolve and complete volume to 100 ml distilled water. Alkaloids were detected when orange to reddish brown ppt bands appeared on the TLC sheets. (Stahl, 1965).

Detection of flavonoids Spraying the TLC bands with diluted NaOH solution (0.085g/ ml) then with 1 ml HCl was added the appearance of a yellow solution that turns white showed being present flavonoids (Egwaikhide and Gimba, 2007).

Detection of anthocyanins by spraying the TLC bands with alcoholic NaOH solution to developed the blue color. (El-Shikh *et al*., 2009).

Detection of tannins by spraying the TLC bands with ferric chloride solution to give greenish-black color showed being present tannins (Trease and Evans, 1989).

Detection of anthraquinones by Spraying the TLC bands with 10 % of NH4OH solution which shows anthraquinones by giving a rose pink color (Wallis, 1967).

Studying the intracellular (Mat) extracellular and secondary metabolites profile for isolates

Toluene, ethyl acetate, and formic acid 90% (5:4:1, v/v/v) (TEF) were used as the migration solvent to separate the secondary metabolites of the most active isolated fungi on TLC sheets.

Characterization of extracellular and intracellular (Mat) metabolites on TLC

On (TLC) chromatography CAMMAG LINOMAT 5 application device was used to apply extracted samples of the selected twelve isolated fungi to (TLC) sheet (silica gel G-60 aluminum sheet, Merck, Germany). (TLC scanner unit at research center, in Al-Azhar, University),s a reference measure, griseofulvin was used. The sheets were created for a (80 mm) size using toluene, ethyl acetate, and 90% (w/v) formic acid (5:4:1, v/v/v) (TEF) as the solvent system. The fluid system for sheets was shut off, and they were allowed to air dry. The sheets were inspected with light that is visible, long (365) nm (UV) light, short (254) nm (UV) light and long (365 nm) (UV) light once more on the reverse.

PCR identification for fungal isolates

Using a Qiagene kit to isolate fungus DNA

Malt extract agar medium (MEA) slopes from colonies that were five to seven days old were brushed using 2 ml of distilled water that sterilized. In a universal 250 ml flask, two ml of spore slurry were used to gently shake (180 rpm at 28 °C for 48 hours) a 100 mL yeast extract agar medium (YES) medium. In a mortar with a clean pestle, the freeze-dried mycelium was ground. In Eppendorf tubes, the sample powder was put. (1.5 ml).

Agarose gel electrophoresis

The gel tray was then put in the electrophoresis vessel with just enough electrophoresis buffers to fill the wells entirely to the top after the agarose (concentration: 2%) had solidified. The comb was then removed. Following the addition of the appropriate DNA ladder as an identifier, the stop loading solution loaded the samples. After the gel was run at the proper voltage level, the DNA was imaged and visible using a gel documentation system transilluminator. (BIO-RAD, Gel Doc 2000).

Chain reaction with polymerase (PCR)

Each primer mixture was mixed with 50µl of the Master Mix (2.5U Taq DNA polymerase + 200µM of each dNTP, 1x Qiagene PCR buffer), and 100µl of the total volume of the reaction). The mixture was then placed directly onto the thermal cycler machine to start the appropriate PCR programme with a universal denaturation step (5 minutes at 94°C), 30 cycles of annealing/extension reactions (20 seconds at 94°C, 30 seconds at an ideal annealing temperature for each amplified fragment, and one minute at 72°C), and perform the last extension stage (for 5 minutes at 72°C), followed by a soak at 4°C.

Purification of DNA fragments from the gel

The band-containing gel segment was then removed from the gel and placed into a dialysis bag that had been refilled with eluting solution (1x TBE, 0.5% SDS).

DNA sequencing

The Cy5/Cy5.5 Sequence kit was used to create sequence of plasmid and PCR fragments from Visible Genetics Inc. (Sanger *et al*., 1977; Tabor and Richardson, 1995).

The A, C, G, and T termination dyes were divided into 3ml portions, and each was then blended into the corresponding designated tube. The tubes were placed into a preheated thermal cycler (gradient Robocycler 96, Stratagene, USA) to run a standard sequencing programme including a universal denaturation cycle (2 minutes at 94°C), 18 cycles of annealing/extension reactions (15 second at 94°C, 15 second at 55°C and one minute at 70°C), and 15 cycles of final extension step (15 second at 94°C and one minute at 70°C) followed by soaking at 4°C. Each tube received 6µl of stop loading dye after the sequencing was finished. 2µl of each sample was placed onto a micro cell cassette after the samples had been heated for 2 minutes at 80 °C and quenched on ice. Filling, casting, polymerizing Micro Cell Cassette, loading the Micro Cell Cassette into Long-Read Tower, and electrophoresis conditions were assigned as stated by Long-Read Tower DNA sequencer System and sample analyses were made by Open Gene software Version 3.1 from Visible Genetics Canada.

GC mass Study for the fungal isolates

Thymus vilgrus plant and T3 fungal isolate were two samples that extracted with ethyl acetate, ethyl acetate layer evaporation, air drying, and residue dissolved with methanol, and mass spectrometric analysis of Shimadzu GC/MS-QP 5050 was subjected to a mass spectral analysis by (RCMB) (Regional center for mycology and biotechnology), Al-Azhar University, Nasr City, Cairo, using a Direct Inlet component DI-50 to mass analyzer. The procedure was as follows: The Column: 30 m, DB5-MS, 0.25 mm ID (J&W scientific), EL is ionization mode. Temperature programmed: 40°C (3 minutes), 150°C (1 minute) at 7°C/ minute, 290°C (1 minute) at 5°C/ minute, 300°C for the detector, 250°C for the injector, Helium and other carrier gases 1.5 ml/ minute is the gas flow per minute. Wily, Nist mass spectral data base was searched in the library.

RESULTS

Fungal colonies were isolated from four medicinal plants and given a preliminary identification

Four medicinal plants provided the twelve fungal strains "*Datura stramonium, Echinacea purpura, Salvia rosmarinus* and *Thymus vilgrus*". The preliminary identification of these isolates by light microscope and image analysis proven that they belong to three genera (*Asprigillus* species, *Alternaria* species and *Fusarium* species) described in (table1) where two *Alternaria* species isolates from *Datura stramonium* are (D1 and D2). Along with two isolates from *Echinacea purpura* are *Fusarium* species from (E1) and *Aspergilus* species from (E2) in comparison three of which isolated fungi from *Salvia rosmarinus* are (R1, R2) and (R3) and all where *Alterinria* species. While the plant *Thymus vilgrus* showed five isolates where three are *Alternaria* species named and two are *Fusarium* species named. In addition to the percentage of isolates that were classified as *Alternaria* species. This was seen in **(Fig 1)** where the *Fusarium* species had a percentage of 25% and the *Aspergilus* species had a percentage of 67%.

Morphological identification of isolated fungi

The twelve fungal isolates were identified under a light microscope using the identification techniques given by (Booth, 1971; Ellis, 1971, 1976; Barron, 1977; Pitt, 1979; Barnett *et al*., 1990; Domsch *et al*., 1993; Kurtzman and Fell, 2000). The fungal structure was identified, measured, and measured using these techniques.

Microscopic identification for twelve fungal isolates

In (Fig 2) the microscopic identification of twelve fungal isolates number (1, 2, 5, 6 ,7 ,8 , 10 and 11) from plants "*Datura stramonium , Salvia rosmarinus* and *Thymus vilgrus*" where identified as *Alternaria* species as it showed powdery appearance ,brown in color culture on MEA and measurements of conidiophores as follow (40 µm long, 4 µm wide ,40.7 µm long, 4.2 µm wide, 40.5 µm long, 4.1 µm wide, 40.3 µm long, 4.1 µm wide, 40.4 µm long, 4.3 µm wide, 40.5 µm long and 4.0 µm wide) which confirm that these isolates are *Alternaria* species .Along with isolates numbers (3, 9 and 12) from plants (*Echinacea purpura* and *Thymus vilgrus*) identified as *Fusarium* species, the characteristic by green culture on MEA and measurement of the macroconidia were septated with (4-septa), fusiform, are (35x3), (35.5x3) and (35.3x3.4)µm. Chains of microconidia with fusoidal shapes and sizes of (8x2.1), (8.1x2.4), and (8.6x2.1) µm were carried on lateral phialides. They were identified as

the *Asprigillus* species from isolate number (4) of the *Echinacea purpura* plant, which had the typical 5.2 cm diameter. The first to form were colonies with radial patterns and yellow-green hues. Conidiophores that were hyaline, roughened, and (0.8 mm) long were seen. The globe-shaped vesicles had a diameter of 38 μm. The sterigmata are of the biseriate type because phialides or the primary sterigmata were born on metulae (secondary sterigmata). The sizes of the phialides and metulae were (8.1x4.6) and (7.8x3.9)μm, respectively. Conidia had an echinulate appearance, a spherical form, and a diameter of (3.5 μm).

Antimicrobial properties against fungal and bacterial strains

According to **tables (2-3)** which summarie the antimicrobial results of the ethyl acetate extracts for the twelve isolated endophytic fungi from four medicinal plants, many of the isolates have antimicrobial activity. Extracellular extracts are also more effective than intracellular extracts against fungi and bacterial strains.

Antibacterial activities of the twelve isolated fungi extracts by agar-well diffusion assay against tested pathogenic bacteria

Table (2) proven that the majority of intracellular (Mat) and extracellular extracts resistance against *Escherichia-coli ATCC 7839* except the isolates (E2 and R3) which give disincentive zone with measurement (12±1 and 8±0.5) mm successively. Furthermore the intracellular (Mat) and extracellular extracts gave disincentive zone ranging from (8±0.5 - 15±0.5) mm with "*Bacillus subtilis ATCC 35021*" and the isolated code (E2) extracellular extract is highest sensitivity with disincentive zone (22±0.5) mm that less than the standard with disincentive zone (22±1) mm. The isolated code (R1) extracellular extract has the highest sensitivity with a disincentive zone (17±0.5) mm, which is less than the standard with a disincentive zone (24±0.5) mm, along with the majority of intracellular (Mat) and extracellular extracts sensitive against "*Staphylococcus aureus ATCC 12600*" with disincentive zones ranging from (4±0.3-15±0.5) mm. In addition to intracellular (Mat) and extracellular extracts that give a disincentive zone ranging from (7±0.5-14±0.4) mm with "*pseudomonas aeruginosa ATCC 15442*" and the isolated code (E2), extracellular extract is the most sensitivity with disincentive zone (26±0.5 mm) that is less than the ascorbic acid stander with disincentive zone (22±0.5 mm) .

Antifungal activity of the twelve isolated fungi used agar-well diffusion method against the tested pathogenic fungi

From **(table 3),** the majority of extracellular and intracellular (Mat) extracts are fungus resistant, along with extracellular extract that have slight sensitivity against "*Candida Albicans ATCC 10231*" that give disincentive zone ranging from (8±0.3-11±0.4) mm. While the intracellular (Mat) and extracellular extracts resistance against "*Fusarium oxysporum DSMZ 62045*".,along with most intracellular extract resistance against "*Aspergillus niger RCMB002033*" except the isolated code (D1, D2 and E1) with disincentive zone (6±1, 6±0.6 and 5±0.5) mm successively, while the most extracellular extract sensitive with disincentive zone range from (4±0.2-5±0.5) mm,along with most extracellular and intracellular (Mat) extract resistance against "*Trycophyton rubrum ATCC 28188*" except the isolated code (E2) that provide better sensitivity with disincentive zone (4±0.3 mm).

Antioxidant activity for fungal extracellular and intracellular (Mat) metabolites

The twelve intracellular (Mat) and extracellular fungal extracts were tested (in vitro) by the DPPH scavenging activities. Results of antioxidant for twelve intracellular (Mat) and extracellular fungal extracts found in **(Figs.3-7)** prove that the intracellular (Mat) extracts have more antioxidant than extracellular extracts.

Antioxidant activity for fungal extracellular metabolites

Fig (3) proven that the isolated (T1) has the most activity of antioxidant with IC_{50} (36.8μg/ml). Along with isolates, (T4) and (T3) have moderate activity of antioxidant with IC_{50} (98.8) and (151.1) μg/ml successively, while the lowest activity of antioxidant was shown by isolate (T2) with IC_{50} (1167) μg/ml.

Fig (4) proven that the isolate (E2) has the most activity of antioxidant with IC_{50} (482.5) μg/ml. Along with isolates (D1) and (D2), they have moderate activity of antioxidant with IC_{50} (1036) and (1059) μg/ml successively. While the lowest isolates (E1, R1 and R2) were IC_{50} (1264, 1258 and 1262) μg/ml successively.

Antioxidant activity for fungal intracellular (Mat) metabolites

Fig (5) proven that the isolates (D2 and E2) have the most activity of antioxidant with IC_{50} (44.7 and 87.2) μg/ml. Along with isolates (D1) and (R2), they have moderate activity of

antioxidant with IC_{50} (159.6) and (210.5) µg/ml successively,while the lowest isolates (E1) and (R1) were IC_{50} (1228 and 1258) µg/ml. successively.

Fig (6) proven that the isolate (T3 and T1) have the most activity of antioxidant with IC_{50} (36.8 and 49) µg/ml successively. Along with isolated code (T2), it has moderate activity of antioxidant with IC_{50} (407.7 µg/ml), while the lowest isolates (T4, T5 and R3) were IC_{50} (1059.9, 1119 and 1160) µg/ml successively.

Fig (7) proven that the isolate (T1) of extracellular extract has the most activity of antioxidant with IC_{50} (36.8) µg/ml and the isolated code (T3) of intracellular (Mat) extract has the most activities of antioxidant with IC_{50} (36.8) µg/ml.

Study of the twelve endophytic fungal isolates extracellular and intracellular (Mat) extracts for tannins, flavonoids, alkaloids, anthocyanins, and anthraquinones

Table (4) proven that the activation of isolated fungi with different phytochemical groups where for tannins group , the fungal isolates (T1, T4) of extracellular and (T3) of intracellular extracts successively give high level of concentration of the examined group, the isolates (D2, R2 and T1) of intracellular (Mat) and (E2) of extracellular extracts give moderate concentration of the examined group, the isolates (D1, R1, R3 and T2) of intracellular (Mat) and (T3) of extracellular extracts successively give low concentration of the examined group while the isolates (D1, D2, E1, R1, R2 , R3, T2 and T5) of extracellular extracts and the fungal isolates (E1, E2, T4 and T5) of intracellular (Mat) extracts did not give any activation. The activation of isolated fungi with flavonoids, the fungal isolates (T1, T4) of extracellular and (T3) of intracellular (Mat) extracts successively give high level of concentration of the examined group, the isolates (D2, R2 and T1) of intracellular extracts give moderate concentration of the examined group, the isolates (D1, E2 and R3) of intracellular (Mat) and (T3) of extracellular extracts successively give low concentration of the examined group while (D1, D2, E1, E2, R1, R2, R3, T2 and T5) of extracellular and (E1,R1, T2, T4 and T5) of intracellular (Mat) extracts successively did not give any activation. The activation of isolated fungi with Alkaloids, the fungal isolates (T1, T4, and E2) of extracellular and (D1, D2, E2 and T3) of intracellular (Mat) extracts successively give low concentration of the examined group while (D1, D2, E1, R1, R2, R3, T2, T3 and T5) of extracellular and (E1, R1, R2, R3, T1, T2, T4 and T5) of intracellular (Mat) extracts successively did not give any activation.

The activation of isolated fungi with Anthocyanins, the all-fungal isolated code of intracellular (Mat) and extracellular extracts did not gives any activation. The activation of isolated fungi with anthraquinones, the isolates (T2 and T5) of intracellular (Mat) and (T2) of extracellular extracts give moderate concentration of the examined group, the isolates (D2, E2, R1, R3 and T3) of intracellular (Mat) and (E2, T1, T4 and T5) of extracellular extracts successively give low concentration of the examined group, while (D1, D2, E1, R1, R2, R3 and T3) of extracellular and (D1, E1, R2, T1 and T4) of intracellular (Mat) extracts successively did not give any activation.

Genomic identification

Figs. (8 and 9) prove the two samples that represent study of genetic three for R2F (isolated fungi number two from *Salvia rosmarinus* plant) and T3F (isolate fungi number three from *thymus vilgrus* plant) successively. The investigation findings support the assertion that the two samples are *Alternaria Alternata*.

GC mass analysis

Table (5) proven that numbers of volatile compounds have been detected from *Thymus vilgrus* plant and (T3) fungal isolate number three from *Thymus vilgrus* plant.

This volatile compounds found in Thymus vilgrus plant alone for instance (Methyl 12,15-octadecadiynoate, 1-Acetoxypropane, Methyl-2-propenoate, 1,3-Dimethylheptane, Glycerol, 1,3,5-Triazine-2,4,6triamine(CAS), Butanedioic acid, monomethyl ester(CAS), 2,3Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, Acetylmonoglyceride, Iso-pinocampheol, trans-2-Decenal, 1-Isopropyl-1,2-cyclohexanediol, 1,5-pentanediol(CAS), Cis-Caryophyllene, Ethanone, 1-(4-hydroxyphenyl), Eicosamethylcyclodecasiloxane, Phytol Isomer, and 2(3H)-furanone,5-heptyldihydro-(CAS) and other found in both Thymus vilgrus plant and T3 fungal isolate for instance (Methyl palmitate, Eicosamethylcyclodecasiloxane, Methyl Oleate, Citroflx A4, Cyclododecasiloxane,tetracosamethyl-(CAS), Cyclononasiloxane, octadecamethyl, Mono(2-ethylhexyl) phthalate and (2-Monopalmitin) and other found in T3 fungal isolate for instance (2,2-dimethoxybutane, Methyl (phosphonomethyl) amino acetic acid,

progesterone, 1-Limonene, Cyclohexasiloxane,dodecamethyl (CAS) ,Tetradecamethylcycloheptasiloxane, Cyclododecasiloxane, tetracosamethyl (CAS), (Cyclononasiloxane, octadecamethyl) and (Hexadecamethylcyclooctasiloxane).Numerous biological characteristics of these substances include immunological function, anticancer, antiseptic, hypocholesterolemic, antihistaminic, antieczemic, nematocidal, antioxidant, antiarthritic, antimicrobial, antidiarrheal, antiproliferative, and pesticide action. The Fig (10) proven that the chromatogram of two samples A) Thymus vilgrus host plant. and B) An endophytic fungus T3 (fungal isolate number three) from Thymus vilgrus plant that contain lot numbers of volatile compounds that have important role in medicinal and chemical field as 1-Limonene , progesterone, Cis-Caryophyllene, Methyl 2-propenoate, Hexadecamethylcyclooctasiloxane, Eicosamethylcyclodecasiloxane and Phytol.

Table (5) proven that many different volatile compounds separated from two samples T3F and *Thymus vilgrus* plant under gas chromatography conditions. Some compounds present individual and other compounds found in both extracted fungi and *Thymus vilgrus* plant. This study proven that there is arelationship between (T3F) isolated fungi and *Thymus vilgrus* plant.

DISCUSSIONS

The relatively understudied resources of medicinal plants and their endophytes can be very helpful for the creation of natural products. Endophytes are fungi or bacteria that can live in healthy plant tissues without causing any obvious symptoms of disease. They might be the source of brand-new natural products with applications in business, farming, and health care (Huang *et al*., 2007). The chemistry, bioactivity, and interactions between endophytes and their host plants were the main topics of our study. The medicinal plants "*Datura stramonium, Echinacea purpurea, Salvia rosmarinus,* and *Thymus vilgrus*" yielded twelve fungal isolates for this inquiry. The first three main taxa to which these isolates were assigned were "*Aspergillus* species, *Alternaria* species, and *Fusarium* species." This outcome is consistent with findings revealed by (Verma *et al*., 2022), who noted the isolation of several endophytes from therapeutic plants. Only two isolates, *Epicoccum* species and *Pleosporales species*, were able to stop *B. cinerea* from spreading by antibiosis. Ten fungi have been found by (Castro *et al*., 2022) from *Baccharis linearis* and *Echinopsis chiloensis*. In this study, twelve intercellular and extracellular fungal endophytes extracts were tested for their antioxidant and antibacterial properties. The results indicated that most fungi extracts exhibit antimicrobial effects, with (E2, *Aspergillus* species) as stated by the MIC. These findings are similar to those of a prior study, which found that some bacteria were resistant to fungi extract (Hateet, 2016). Based on past investigations, grass endophytes produce substances that have antibacterial and other pharmacological properties. Some theories contend that endophytes might serve as a new supply for antibiotics. According to Saleem *et al*., (2012), these investigations clearly establish the detected fungal endophytes from medicinal plants as potential sources of antibacterial activity. The fungal endophytes *Acremonium coenophialum*, which was isolated from the medicinal plant *Myrtus communis*, were the main focus of (Hateets, 2020) investigation into the extracts antibacterial and anti-diabetic properties. The current study findings also show that the fungal extracts exhibit potential in vitro antioxidant activity against the DPPH free radical, when compared to the external metabolites; the examined fungal endophytes intracellular metabolites generally displayed promising antioxidant potential, whereas the IC_{50} values of the extracts ranged from (36.8) µg/ml for (T1, *Altrnaria* species) extract to (1264) µg/ml for (E1, *Fusarium* species) extract. Another experiment into the antioxidant activity of endophytes was carried out concurrently by (Gautam, 2022). Antioxidants counteract a variety of disorders brought on by oxidative stress by absorbing free ions from the body. Hafsan *et al*., (2018) suggest that fungal endophytes are drenched in novel active substances like antioxidants and anticancer drugs. The results of our analysis revealed that phenolics are present in significant concentrations both extracellularly and intracellularly among the secondary metabolites of the studied organisms. Twelve fungal endophyte isolates and the host medicinal plants were subjected to phytochemical screening. The medicinal plants that may absorb free ions contain a variety of active chemicals, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and other endogenous metabolites. Flavonoids, quinones, lignans, lignin, stilbenes, phenolic acids, coumarins and tannins are a few examples of phenolic chemicals (Huang *et*

al., 2007). The antibacterial and antioxidant effects of the study could be caused by the presence of active phytochemical substances. This perspective is supported by a number of stories. The inhibition of secondary metabolites by (Bassole *et al*., 2003; Viljoen *et al*., 2003; Erasto *et al*., 2004; Krishnaraju *et al*., 2005) provides support for the idea that plants have antibacterial properties because of the presence of essential oils or tannins, alkaloids, flavonoids, and terpenoids. Numerous investigations of medicinal plants phytochemical composition and biological activity by (Ibrahim *et al*., 2005; Parekh and Chanda, 2007). The *Plectranthus glandulosis* plant was shown to include tannins, alkaloids, anthraquinones, glycosides, flavonoids, phlobatanins, steroids, and terpenoids by Egwaikhide and Gimba (2007) study, which also demonstrated the plants antibacterial characteristics. It is possible for tannin to affect yeast, filamentous fungus, and bacteria. The antibacterial actions of tannins are effective against all of the test organisms, according to Banso and Adeyemo (2007). Secondary metabolites have disincentive properties frequently lend credence to this assertion. Secondary metabolites are a chemical compounds generated by related fungal species, according to this studies. TLC characterization of the twelve secondary metabolites generated by fungi and GC analysis of the T3 fungus and its host *thymus vilgrus* plant. According to Frisvad *et al*., (2008), secondary metabolites are chemical substances produced by smaller members of a genus, order, or even phylum of fungi. Our studies have also demonstrated that endophytes may synthesize compounds from fatty acids, fatty acid esters, and sterols, like methyl palmitate and methyl oleate, that plants can use to fend off ailments and stimulate development. These substances also exhibit a variety of biological behaviours. Hexadecenoic acid two biological behaviours are the prevention of cancer and the reduction of inflammation, methyl linoleate exhibits hepatoprotective, eczema-relieving, and hypocholesterolemic characteristics (Chenniappan *et al*., 2020).

CONCLUSION

Studies suggest that endophytes play important roles in a variety of aspects of life, from their effects on host plants to the implications for how people interact with their environment. Endophytic fungi are rich in fatty acids, phenolic compounds, alkaloids, tannins, and anthraquinones, to mention a few. In the future, biotechnology may use fungal endophytes to extract various industrial and therapeutic bioactive compounds from plants. These substances, such as fatty acids and volatile oils that act as antibacterial, antioxidant, antiviral, and anticancer agents, are crucial to the pharmaceutical industry. Finally, endophytic fungi collaborate with their host plant to create secondary metabolites as a type of defense against other living things and adverse environmental conditions. Secondary metabolites extend the life of the host plant and fungus by protecting them from the harmful effects of oxidation. Endophytes, particularly if they produce phytotoxic chemicals, can boost host competition.

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Table 1: Fungal colonies were isolated from pharmaceutical plants and given a preliminary identification.

No.	Isolated Code [1]	Plant Source	FR % [2]	Preliminary identification
1	D1	*Datura stramonium*	10	*Altrnaria* species
2	D2	*Datura stramonium*	15	*Altrnaria* species
3	E1	*Echinacea purpura*	20	*Fusarium* species
4	E2	*Echinacea purpura*	30	*Aspergillus* species
5	R1	*Salvia rosmarinus*	15	*Altrnaria* species
6	R2	*Salvia rosmarinus*	25	*Altrnaria* species
7	R3	*Salvia rosmarinus*	10	*Altrnaria* species
8	T1	*Thymus vilgrus*	15	*Altrnaria* species
9	T2	*Thymus vilgrus*	10	*Fusarium* species
10	T3	*Thymus vilgrus*	15	*Altrnaria* species
11	T4	*Thymus vilgrus*	20	*Altrnaria* species
12	T5	*Thymus vilgrus*	30	*Fusarium* species

(D1and D2) are the fungal isolates number 1 and 2 from *Datura sramonium* plant , (E1and E2) are the fungal isolates number 1 and 2 from *Echinacea purpura* plant , (R1,R2and R3) are the fungal isolates number 1 , 2 and 3 from *Salvia rosmarinus* plant and (T1,T2,T3,T4 and T5) are the fungal isolates number 1 , 2 ,3,4 and 5 from *Thymus vilgrus* plant and [2] FR% is frequency percent.

Table 2: Antibacterial activities of the twelve fungal isolates extracts by agar-well diffusion assay against tested pathogenic bacteria.

NO.	Tested bacteria Fungal Isolated	Inhibition zone (mm)							
		Bacillus subtilis ATCC 35021		*Escherichia- coli ATCC 7839*		*Staphylococcus aureus ATCC 12600*		*pseudomonas aeruginosa ATCC 15442*	
		Intra [2]	Extra	Intra	Extra	Intra	Extra	Intra	Extra
1	D1	10±1.1	12±1	-	-	10±0.2	11±0.5	7±0.5	11±1
2	D2	8±0.5	9±1	-	-	12±0.3	10±0.8	8±1	10±0.5
3	E1	9±0.5	8±10.5	-	-	-	10±0.7	11±1	11±1
4	E 2	-	22±0.5	-	12±1	5±0.5	13±1	6±0.7	26±0.5
5	R1	9±0.3	10±1	-	-	15±1	17±0.5	14±1	14±0.4
6	R2	8±0.5	8±1	-	-	15±1	-	9±0.7	13±1
7	R3	8±1	9±1	8±0.5	-	9±1	-	8±1	13±0.8
8	T1	8±0.5	11±0.3	-	-	10±0.3	9±0.5	7±1	11±0.5
9	T 2	8±0.5	9±0.6	-	-	-	4±0.4	-	7±1
10	T3	15±0.5	8±1	-	-	8±0.9	4±0.3	11±1	8±1
11	T4	-	-	-	-	10±1	8±0.4	8±1	-
12	T5	-	-	-	-	15±0.5	8±0.5	-	7±1
Chloramphenicol [3]		22±1		16±0.5		24±0.5		22±0.5	

The data represent the mean diameter of inhibition zone ± SD using well diffusion agar method; [2] Intra and extra are intracellular and extracellular ethyl acetate extracts of the twelve entophytic fungal isolates respectively, [3]chloramphenicol used as stander.

Table 3: Antifungal activities of the twelve fungal isolates extracts by agar-well diffusion method against tested pathogenic fungi.

NO.	Tested bacteria Fungal Isolated	Inhibition zone (mm)							
		Candida Albicans ATCC 10231		*Fusarium oxysporum DSMZ 62045*		*Aspergillus niger RCMB002033*		*Trycophyton rubrum ATCC 28188*	
		Intra [2]	Extra	Intra	Extra	Intra	Extra	Intra	Extra
1	D1	-	-	-	-	6±1	-	-	-
2	D2	-	-	-	-	6±0.6	-	-	-
3	E1	-	-	-	-	5±0.5	5±0.5	-	-
4	E 2	-	11±0.4	-	-	-	4±0.4	-	4±0.3
5	R1	-	-	-	-	-	4±0.2	-	-
6	R2	-	-	-	-	-	5±0.3	-	-
7	R3	-	8±0.3	-	-	-	5±1	-	-
8	T1	-	-	-	-	-	4±0.3	-	-
9	T 2	-	-	-	-	-	5±0.5	-	-
10	T3	-	-	-	-	-	4±0.5	-	-
11	T4	-	9±0.2	-	-	-	-	-	-
12	T5	10±0.5	-	-	-	-	-	-	-
Ketoconazole [3]		11±1		11±0.5		18±0.6		14±0.4	

The data represent the mean diameter of inhibition zone ± SD using agar-well diffusion method; [2]Intra and extra are intracellular and extracellular ethyl acetate extracts of the twelve entophytic fungal isolates respectively, [3]ketoconazole used as stander.

Table 4: Study of tannins, flavonoids, alkaloids, anthocyanins, and anthraquinones, in the intracellular and extracellular extracts of the twelve entophytic fungal isolates.

NO.	fungus isolates	Phytochemical groups									
		Tannins		Flavonoids		Alkaloids		Anthocyanins		Anthraquinones	
		Extra	Intra	Extra	Intra	Extra	Intra	Extra	Intra	Extra	Intra
1	D1	-	+	-	+	-	+	-	-	-	-
2	D2	-	++	-	++	-	+	-	-	-	+
3	E1	-	-	-	-	-	-	-	-	-	-
4	E 2	++	-	-	+	+	+	-	-	+	+
5	R1	-	+	-	-	-	-	-	-	-	+
6	R2	-	++	-	++	-	-	-	-	-	-
7	R3	-	+	-	+	-	-	-	-	-	+
8	T1	+++	++	+++	++	+	-	-	-	+	-
9	T 2	-	+	-	-	-	-	-	-	++	++
10	T3	+	+++	+	+++	-	+	-	-	-	+
11	T4	+++	-	+++	-	+	-	-	-	+	-
12	T5	-	-	-	-	-	-	-	-	+	++

Table 5: Secondary metabolites of T3 isolate and its host plant separated by GC mass chromatography and its predictable identification.

NO.	Retention time	Area under peak		compound name
		(T3) Fungi	*Thymus plant*	
1	6.065		1147032	Methyl 12,15-octadecadiynoate
2	6.086	510365		Methyl (phosphonomethyl) amino acetic acid
3	6.182		1667842	1-Acetoxypropane
4	6.297	227566		2,2-dimethoxybutane
5	6.541		797092	Methyl 2-propenoate
6	7.117		1210276	1,3-DIMETHYLHEPTANE
7	13.723		4794789	Glycerol
8	13.861	172229		1-Limonene
9	15.334		1833841	1,3,5-Triazine-2,4,6triamine(CAS)
10	15.914		1480809	Butanedioic acid ,monomethyl ester(CAS)
11	17.485		2130538	2,3 Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
12	20.33		689931	Acetyl monoglyceride
13	20.433		1048425	Bicyclo(3,1,1)Heptan-3-Ol,2,6,6-Trimethyl- ,(a.alpha,2.beta,3alpha,5.alpha)or Iso-pinocampheol
14	20.847		3292223	trans-2-Decenal
15	22.485	148353		Cyclohexasiloxane,dodecamethyl(CAS)
16	26.517		592590	Cis-Caryophyllene
17	26.92		477229	Ethanone, 1-(4-hydroxyphenyl)
18	28.44	49898		Tetradecamethyl cycloheptasiloxane
19	32.058		647703	1-Isopropyl-1,2-cyclohexanediol
20	32.732		508562	1,5-pentanediol (CAS)
21	34.533	364876		Hexadecamethylcyclooctasiloxane
22	40.117	283770		Cyclononasiloxane, octadecamethyl
23	43.611	402294	611153	Methyl palmitate
24	43.789	99259		Progesterone
25	45.198	231323	283597	Eicosamethylcyclodecasiloxane
26	48.784		490304	Phytol Isomer
27	49.203	55336	413280	METHYL OLEATE
28	49.31		757711	2(3H)-furanone,5-heptyldihydro-(CAS)

29	49.872	199707	680432	Cyclododecasiloxane, tetracosamethyl- (CAS)
30	54.064	477772	5150575	Citroflx A4
31	55.999	64135		Cyclododecasiloxane, tetracosamethyl- (CAS)
32	60.766	24064	1437092	(2-Monopalmitin)
33	61.71	369236	1122821	Cyclononasiloxane , octadecamethyl
34	61.857	86857	1186540	Mono(2-ethylhexyl) phthalate

(-) means not present ,(+) mean low concentration, (++) is moderate concentration and (+++) is high concentration of the tested compound groups respectively depending on the intensity of the color produced in different tests where Tannins tested by spraying the TLC bands with ferric chloride , flavonoid tested by spraying the TLC bands with diluted NaOH then with 1 ml HCl was added, Alkaloids tested by spraying the TLC bands with Dragendorff's reagent, Anthocyanin tested by Spraying the TLC bands with alcoholic solution of NaOH and Anthraqunone tested by Spraying the TLC bands with 10 % NH4 OH .

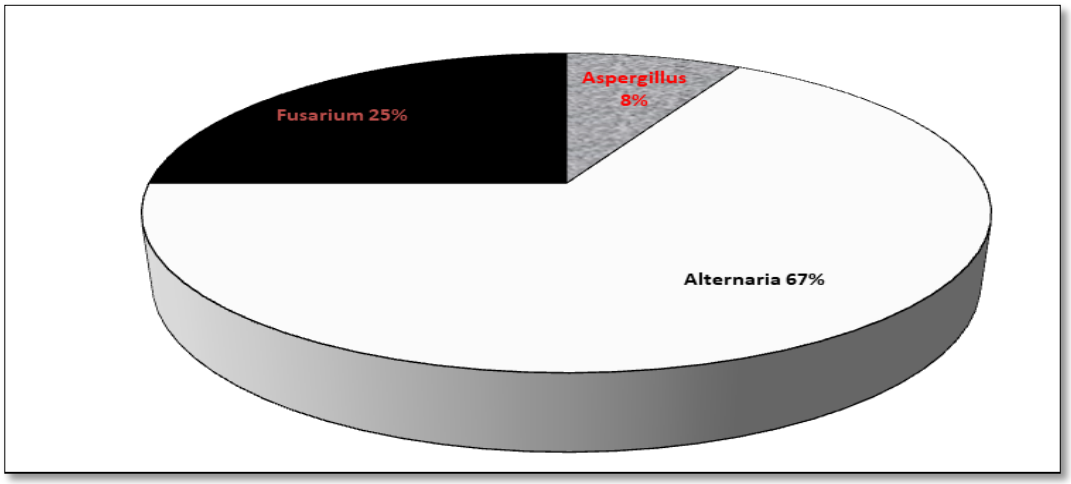

Figure 1: Percentages of the isolated endophytic fungal groups from four medicinal plants.

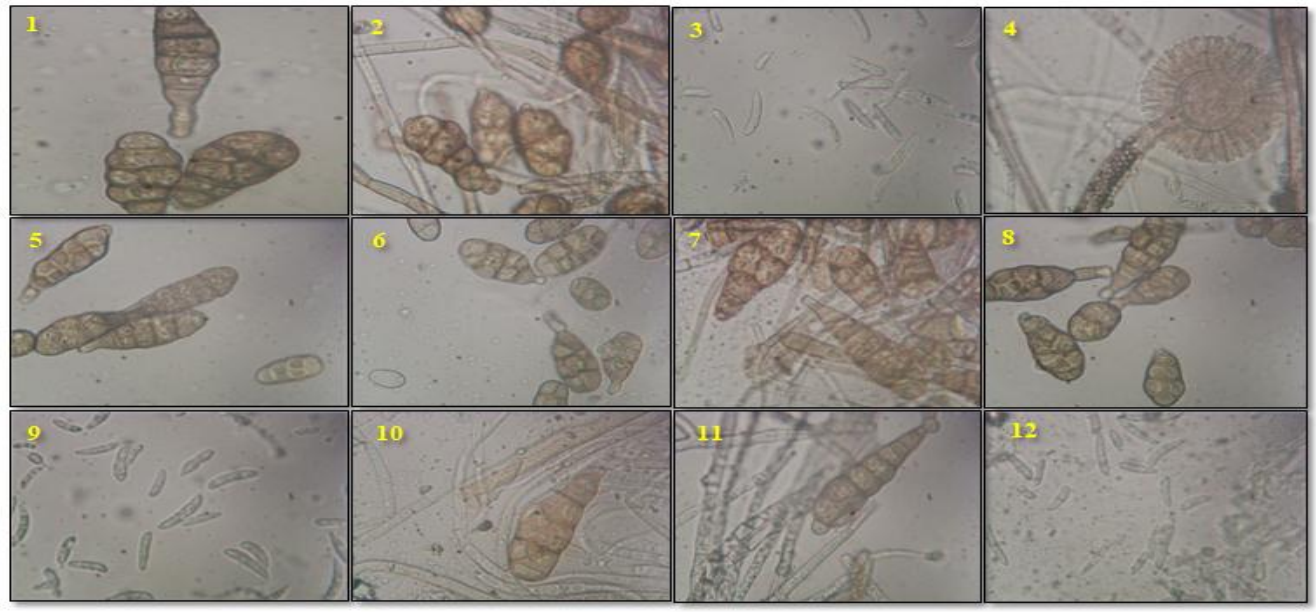

Figure 2: Microscopic identification of the twelve endophytic isolates where (1, 2,5,6,7,8,10 and 11) is *Altrenaria* species where it showed the characterized conidiophores and conidia in short chain for *Altrenaria*. While (3, 9 and 12) is *Fusarium* species where it showed the characterized micro- and macroconidia for *Fusarium*. Photo (4) is *Asprigillus* species where it showed the conidiophores and vesicle structure for *Asprigillus*.

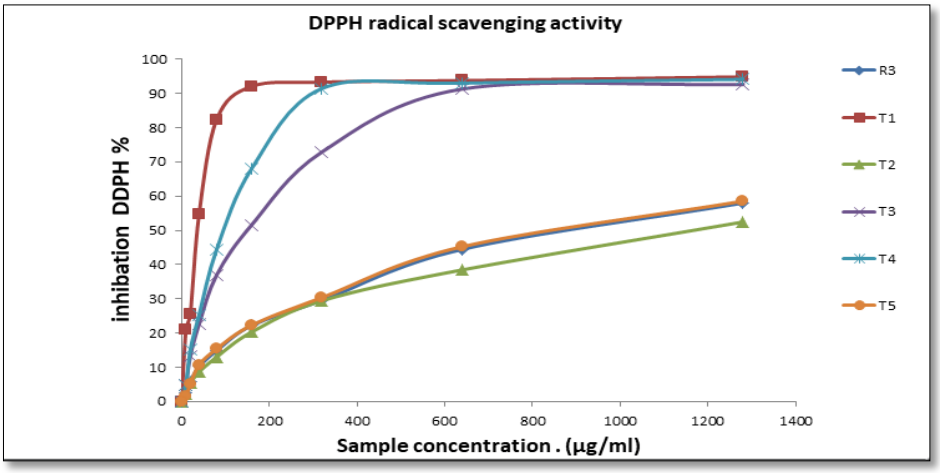

Figure 3: The antioxidant activity of six extracellular endophytic fungal ethyl acetate extracts (R3, T1, T2, T3, T4 and T5) at concentrations (10-1280 µg/ml) using the DPPH radical scavenging activity.

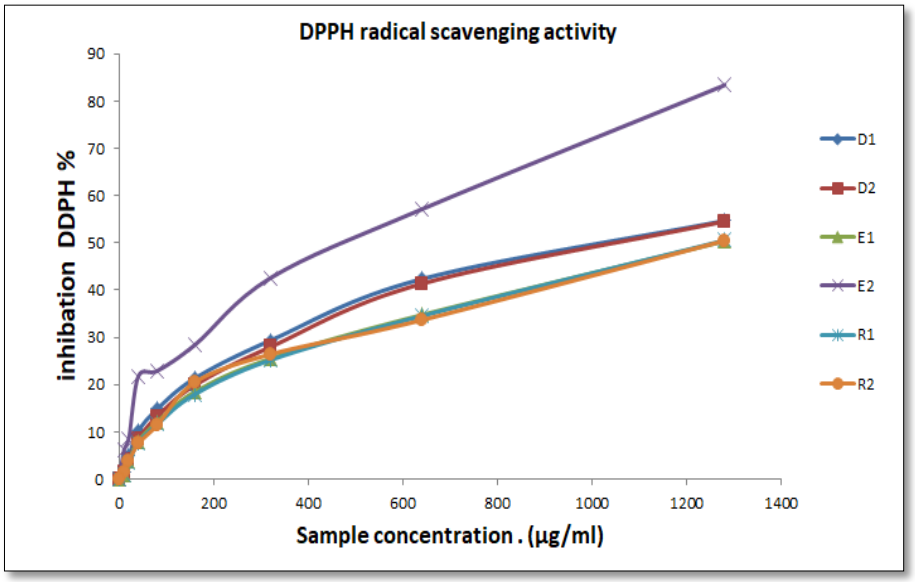

Figure 4: The antioxidant activity of six extracellular endophytic fungal ethyl acetate extracts (D1, D2, E1, E2, R1 and R2) at concentrations (10-1280 µg/ml) using the DPPH radical scavenging activity.

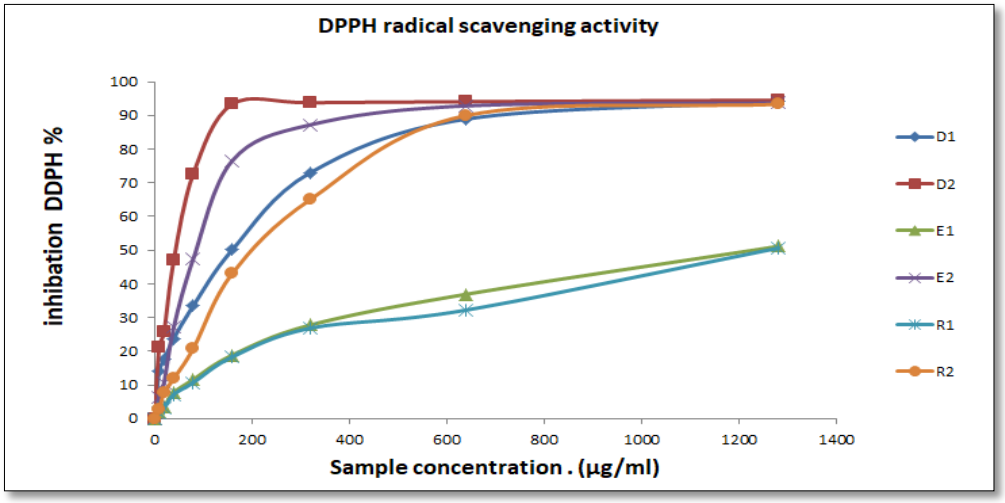

Figure 5: The antioxidant activity of six intracellular endophytic fungal ethyl acetate extracts (D1, D2, E1, E2, R1 and R2) at concentrations (10-1280 µg/ml) using the DPPH radical scavenging activity.

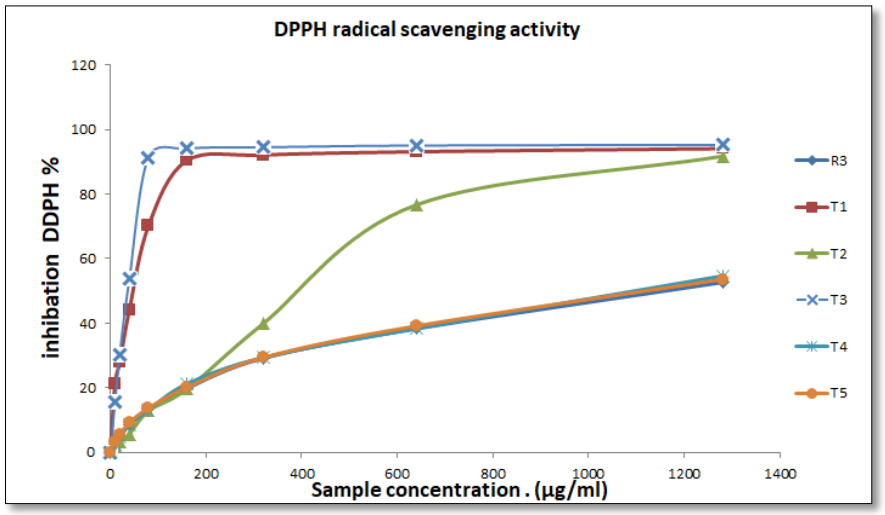

Figure 6: The antioxidant activity of six intracellular endophytic fungal ethyl acetate extracts (R3, T1, T2, T3, T4 and T5) at concentrations (10-1280 µg/ml) using the DPPH radical scavenging activity.

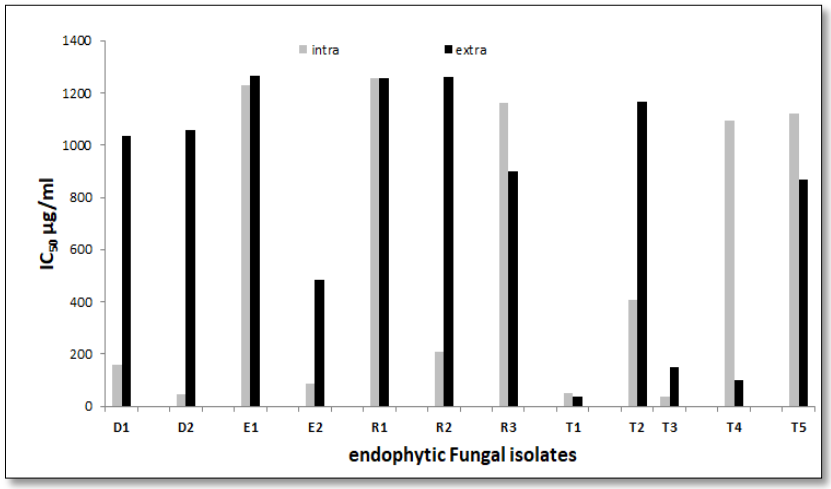

Figure 7: The IC_{50} in µg/ml of DPPH Scavenging activity for the twelve endophytic fungal extracellular and intracellular ethyl acetate extract.

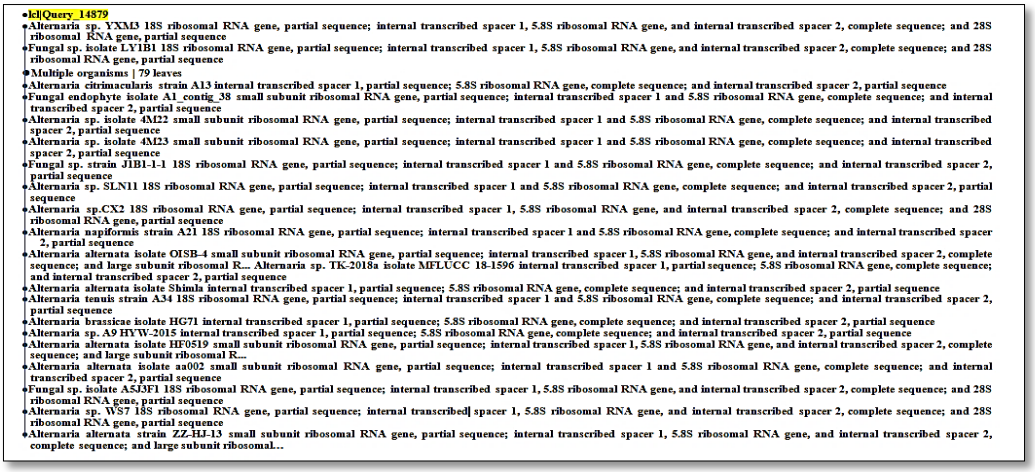
lcl|Query_14879
Alternaria sp. YXM3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Fungal sp. isolate LY1B1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Multiple organisms | 79 leaves
Alternaria citrimacularis strain A13 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Fungal endophyte isolate A1_contig_38 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria sp. isolate 4M22 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria sp. isolate 4M23 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Fungal sp. strain J1B1-1-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria sp. SLN11 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria sp.CX2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Alternaria napiformis strain A21 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria alternata isolate OISB-4 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 R... Alternaria sp. TK-2018a isolate MFLUCC 18-1596 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria alternata isolate Shimla internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria tenuis strain A34 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria brassicae isolate HG71 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria sp. A9 HYW-2015 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Alternaria alternata isolate HF0519 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 R...
Alternaria alternata isolate aa002 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence
Fungal sp. isolate ASJF1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Alternaria sp. WS7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence
Alternaria alternata strain ZZ-HJ-13 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...

Figure 8: Genomic identification for R2F endophytic fungal isolated from *Salvia rosmarinus* and identified by using PCR and DNA sequencing confirmed that the isolate is *Alternaria alternata*.

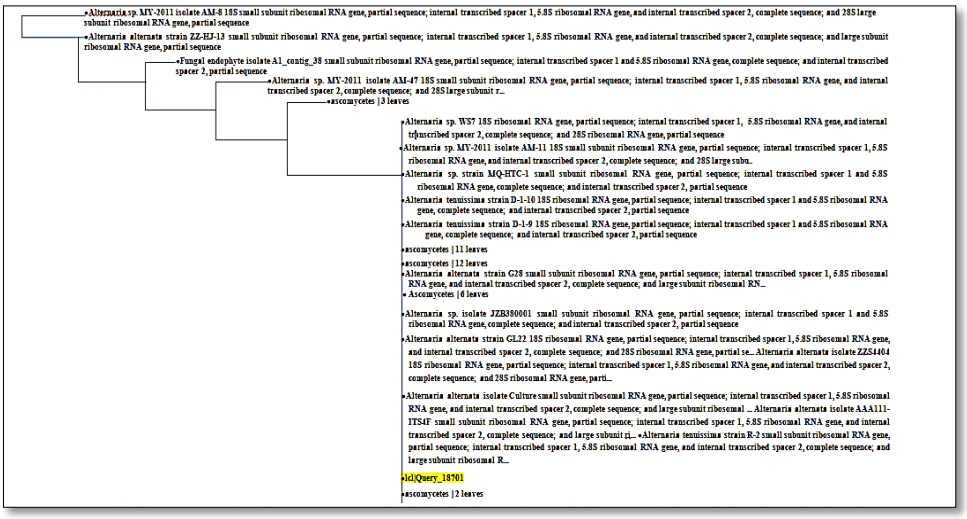

Figure 9: Genomic identification for T3F endophytic fungal isolated from *Thymus vilgrus* and identified by using PCR and DNA sequencing confirmed that the isolate is *Alternaria alternate*.

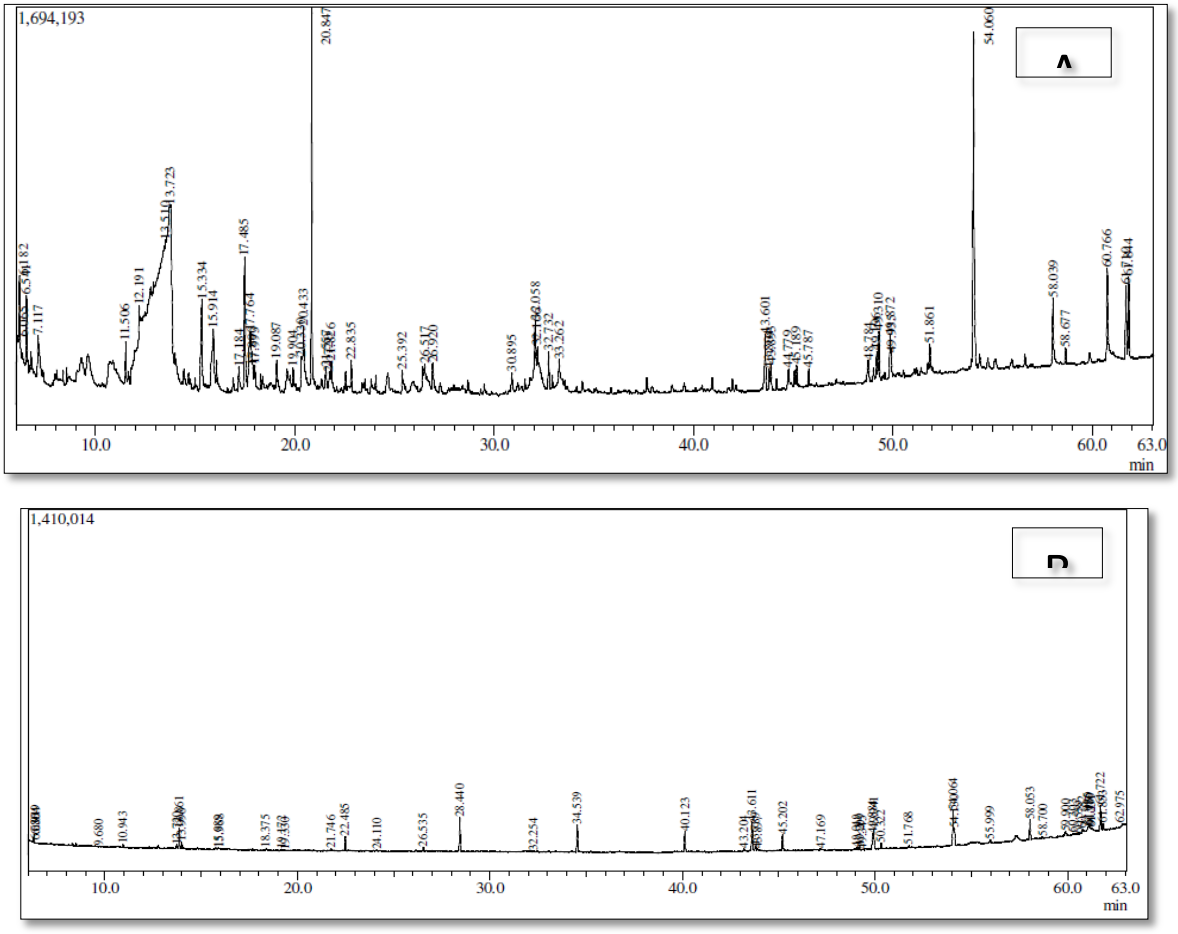

Figure 10: GC Chromatograms of volatile compounds from: A) *Thymus vulgaris* host plant. B) An endophytic fungus (T3)

تنوع الأنشطة الحيوية والخواص الكيميائية لنواتج الأيض الثانوية المنتجة بواسطة فطريات معزولة من داخل الأنسجة الحية لبعض النباتات الطبية

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الملخص العربي

من الأهمية دراسة الفطريات الداخلية لأنها تعزز الإنتاج النباتي الذي يفيد الناس والحيوانات ويلبي الاحتياجات الغذائية. فى هذه الدراسة تم عزل اثنتى عشرة عزلة فطرية من نباتات طبية متنوعة ,وتبين أن هذه العزلات تنتمى إلى ثلاثة أجناس مختلفة شملت: *Asprigillus* ، وأنواع *Alternaria* ، وأنواع *Fusarium*. تم إجراء نشاط مضاد للميكروبات للمستخلصات داخل الخلايا وخارج الخلية لجميع الفطريات المعزولة وأظهر أن المستخلصات خارج الخلية كانت لها نشاط أعلى من المستخلصات داخل الخلايا . كانت "*Candida albicans* " أكثر الأنواع الفطرية حساسية ، حيث خلقت منطقة مثبطة (11 ± 0.4 مم) مع عزلة فطرية (E2) ، وكانت "*Pseudomonas aurogonosa*" أكثر الأنواع البكتيرية حساسية ، حيث أنتجت منطقة مثبطة (26 ± 0.5 مم) مع عزل فطري داخلي (E2). بالإضافة إلى ذلك ، باستخدام (1 ، 1) diphenyl-2-picrylhydrazyl (DPPH) نشاط الكسح لتقييم أنشطة مضادات الأكسدة للعزلات الفطرية الاثنتي عشر ، فقد وجد أن الفطريات المعزولة (T1) و (T3) لديها أعلى أنشطة مضادات الأكسدة ، مع قيم IC_{50} من 36.8 و 36.8 ميكروغرام / مل ، على التوالي. تم العثور على القلويات والفلافونويد والأنثوسيانين والعفص والأنثراكينون في المستخلصات الفطرية خارج الخلية وداخل الخلايا ، والتي تم فصلها باستخدام كروماتوجرافيا الطبقة الرقيقة (TLC) ، مما يدل على أن مستخلصات الخلايا خارج الخلية لديها تركيز أعلى من هذه المجموعات الكيميائية النباتية من الخلايا داخل الخلايا. بالإضافة إلى ذلك، تم استخدام تفاعل البوليميراز المتسلسل (PCR) للتعرف على أكثر الفطريات المعزولة نشاطًا (R2 و T3) ، وتحديد العزلات على أنها *Alternaria Alternata*.

الكلمات الاسترشادية:النباتات الطبية, الفطريات، النشاط المضاد لميكروبات.