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Antimicrobial and Antioxidant Activities of Endophytic *Talaromyces verruculosus* (AUMC15459) Strain Isolated from *Catharanthus roseus* Plant in Libya

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A NOVEL endophytic fungal strain, *Talaromyces verruculosus* (AUMC15459), was successfully isolated from leaves of the medicinal plant *Catharanthus roseus* in Tripoli, Libya. The potential antimicrobial and antioxidant activity of the fungus was investigated. The ethyl acetate crude extract was assessed for its antibacterial activity against four standard pathogenic microbial strains: *Bacillus subtilis* (ATCC® 6633), *Staphylococcus aureus* (ATCC® 6538), *Escherichia coli* (ATCC® 25922), and *Candida albicans* (ATCC® 10231). Furthermore, the ethyl acetate crude extract showed an *in vitro* growth inhibition of all tested pathogenic bacteria and Candida species. Moreover, the antioxidant activity was assessed using a DPPH radical scavenging assay with a recorded IC_{50} of 277.9 µg/mL. Additionally, GC-MS was conducted to identify the effective compounds in the fungal ethyl acetate crude extract. The most predominant compounds found in the ethyl acetate crude extract, Butyl-2-ethylhexyl phthalate and Heptadecene-(8)-carbonic acid-(1), are known for their antibacterial and antioxidant activities. The endophytic *Talaromyces verruculosus* (AUMC15459) was submitted to GenBank under the accession code OR755888. The identified isolate is a prospective strain with efficient metabolites. To the best of our knowledge, this study represents the initial discovery of the broad-spectrum antimicrobial and antioxidant efficacy of the endophytic *T. verruculosus* AUMC15459 crude extract for potential applications in the medicinal field.

Keywords: *Catharanthus roseus*, Endophytes, Fungal metabolite, *Talaromyces verruculosus*.

Introduction

Microorganisms, known as endophytes, live in various plant sections, usually without displaying any outward signs of illness. Endophytes have a variety of connections with their host plants, including symbiosis, pathogenesis, antagonistic relationships, and more. They can counteract stress on the plants, encourage plant development, and even trigger plant defense against pathogenic invasions (Khare et al., 2018). They produce similar secondary metabolites, enabling them to strengthen their host plants' defense mechanism and are, therefore, essential for the ex-planta synthesis of these substances (Fadiji and Babalola 2020). Moreover, these microbes are recognized for producing bioactive secondary metabolites, some of which hold potential applications as antioxidants, antifungals, antibacterials, and anticancer agents, thereby underscoring their significance in nature (Pham et al., 2020; Tiwari et al., 2017).

Notably, these substances are pivotal in the medical sector and mirror those produced originally by the host plants. Often, these

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endophytes function as elicitors for the *in vivo* augmentation of bioactive secondary metabolites that their host plants generate, raising the rate at which these compounds are produced (Pandey et al., 2016). There are hundreds of these endophytic bacteria in *Catharanthus roseus*. Alternate names for it include Vinca rosea, Old Maid, Sadabahar, Cape periwinkle, and Madagascar periwinkle. It is a member of the Apocynaceae family, whose members are primarily utilized for their therapeutic qualities (Ayob and Simarani 2016).

Additionally, it is recognized for producing a wide range of terpenoid indole alkaloids, which are extremely significant in the medical field and include vincristine, vinblastine, catharanthine, ajmalicine, and vindoline (Almagro et al., 2015). One significant alternative strategy to combat the rising drug resistance of specific plant and human pathogens is the discovery of novel antimicrobial compounds from endophytes (Song, 2008; Yu et al., 2010). These substances can infiltrate and eliminate pathogenic microorganisms that impact humans and animals (Strobel et al., 1999a, 1999b). Bioactive materials are known as antioxidant chemicals that stop oxidation. Bioactive compounds derived from endophytic fungi can efficiently scavenge reactive oxygen species (ROS) and superoxide radicals. It has been discovered that a wide variety of fungal endophytes generate potent oxidation inhibitors (Khan et al., 2017).

This study aimed to identify a new endophytic fungal strain from the medicinal plant *Catharanthus roseus* cultivated in a different geographic region, such as Tripoli, Libya, and investigate its prospective antimicrobial and antioxidant potentiality.

Material and Methods

Collection of C*atharanthus roseus* samples

Healthy plant parts, including roots, stems, leaves, and flowers, were collected from *Catharanthus roseus* plants cultivated in Tripoli, Libya.

Sample processing and endophytic isolation

All selected healthy plant parts were washed thoroughly in running tap water, followed by double distilled water before surface sterilization processing. *Catharanthus roseus* plants were surface sterilized using a previously reported protocol (Chen et al., 2013; Thongsandee et al., 2013) with some modifications to isolate fungal endophytes. The plant parts were separated and subjected to subsequent steps of rinsing and washing for surface sterilization in an air laminar flow as follows: rinsing in 70% ethanol for 60 s, 3.4% sodium hypochlorite (NaClO) for 30 s, and washing two or three times with sterile distilled water. The washed parts were dried using sterile filter papers and left until thoroughly dried. Isolation of endophytes from plant samples was processed by two methods. The first method was the surface-inoculation method, in which the sterilized plant tissues were cut into 1.0 × 1.0 cm pieces and placed on the surface of Sabouraud agar medium, supplemented with 50 µL mL^{-1} amoxicillin to prevent bacterial contamination. The second method included grinding the surface-sterilized plant tissues with 10 mL of sterile distilled water with a sterile mortar and pestle. Then, 1 mL of the obtained suspension was serially diluted, and 100 µL from each dilution was distributed on the same agar media. The inoculated plates from the two methods were incubated at 28°C ± 2.0 till the emergence of fungal hyphae from plant tissues. 100 µL water sample from the last wash was inoculated on the prepared media to ensure the plant tissues were successfully surface sterilized. The obtained fungi were then purified and preserved on Sabouraud agar slants in a refrigerator for further experiments.

Fungal cultivation and preparation of the crude extract

For each isolated strain, 3 discs of 5 mm size were inoculated in a 250 mL flask containing 100 mL of Sabouraud dextrose broth medium. The cultures were incubated at 28°C for 2 weeks. After incubation, the culture media of fungal isolates were filtered through Whatman filter paper No.1 to harvest the mycelia. The supernatant was collected and used to extract the metabolites (Shams et al., 2009; Aslam et al., 2011). As a result, a 100 mL petroleum ether was used to extract the supernatant, and the pH was adjusted to 2.0 using 0.1 M HCL. In a separating funnel, two phases were formed and left to stabilize. The organic phase was eliminated by removing the petroleum ether layer. Subsequently, it was transferred to a conical flask and treated with 25% ammonium hydroxide to adjust the pH to 8.5. The lower aqueous layer was extracted using an equal volume of ethyl acetate.

After extraction, two phases were segregated using a separating funnel. The ethyl acetate layer was subsequently dehydrated and evaporated utilizing a rotary evaporator (IKA, RV10,

Germany) under vacuum at 150 rpm and a 45 °C water bath until complete dryness, adhering to the methodology outlined by Zhu et al. (2010). The dried crude extracts were dissolved in methanol for further studies (Chandrasekaran et al., 2014).

Investigation of the biological activities of the fungal endophyte crude extract

Antimicrobial activity test

Three pathogenic bacterial strains, *Escherichia coli* (ATCC® 25922), *Staphylococcus aureus* (ATCC® 6538), and *Bacillus subtilis* (ATCC® 6633), were obtained from the Microbiology Unit of the Central Laboratory of the Faculty of Science, Ain Shams University, Cairo, Egypt. Meanwhile, the pathogenic yeast strain of *Candida albicans* (ATCC® 10231) was obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). A disk diffusion test (Zaidan et al., 2005) was conducted to assess the antimicrobial activity of the ethyl acetate crude extract. 100 µL of test inocula at a concentration of 10^8 cfu. mL^{-1} were distributed using sterile cotton swabs onto Muller-Hinton agar media (for the tested bacterial strains) and Sabouraud dextrose agar (for the *Candida albicans* strain). 100 µL of the crude alkaloid extract was loaded onto filter paper discs (1.5 cm diameter). The discs were air-dried and transferred to the surface of the agar medium. The plates were left in a refrigerator for 2 h (to allow for diffusion) and then incubated at 37°C for 24 h. The appearance of the inhibition zone (IZ) around the discs was recorded and measured. All antimicrobial tests were applied in triplicates, and the mean IZ in mm was taken.

Antioxidant activity test

The antioxidant activity of the ethyl acetate crude extract was assessed using the DPPH (2, 2-dipheny-l- picrylhydrazyl) radical scavenging assay. DPPH is a purple-colored stable free radical. The tested extract is incubated with DPPH, and the antioxidant molecules in the extract transform the DPPH into yellowish 2,2- diphenyl-1- picrylhydrazine. The level of purple-to-yellow transformation reflects the scavenging capability of extracts that could be detected at 540 nm. To conduct the DPPH assay, fungal crude extract was prepared at a final concentration of 200, 300, 400, 500, and 750 µg mL^{-1} in methanol. A trolox standard solution was prepared at 6 concentrations of 1.25, 2.5, 5, 6.25, 7.5, and 10 µg mL^{-1} in methanol. In a 96-well plate, 100 µL of fresh DPPH reagent (0.1% in methanol) and 100 µL of the tested crude extract were added at each tested concentration. The reaction was then allowed to run at room temperature for 30 min in the dark. The subsequent decrease in DPPH color intensity was measured at 540 nm. Results were expressed as means ± SD through the following equation (Zheng et al., 2013; Boly et al., 2016):

$$\text{percentage inhibition} = \frac{(\text{Average absorbance of blank} - \text{average absorbance of the test})}{\text{Average absorbance of blank}} \times 100$$

Microsoft Excel® was applied for data analysis. Besides, Graph pad Prism 6® was applied to estimate the IC_{50} value by converting the concentrations to their logarithmic value and selecting a non-linear inhibitor regression equation (log (inhibitor) vs. normalized response – variable slope equation) (Zheng et al., 2013; Boly et al., 2016).

Identifying the fungal endophytic strain

The most potent endophytic fungal strain that showed antimicrobial activity against all tested pathogenic strains and antioxidant activity was identified based on cultural morphology and microscopic characteristics (Moubasher, 1993). In addition, molecular identification was conducted as follows: a 7-day-old culture of the fungal strain was prepared on Czapek's yeast extract agar (CYA) at 28°C. Fungal DNA was extracted using a Patho-gene-spin DNA/RNA extraction kit (Intron Biotechnology Company, Korea). PCR was carried out using the following primers: forward ITS1 primer 5' - TCC GTA GGT GAA CCT GCG G – 3' and reverse ITS4 primer 5'- TCC TCC GCT TAT TGA TAT GC - 3'. Then, the recovered amplicons were sequenced (White et al.,1990). The resulting sequence was submitted to the BLAST tool on the website https://blast.ncbi.nlm.nih.gov. The nucleotide sequences were aligned, and phylogenetic analysis was conducted using MegAlign software version 5.05. The endophytic *Talaromyces verruculosus* (AUMC15459) was deposited in the GenBank under accession no. OR755888.

Gas chromatography-mass spectrometry analysis (GC-MS) methodology

The GC-MS system (Agilent Technologies) was equipped with a gas chromatograph (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo, Egypt. The GC had an HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Analyses were conducted using helium as the carrier gas at a 2.0 mL/min flow rate at a splitless injection volume of 2 µL. The temperature program consisted of an initial 2-minute period at 45°C, followed by a 10°C/min increase to 310°C, held for 10 minutes; further elevated at 10°C/min to 325°C, and maintained for 5 minutes. The injector and detector were held at 280°C and 280°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 50-550. Different constituents were identified by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data (El-Hagrassi et al., 2020). GC-MS was employed to identify the compounds responsible for the broad biological spectrum.

Statistics

All tests were performed in triplicates, statistical significance was assessed using a one-way ANOVA (analysis of variance, SPSS software v.18) test, and the means were compared with Duncan's test at 0.05 level.

Results

Endophytic isolation from *Catharanthus roseus*

36 fungal isolates were purified from *Catharanthus roseus* collected samples: 13 isolates from roots, 9 from stem, 4 from leaves, and 10 from flowers.

Investigation of the biological activities of the fungal endophyte crude extract

Antimicrobial activity of crude extract of *Talaromyces verruculosus*

As presented in Figure 1 and Table 1, the tested crude extract displayed a significant ($P \leq 0.05$) capability in controlling the tested pathogenic bacterial and yeast strains. The crude extract of *T. verruculosus* presented a broad antimicrobial spectrum. It formed a maximum significant clear inhibition zone of 44 mm ± 0.0000[a] towards the Gram-positive bacterial strain of *S. aureus* ATCC® 6538. Moreover, close mean inhibition zones' values of 38.6 mm ± 0.5507[b] and 37 mm ± 0.1000[b] were recorded for the Gram-negative bacterial strain of *E. coli* ATCC® 25922 and *B. subtilis* ATCC® 6633, respectively, with no significant differences between them. In addition, the crude fungal extract showed a lower significant ($P \leq 0.05$) clear inhibition zone of 19.3 mm ± 0.0577[c] for the yeast strain of *C. albicans* ATCC® 10231.

Antioxidant activity of crude extract of *Talaromyces verruculosus*

The ability of the extract to scavenge free radicals was investigated using DPPH, a stable free radical with a distinctive absorbance at 540 nm. The absorption diminishes as antioxidants give protons to this radical. The sample was evaluated against this radical at various concentrations, and readings were recorded by observing a decrease in absorbance, indicating the degree of radical scavenging property. The sample's scavenging abilities were assessed alongside the reference "trolox." The recorded IC_{50} value was 277.9 ± 2.63 µg mL^{-1} (Figure 2).

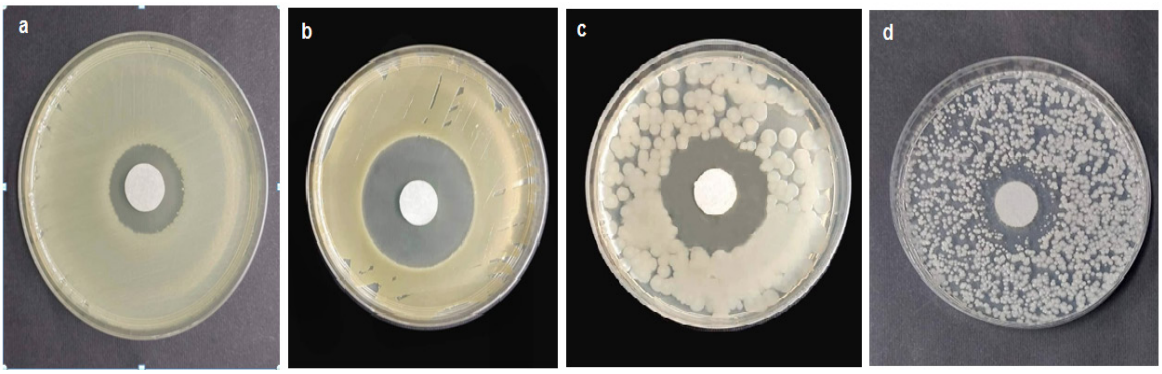

Fig.1. Antimicrobial activity of the crude extract of *Talaromyces verruculosus* (AUMC15459) against tested strains, *(a) E. coli, (b) S. aureus, (c) B. subtilis, and (d) C. albicans.*

TABLE1. Mean values of inhibition zones (mm) ± standard deviation (SD) recorded from the different tested microbial strains after treatment by the crude extract of *Talaromyces verruculosus.*

Microbial Strains	Inhibition Zones (mm) ± (SD)
E. coli	38.6 ± 0.5507^{b}
S. aureus	$44\pm0.000^{0}a$
B. Subtilis	37 ± 0.1000^{b}
C. albicans	19.3 ± 0.0577^{c}

Data are shown as the mean ± SD of triplicate measurements from independent experiments. a-c means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$).

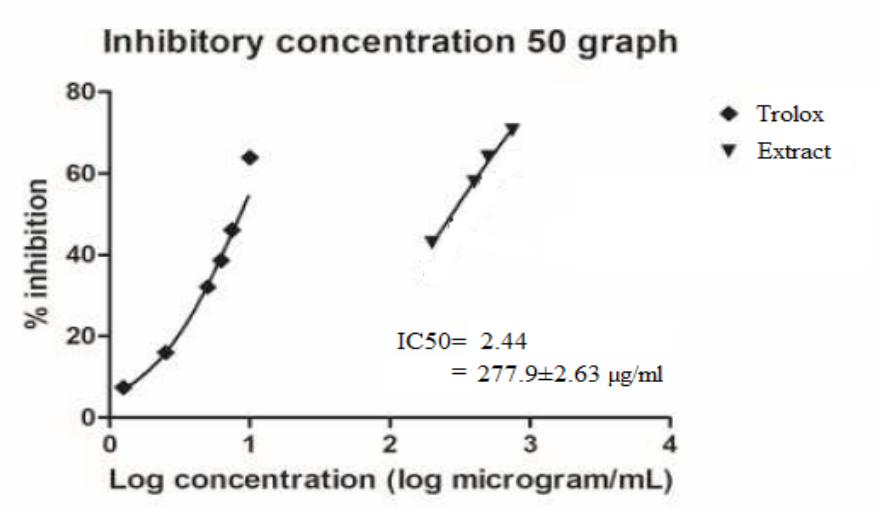

Fig.2. DPPH scavenging activity inhibition percentages exhibited by crude extract of *T. verruculosus* with standard trolox.

Fungal identification

Macroscopic and microscopic criteria of the most potent endophyte fungus with positive antimicrobial, and antioxidant activity is represented in Figure 3 (a, b). The cultural morphology on Czapek`s yeast agar medium at 28 °C for 15 days. Colony surface showing green to olive green color and brown reverse. The microscopic characteristics after staining with lactophenol cotton blue stain showed pigmented conidiophores. Conidiophores terminating in metulae and lanceolate phialides which produce chains of spheroidal unicellular conidia. The nucleotide sequence of the fungal partial sequence of the internal transcribed spacer 1 coding gene sequence (556 bp) showed 99.64% - 100.0% identity and 99%-100% coverage with several strains of *Talaromyces verruculosus*. The nucleotide sequence was submitted at the GenBank database under accession number OR755888. Phylogenetic tree was constructed to illustrate the phylogenetic relation between *Talaromyces verruculosus* (AUMC15459) and related strains in the GenBank. Figure (4)**.**

GC-MS analysis of the *T. verruculosus* extract

Using GC-MS to analyze the crude extract of *Talaromyces verruculosus* (AUMC15459), it was possible to identify the chemical components of the fungal extract. The n-hexane fraction of the tested fungal extract revealed the existence of a diverse range of chemicals. The total peak areas, the molecular weights, the molecular formulas, and the retention times of the discovered compounds included in the extract are listed in Table 2. The total peak area of the detected compounds is 90.13 %. The obtained GC-MS chromatogram is displayed in Figure 5. In the *T. verruculosus*

extract, 15 compounds were detected. The major compounds were butyl-2-ethylhexyl phthalate (52.99%), heptadecene-(8)-carbonic acid (21.69%), and triethyl phosphate (4.54%), which represented 79.22 % of the total peak areas. The rest of detected compounds were benzoic acid, 4-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-NNO-azoxy]-, methyl ester (2.72%), 10-methoxy-nb-alphamethyl corynantheol (2.21%), methyl 12,13-tetradecadienoate (1.04%), 2-cinnamoyl-2-(N-phenylamino) ethyl t-butyl ketone (1.00%), glycerine-1,3-diolein (0.84%), cyclopentyl-methyl phosphonic acid, 2-isopropyl-5-methylcyclo hexyl ester (0.64%), (7-Oxo-2-oxa-7-thiatricyclo [4.4.0.0 (3,8)] decan-4-ol (0.47%), methyl 9,10-octadecadienoate (0.47%), 1,1-dideuterio-hexadecanyl methane sulfonate (0.38%), and hexyl(methylidyne) ammonium (0.29%), for which represented 20.78% of the total peak areas.

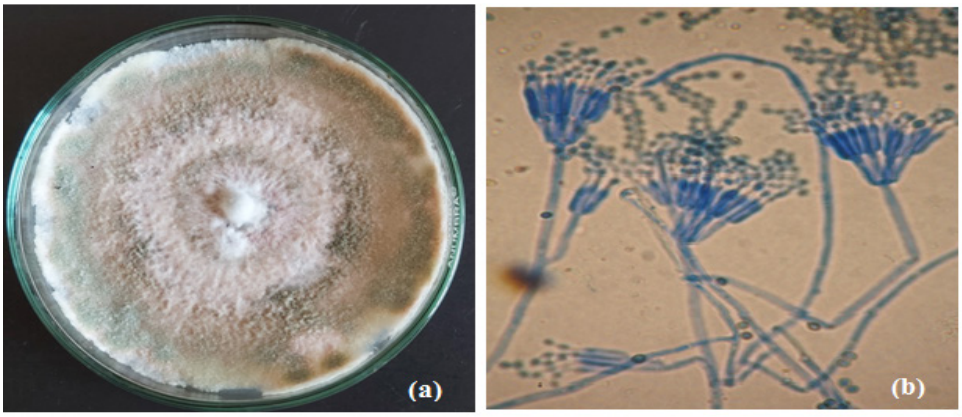

Fig.3. The macroscopic characteristics of *Talaromyces verruculosus* AUMC15459 Colony appearance on Czapek`s yeast agar after 15 days at 28ºC (a) and the microscopic examination (b).

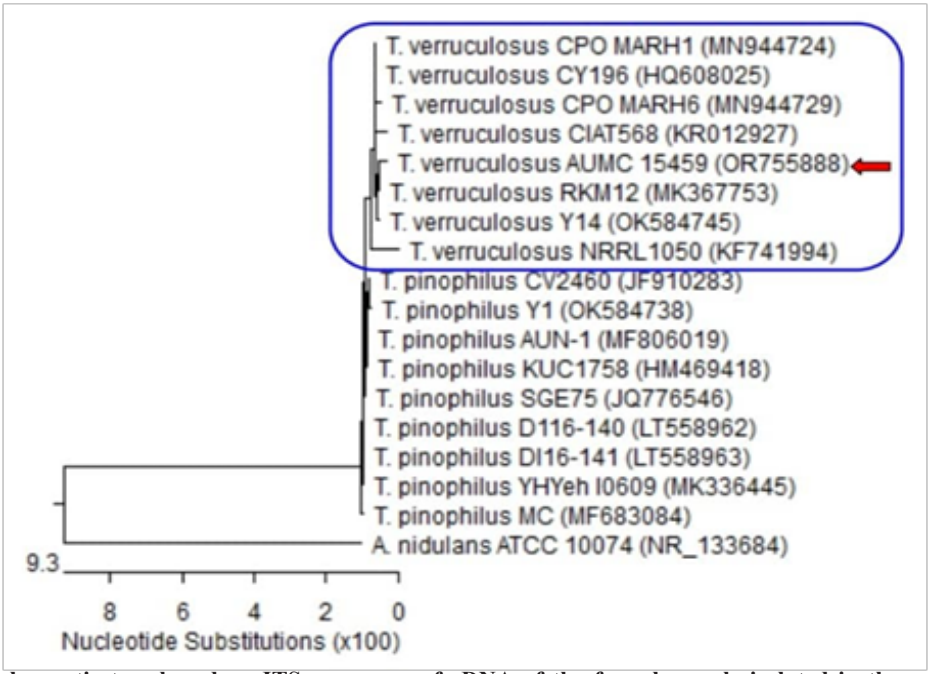

Fig. 4. Phylogenetic tree based on ITS sequences of rDNA of the fungal sample isolated in the present study (*Talaromyces verruculosus* AUMC 15459, GenBank accession OR755888, arrowed) aligned with sequences of closely related strains in the GenBank. This strain showed 99.64% - 100.0% identity and 99%-100% coverage with several strains of *T. verruculosus* including the type strain NRRL1050 (KF741994). *Aspergillus nidulans* is included as outgroup strain. *A.*= *Aspergillus*, and *T.* = *Talaromyces*.

TABLE 2. GC/MS analysis of the extracted crude from the identified fungal strain.

Peak No.	R_t (min.)	MF	Area %	Identified compounds
1	3.184	C7H13N	0.29	Hexyl(methylidyne) ammonium
2	7.459	C6H15O4P	4.54	Triethyl phosphate
3	16.746	C16H11N3O5	2.72	Benzoic acid, 4-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-NNO-azoxy]-, methyl ester, (Z)-;
4	17.564	C20H26O4	52.99	Butyl-2-ethylhexyl phthalate
5	17.678	C21H29N2O2	2.21	10-Methoxy-nb-alphamethyl corynantheol
6	19.412	C18H34O2	21.69	Heptadecene-(8)-carbonic acid-(1)
7	19.527	C21H29N2O2	0.57	10-Methoxy-nbalpha methyl corynantheol
8	21.861	C15H26O2	1.04	Methyl 12,13-tetradecadienoate:
9	22.027	C8H12O3S	0.47	(7-Oxo-2-oxa-7-thiatricyclo [4.4.0.0 (3,8)] decan-4-ol
10	22.422	C21H25NO	1.00	2-Cinnamayl-2-(N-phenylamino) ethyl t-butyl ketone
11	22.788	C17H34D2O3S	0.38	1,1-Dideuterio-hexadecanyl methane sulfonate
12	24.156	C16H31O2P	0.64	Cyclopentyl-methylphosphinic acid, 2-isopropyl-5-methylcyclo hexyl ester
13	29.54	C19H34O2	0.47	Methyl 9,10-octadecadienoate
14	32.321	C19H34O2	0.28	Heptadecene-(8)-carbonic acid-(1)
15	38.289	C39H72O5	0.84	Glycerine-1,3-diolein

R_t: Retention time MF: Molecular formul

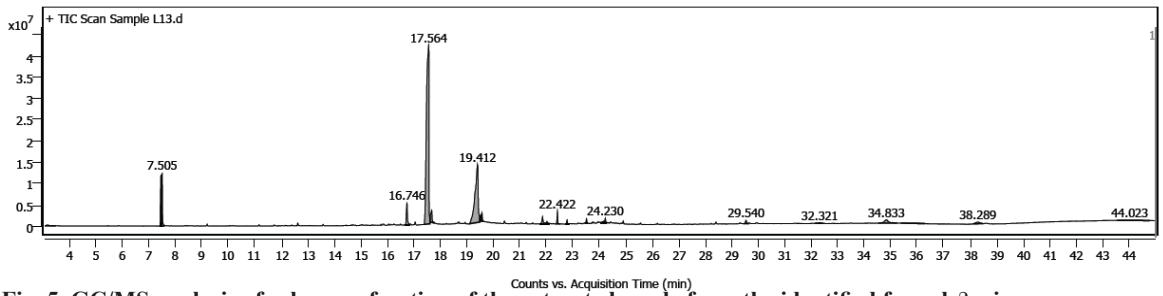

Fig. 5. GC/MS analysis of n-hexane fraction of the extracted crude from the identified fungal strain.

Discussion

Medicinal plants provide people with medicines to prevent disease, maintain health, or cure ailments (Balick *et al*., 1996). *Catharanthus roseus* is an important medicinal plant that is a vital source of alkaloids, indole, phenolics, and other compounds. It is an essential herbal drug because different plant parts are used as antimicrobial agents and antioxidants (Patil and Ghosh, 2010; Bhutkar and Bhise, 2011). Furthermore, it has been used to treat various diseases, such as cancer, diabetes, hypertension, and menstrual disorders (Hisiger and Jolicoeur, 2007). Endophytic fungi have been found in each plant species examined, and it is estimated that over one million fungal endophytes exist in nature (Petrini, 1991). Fungi are the most frequent endophytes as they can survive asymptomatically in the tissues of plants

aboveground and belowground as roots, stems, and leaves (Staniek *et al*., 2008). The relationship of fungal endophytes with their host plants depends upon the host genotype, the genotype of the endophyte, and the environment in which they grow (Unterseher and Schnittler, 2010; Salam et al., 2017). Horizontal gene transfer (HGT) refers to the transmission of genetic material across the genomes of biological organisms by processes other than fertilization. HGT is a universal phenomenon observed in bacterial, fungal, and eukaryotic genomes (Bansal and Meyer, 2002; Vos *et al*., 2015). Because natural selection favors the evolution of beneficial endophytic strains, several endophytes have been identified for secreting secondary metabolites that protect plants against insect pests, pathogenetic organisms, and herbivores (Saikkonen *et al*., 2004). Thus, endophytes represent a promising source of novel, biologically active metabolites for pharmacological and agricultural applications (Dreyfuss and Chapela, 1994; Schulz *et al*., 2002). In the current study, crude extract of *Talaromyces verruculosus* exhibited a broad, significant ($P \leq 0.05$) antimicrobial activity against all tested microbial pathogens *(Escherichia coli* ATCC®25922, *Staphylococcus aureus* TCC® 6538, *Bacillus subtilis* ATCC® 6633, and *Candida Albicans* ATCC® 10231). Previous studies showed that fungal endophytic *Alternaria alternata* crude extract displayed significant biological activities as an antimicrobial and anti-mycotoxigenic agent for a bioactive chemical extracted from *Catharanthus roseus* (leaf tissues) (Sudharshana et al., 2019). Both endophytic fungi, *Alternaria* sp. (plant host; *Withania somnifera*) and *Colletotrichum* sp. (plant host; *Moringa oleifera*), have demonstrated antibacterial activity towards *E. coli* and *Staphylococcus aureus* in their ethanolic extract (Atri et al., 2020). In agreement with this study, *Talaromyces* sp. (plant host; *Kandelia candel* L. Druce) has biological activities as an antifungal and antibacterial agent towards *Pseudomonas aeruginosa*, *Sarcina ventriculi*, and *E. coli* (Liu *et al*., 2010). In the current study, the antimicrobial activity of crude extract of *Talaromyces verruculosus* against the tested microbial pathogens was studied by measuring the inhibition zone by the disc diffusion assay. The crude extract of *Talaromyces verruculosus* exhibited considerable microbial growth controlling against the tested microbial pathogens: *Escherichia coli* ATCC®25922, *Staphylococcus aureus* ATCC®6538, *Bacillus subtilis* ATCC®6633, and *Candida Albicans* ATCC®10231.

In the present study, the crude extract of *Talaromyces verruculosus* displayed significant biological activity with its antioxidant behavior through DPPH scavenging. It was reported that the ethyl extract from *C. roseus*-associated *Chaetomium nigricolor* had been found to have antioxidant, cytotoxic, and apoptotic properties (Dhayanithy et al.,2019). Endophytic fungi (*Arthinium* sp., *Colletotrichum* sp., and *Diaporthe* sp.) related to *Aquilaria subintegra* tend to produce a wide range of bioactive compounds with potent antioxidant effects estimated through DPPH scavenging assay (Monggoot et al., 2017). According to an *in vitro* study, the endophytic fungal strains of the genus *Fusarium* sp. that host *Fritillaria unibracteata* are potent at producing antioxidants, such as gallic acid, rutin, and phlorizin (Pan et al., 2017).

Concerning the results of gas-chromatography/mass-spectrometry (GC-MS) analysis of the fungal extract, which contains the bioactive constituents that might be responsible for the antimicrobial and antioxidant activities, their compatibility with relevant studies was explored (Table 1). Some previous studies detected bioactive components such as alkaloids, acids, phosphates, aldehydes, ketones, flavonoids, hydrocarbons, phenolics, esters, and additional compounds in other endophytic fungal species. In this study, 15 constituents were detected by GC-MS analysis in the fungal crude extract. The major constituents have been detected in *Talaromyces verruculosus* crude extract were; butyl-2-ethylhexyl phthalate (52.99 %), heptadecene-(8)-carbonic acid (21.69%), triethyl phosphate (4.45 %), and benzoic acid, 4-[(1,3-dihydro-1,3-dioxo-2H-isoindol-2-yl)-NNO-azoxy]-, and methyl ester (2.72 %). The findings of this study agree with numerous previous scientific studies (Habib and Karim, 2009; Tarik et al., 2019; Bratty et al., 2020; Siswadi and Saragih, 2020). Certain hexyl-ammonium compounds exhibit antimicrobial properties and can be used as antimicrobials in various applications. Depending on the specific microorganism targeted, these compounds may have bactericidal or fungicidal effects. Quaternary ammonium compounds (QACs), particularly those bringing long hydrocarbon chains, show a broad-spectrum antimicrobial activity to gram-positive and gram-negative bacteria, fungi, and some viruses (Simoncic and Tomsic, 2010).

Several prior studies have examined benzoic acid's antioxidant properties, antifungal activity, and its derivatives against *Candida albicans* (Hsuan et al., 2021). Furthermore, recent research has examined the antioxidant and antimicrobial properties of butyl-2-ethylhexyl phthalate (Singh et al., 2021). The antioxidant activity properties of methyl 12,13-tetradecadienoate (Gupta et al., 2023; Oli et al., 2023), in addition to the antioxidant and antimicrobial potentials of heptadecene-(8)-carbonic acid compound (Iqbal et al., 2024) were reported. All these pertinent results contribute to the significance of this study. In summary, most of these outcomes align with the findings of the present investigation. This study proved the antimicrobial and antioxidant activities of the fungus *Talaromyces verruculosus*, which should be considered a novel and prospective source for antimicrobial and antioxidant producers. Optimizing the growing conditions of endophytes is essential for the large-scale production of these noteworthy bioactive secondary metabolites and for evaluating the cytotoxicity of the compounds.

Conclusion

This study proved the potential biological activities of the novel identified fungus, *Talaromyces verruculosus* AUMC15459, isolated from *the Catharanthus roseus* plant in Libya, which should be considered a prospective alternative source of antimicrobial and antioxidant compounds. In future research, it is imperative to optimize fungal cultivation conditions and investigate the cytotoxicity of the fungal crude extract to facilitate the application of these outstanding bioactive secondary metabolites on a potential large-scale industrial level.

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*Research involving Human Participation and/or Animals***:** Not applicable

Competing interests: The authors declare no conflict of interest

Authors contribution: SJ article investigation, conducting the experiments, recording the obtained results, data analysis, providing resources, and contribution in manuscript writing, reviewing and editing. **AGZ** article investigation, designing and conducting experiments, data analysis, designing the statistical modeling, data representation, preparing the original draft, manuscript revising and editing. **EHE and ST** suggested the research point, providing supervision, designing experiments, and participated in manuscript writing, reviewing, and editing. All authors reviewed the article.

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Availability of data and materials: All data generated or analyzed during this study are included in this published article.

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في هذه الدراسة تم عزل الفطريات الحية الدقيقة الداخلية من الجذور والسيقان والأوراق والأزهار السليمة لنبات الكاثارانثوس روزيوس الطبية المزروعة في طرابلس ، ليبيا كتضاد ميكروبي ومضاد للأكسدة ، من بين هذه العزلات :AUMC15459 *Talaromyces verruculosus* والذي تم تحديده شكلياً وجزيئياً وتسجيله في بنك الجينات العالمي NBCI. اظهرت نتجية مضادات الميكروبات في المستخلص *T. verruculosu* تجاه الميكروبات الاربعة الممرضة التي تم اختبارها (ايشيريشيا كولاى ، أستافيلوكوكاس أورياس، باسيلاس سبتيلس ،وكانديدا البيكانس) تاثيرا قويا وفعالا على الميكروبات الممرضة المستخدمة كما اظهر كمضاد للأكسدة عبرمقايسة الكسح الجذري DPPH لمستخلص AUMC15459 *T. verruculosus* نتيجة (IC50، 277.9 ميكروغرام/مل) ، وأخيراً تم إجراء GC-MS للتعرف على المركبات الفعالة للمستخلصات الفطرية *Talaromyces verruculosus*. اكتشفت هذه الدراسة فعالية الكائنات الحية الدقيقة الداخلية للسلالات فطرية جديدة لنبات الكاثارانثوس روزيوس البنفسجية ذات التطبيقات المحتملة على نطاق واسع في المجال الطبي.