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Isolation, Identification and Biological Applications of Endophytic Fungi from *Ocimum basilicum* and *Ammi visanga*

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MEDICINAL plant-associated fungi called endophytes may have a role in promoting plant growth via multiple pathways. More people are becoming aware of endophytic fungi as sources of bioactive asubstances with beneficial medicinal properties. In the present study, two endopytic fungi were isolated from two medicinal plants, *Ocimum basilicum* and *Ammi visanga*, and were identified as *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441. Petroleum ether and ethyl acetate were used to extract secondary metabolites from these fungi. The petroleum ether and ethyl acetate extracts of these endophytic fungi exhibit potent antimicrobial activity against human pathogenic microbes such as *Escherichia coli*, *Bacillus subtilis*, *Aspergillus chrysogenium*, and *Aspergillus fumigatus*. Moreover, *Curvularia pseudobrachyspora* AUMC16441 and *Alternaria alternata* AUMC16440 were able to produce indole acetic acid with concentrations of 9.62 and 5.7 µg/mL, respectively, when tested with 5 µg/mL tryptophan and had antidiabetic activities with percentages of 94% and 82%, respectively. The bioactive substances of the fungal and plant extracts were identified using gas chromatography-mass spectrometry analysis (GC-MS) that revealed the presence of different compounds belonging to different groups, such as fatty acids, flavonoids, hydrocarbons, phenolics and alcohols. The most relevant compounds were hexadecanoic acid, tetradecane, phenol 2, 2'-methylenebis [6-(1, 1-dimethylethyl)-4-methyl, 13-docosenamide, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, ethyl iso-allocholate, cyclooctasiloxane hexadecamethyl, cycloheptasiloxane tetradecamethyl, and cyclohexasiloxane dodecamethyl. These substances are recognized to have a number of advantageous biological characteristics. In addition, the GC-MS result revealed that many compounds are common between endophytic fungal isolates and their host, confirming the role of endophytic fungi in protecting their host and vice versa.

Keywords: Medicinal plants, fungal extract, *Curvularia pseudobrachyspora*, *Alternaria alternata*, secondary metabolites.

Introduction

Microorganisms such as bacteria, fungi, and actinomycetes were referred to as endophytes when they spend all or a portion of their lives inside or between plant cells. Endophytes can colonize the internal plant tissues such as leaves, petioles, stems, twigs, bark, roots, fruit, flowers, and seeds without showing to harm or infect their host plants in any way **(Fouda *et al.*, 2015)**.

In a symbiotic relationship, plants benefit fungal endophytes by giving them nutrients, shelter, and a way to spread their spores, while fungal endophytes convert the bioactive substances that the host plant produces into products with multiple uses **(Schouten, 2019)**. It is also known that fungal endophytes can affect how plants produce their own enzymes, phytohormones, and bioactive substances **(Khan *et al.*, 2016 and Satheesan & Sabu, 2020)**.

All plants have an endophytic relationship with one or more fungi **(Haddadderafshi, 2015)**. Members of the Ascomycota as well as a few species from the Basidiomycota, Zygomycota, and Oomycota form the majority of endophytic fungi **(Rajamanikyam *et al.*, 2017)**. The bioactive substances produced by these plant-associated microbes may function as an antibiotics, inducers, and regulators **(Alsheikh *et al.*, 2020; Erb & Kliebenstein, 2020; and Funes *et al.*, 2020)**. Fungal endophytes are a natural source of new bioactive substances that are important for medicine **(Newman and Cragg, 2016)**.They produce natural bioactive chemicals or their

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derivatives that make up more than 70% of the anticancer and antibacterial drugs **(Newman and Cragg, 2020)**. Endophytic fungi produce a wide range of bioactive secondary metabolites, including phenols, alkaloids, steroids, peptides, flavonoids, quinones, and terpenoids, that may be used to promote plant development or treat diseases in both plants and humans **(Zhao *et al*., 2019)**.

Diabetes is a chronic metabolic disease that disrupts the metabolism of carbohydrates, fats, proteins, water, and electrolytes **(Agnaniet *et al*., 2016)**. The goal of diabetes treatment is to get patients' blood sugar levels back to normal by controlling the action and network of carbohydrate-degrading/hydrolyzing enzymes as well as monosaccharide absorption **(Alqahtani *et al*., 2020)**. Amylase and glucosidase, two key digestive enzymes, are in the role of converting complex dietary carbs into sugar in the digestive system. By controlling these enzymes, it is possible to reduce the postprandial rise in blood glucose levels, which will limit insulin action and regulation **(Alqahtani *et al*., 2020)**.

Endophytic fungi can play a role in treatment of diabetes mellitus through the production of enzyme inhibitors for two digestive enzymes, amylase and glucosidase, which are responsible for the breakdown of complex dietary carbohydrates into sugar in the digestive tract **(Lee *et al*., 2013)**. PPHG (postprandial hyperglycemia) is reduced by synthetic inhibitors such as voglibose, miglitol and acarbose by interfering with the function of carbohydrate-digesting enzymes, hence reducing glucose absorption. Endophytic fungi have natural antidiabetic metabolites that can be used as safe medications in place of these synthetic inhibitors **(Khan *et al*., 2019)**.

Plant growth-promoting fungi (PGPF) can directly stimulate plant growth by producing phytohormones like indole-3-acetic acid (IAA) and gibberellic acid **(Hashim *et al*., 2020)**. IAA is a hormone found in plants that is known to be essential in cell elongation division and development **(Khan *et al*., 2018)**. Together, endogenous plant indole-3-acetic acid and fungal indole-3-acetic acid have a synergistic reaction that promotes plant growth. Therefor the aim of this study is to collect different medicinal plants, isolate and identify fungal endophytes that survived inside different parts of plants (leaves, stems and roots), to investigate the distribution and diversity of endophytic fungal flora of plants. In addition to determining their antimicrobial, antidiabetic activities, and assay the capacity of these endophytes to produce indole acetic acid.

Material and Methods

Collection of medicinal plant samples

Ten medicinal plants were collected from MEPACO-MEDIFOOD (Arab Company for Pharmaceutical and Medicinal Plants), Inshas Al-Raml, Belbeis Center, Sharkia Governorate, Egypt. (Table, 1). Plant samples were mature, healthy, and did not have any disease symptoms based on the visual investigation of plant organs (leaves, stem, and root). Each plant sample was placed in a sterile bag and sealed well, then labeled and taken to the laboratory.

Table 1. Collected medicinal plants and their families.

Plant	Family
Ammi visanga	*Apiaceae*
Camellia sinensis	*Theaceae*
Datura stramonium	*Solanaceae*
Lavandula angustifolia	*Lamiaceae*
Melisa officinalis	*Lamiaceae*
Mentha piperita	*Lamiaceae*
Ocimum basilicum	*Lamiaceae*
Origanum majorana	*Lamiaceae*
Salvia officinalis	*Lamiaceae*
Tanacetum parthenium	*Asteraceae*

Isolation of fungal endophytes from medicinal plants

Following the collection of plant samples, plants were cleaned with running water. Using a sterile scalpel, the mid portions of leaves, stems, and roots were cut into equal pieces, including the midrib, and surface-sterilized by a series of solutions **(Khalil *et al*., 2021)**. The sterilized plant parts were divided, then four segments per plate were cultured on potato dextrose agar (PDA) and malt extract agar media (MEA), and incubated at a temperature of 28 °C. Every day, the plates were checked for fungal growth (**ALKahtani *et al*., 2020**).

Preparation of the plant extract

The leaves of *Ocimum basilicum* and stems of *Ammi visanga* extracts were obtained using a method similar to that reported by **(Talita *et al*., 2017)**.

Crude extract preparation of isolated endophytic fungi

Crude extract preparation of each fungal isolate was prepared using a method similar to that reported by (**An *et al*., 2020 and Khalil *et al*., 2021)** with a few minor modifications; cell-free supernatant (CFS) of fungal isolates was extracted by two solvents, ethyl acetate and petroleum ether.

Antimicrobial testing of endophytic fungal crude extracts

The antimicrobial screening was carried out using the well agar diffusion method against two human bacterial pathogens, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, and two human fungal pathogens, *Aspergillus fumigatus* MT 103088 and *Aspergillus chrysogenum* MT 103060 **(Yadav *et al*., 2010 and Fouda *et al*., 2020)**. According to the Clinical and Laboratories Standards Institute (CLSI), the bacterial inoculum was adjusted to 1-2 10^8 CFU/mL and to 1-5 10^6 CFU/mL for *Aspergillus* spp. **(Petrikkou *et al*., 2001).** On Muller-Hinton agar (MHA), the prepared inoculums of the tested pathogens were surface inoculated using sterile swaps, then wells (6 mm in diameter) were made per petri-dish and loaded with 100 µL of the following: ethyl acetate and petroleum ether extracts of endophytic fungi, a water solution of cefadroxil antibiotic (100 µg/mL) as a positive control for bacterial pathogens, or dimethyl sulfoxide itraconazole solution (100 µg/mL) as a positive control for fungal pathogens, and DMSO as a negative control. The inoculated plates were put in the refrigerator for 1 hour to allow diffusion of the extract, and then incubated for 24 h at 37 °C in the case of bacteria and at 28 °C for 48 h in the case of fungi. The assay was carried out in duplicate, and inhibition zones were measured (**Penido *et al*., 2013**).

Screening and quantification of Indole-3-Acetic Acid (IAA) production by endophytic fungal isolates.

The ability of isolated endophytic fungi to produce IAA was determined using a method similar to that reported by **(Ahmad *et al*., 2005)** with a few minor modifications. Following incubation, the cultures were filtered, and then 5 mL of each culture were centrifuged. 1 ml of supernatant was then combined with 2 mL of Salkowski's reagent. IAA generation was indicated by the emergence of a pink color **(Mehmood *et al*., 2018)**; optical density was determined at 530 nm using a spectrophotometer.

Production of anti-diabetic metabolites by endophytic fungal isolates

One disc (6 mm) of each fungal culture was inoculated in 50 ml of MEA media. After that, the flasks were kept in a shaker at 30 °C and 200 rpm for ten days. After that, ethyl acetate (50 ml) was added to each flask, and it was shaken one more at 150 rpm at 45 °C **(Singh and Kaur, 2016)**. The antidiabetic assay was carried out using the alpha-amylase inhibitory test **(Khan *et al*., 2019)**. Before adding the starch solution, blanks were prepared by adding DNSA reagent to denature the enzyme **(Kumari and Silva, 2018)**. As a positive control, metformin/acarbose was utilized **(Ahmed, 2018)**. This formula was used to calculate the inhibitory activity of alpha-amylase **(Kazeem *et al*., 2013)**.

%inhibition= [(Ac-Ae) /Ac] x 100

Where Ae stands for extract absorbance and Ac for control absorbance.

Identification of the endophytic fungal isolates

Based on the macroscopic and microscopic features of the fungal cultures, the most potent fungal isolates were recognized at the species level in accordance with **(Ellis, 1971 and 1976** & **De Hoog, 1995).**

Molecular identification of the most potent endophytic fungal isolates

Molecular identification of fungal isolates was carried out at the Molecular Biology Research Unit, Assiut University, according to **(Pitt & Hocking, 2009 and White *et al*., 1990)**.

Diversity of identified fungal isolates among different plant segments:

Three parameters including, colonization rate, isolation rate and colonization frequency, were determined according to the following equations **(Musavi and Balakrishnan, 2013)**.

Colonization rate (CR%)

$$= \frac{number\ of\ sgments\ colonized\ with\ isolated\ fungi}{total\ number\ of\ segment\ investigated} \times 100$$

Isolation rate (IR%)

$$= \frac{number\ of\ isolated\ fungi\ recovered\ from\ tissue}{total\ number\ of\ tissue\ segments} \times 100$$

Colonization frequency (CF%).

$$= \frac{\text{The number of plant segments colonized by a single endophyte}}{total\ number\ of\ segment\ \text{observed}} \times 100$$

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical composition of ethyl acetate and petroleum ether extracts of *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441, as well as the methanolic extracts of *Ammi visanga* stem and *Ocimum basilicum* leaves, were analyzed according to **(Hashem *et al*., 2022)**.

Results

Isolation and diversity of endophytic fungal isolates

A total of 80 fungal isolates were obtained using various media (PDA and MEA) from various sections: roots, stems, and leaves of ten medicinal plant samples. The highest number of endophytic fungal isolates (six isolates) were isolated from the leaves and root of *Datura stramonium*, the root of *Ammi visanga*, the stem of *Menthe piperita*, and the leaf of *Camellia sinensis*. Followed by leaves of *Salvia officinalis*, stems of *Lavandula angustifolia*, leaves and stems of *Ammi visanga*, leaves and stems of *Origanum majorana*, leaves of *Mentha piperita*, leaves and stems of *Melisa officinalis*, and leaves of *Tanacetum parthenium* with four or three isolates. The lowest number of fungal isolates (1 & 2 isolates) are obtained from tissues of the leaves and stem of *Ocimum basilicum*, the stem of *Salvia officinalis*, the stem of *Datura stramonium*, the leaves of *Lavandula angustifolia*, the stem of *Camellia sinensis,* and the stem of *Tanacetum parthenium* (Table 2).

Table 2. Endophytic fungi isolated from different tissues of medicinal plants.

Medicinal plant	Number of endopytic fungal isolates/ Plant tissues		
	Root	Stem	Leaves
Ocimum basilicum	_	1	2
Salvia officinalis	_	2	4
Datura stramonium	6	2	6
Ammi visanga	6	3	3
Lavandula angustifolia	_	4	2
Origanum majorana	_	4	3
Mentha piperita	_	6	4
Camellia sinensis	_	2	6
Tanacetum parthenium	_	1	4
Melisa officinalis	_	3	4

Antimicrobial testing of crude extracts of endophytic fungal isolates

The antimicrobial activity of the 80 endophytic fungal isolates were evaluated against four human pathogens; it was found that only 19 fungal isolates have antimicrobial activity, and isolate NO. 38 and isolate NO. 1 exhibited the most potent antimicrobial activity against the tested human pathogens (Table 3, Fig. 1 A-D and Fig. 2 A-D). Data revealed that the ethyl acetate extract of isolate NO. 1 is more effective on the tested pathogens. It had antibacterial activity on *Escherichia coli* and *Bacillus subtilis* with inhibition zones of 31 mm and 29 mm, respectively (Fig. 1A, 1B); also, it had antifungal activity only on *Aspergillus fumigatus* with inhibition zones of 23 mm (Fig. 1C). However, the petroleum ether extract was less effective; it had antifungal activity only on *Aspergillus fumigatus* with inhibition zones of 10 mm and had antibacterial activity against *E. coli* and *Bacillus subtilis* with inhibition zones of 10 mm and 12 mm, respectively (Fig. 1A).

On the other hand, the petroleum ether extract of isolate NO. 38 exhibited a wide range of activity. It had antifungal activity against *Aspergillus fumigatus* and *Aspergillus chrysogenum* with inhibition zones of 47 mm and 25 mm, respectively (Fig. 2C, D). Also, it exhibited antibacterial activity against *Bacillus* and *E. coli*, with inhibition zones of 22.5 mm and 25.3 mm, respectively (Fig. 2B, A). Ethyl acetate extract of isolate NO. 38 exhibited antibacterial activity only and was more efficacious on *Bacillus subtilis* than *Escherichia coli*, with inhibition zones of 55 mm and 37.6 mm, respectively (Fig. 2B, A).

Table 3. Antimicrobial activity of crude extracts of endopytic fungal isolates.

Isolated Endophytic Fungi	Diameter of inhibition zone (mm)							
	Tested pathogens / Extracts							
	Aspergillus chrysogenum		*Aspergillus fumigatus*		*Bacillus subtilis*		*Escherichia coli*	
	Et.	Pet.ether	Et.	Pet. ether	Et.	Pet. ether	Et.	Pet. ether
Isolate NO. 1	- ve	- ve	23	10	29	12	31	10
Isolate NO. 38	- ve	25	- ve	47	55	22.5	37.6	25.3

Et: Ethyl acetate, Pet: Petroleum

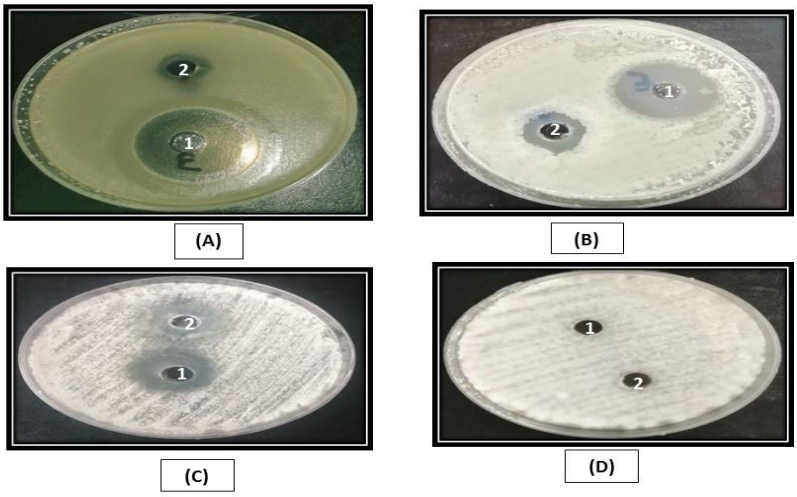

Fig. 1. Antimicrobial activity of isolate NO. 1on (A) *E.coli*, (B) *Bacillus subtilis*, (C) *Aspergillus fumigatus* (D) *Aspergillus chrysogenium* (1) Ethyl acetate (2) Petroleum ether

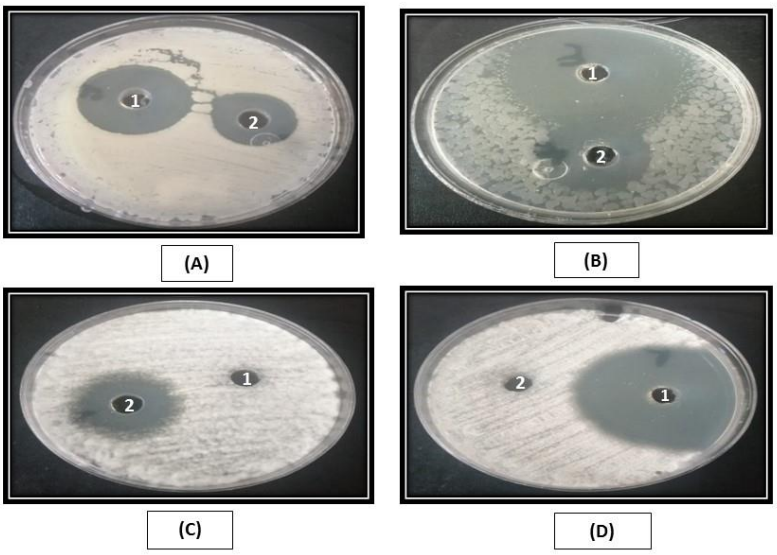

Fig. 2. Antimicrobial activity of isolate NO. 38 on (A) *E.coli*, (B) *Bacillus subtilis*, (C) *Aspergillus chrysogenium*, (D) *Aspergillus fumigatus*, (1) Ethyl acetate, (2) Petroleum ether

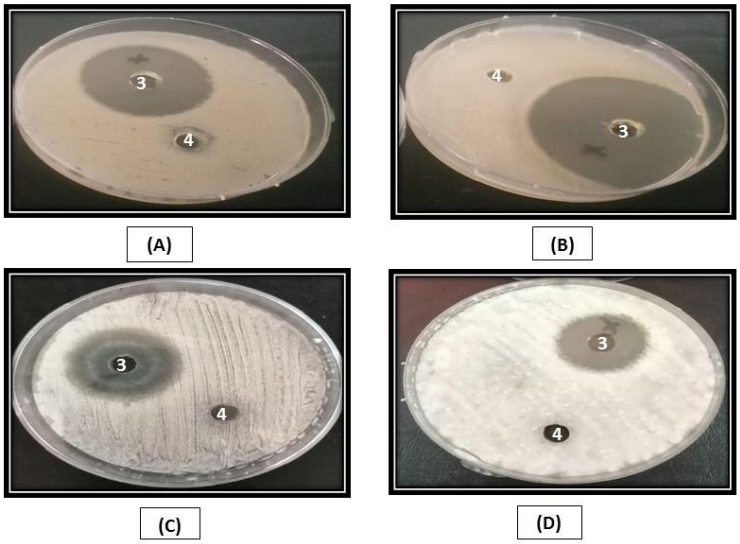

Fig. 3. Positive control (A) on *E.coli*, (B) on *Bacillus subtilis*, (C) on *Aspergillus fumigatus* (D) on *Aspergillus chrysogenium* (3) Positive control (4) Negative control

Indole acetic acid production

The result showed that 25 endopytic fungal species were able to produce IAA and give a pink color when adding Salkowski's reagent and the analysis of the result revealed a variation in IAA production among endophytic fungal isolate species. Isolate NO. 38 and isolate NO. 1 were the most active isolates that were able to synthesize high concentrations of IAA with concentrations of 9.62 µg/mL and 5.7 µg/mL, respectively.

Alpha amylase inhibition assay

The endophytic fungal extracts were evaluated for their anti-diabetic properties using the α-amylase inhibition assay. The results demonstrated that the extract of isolate NO. 38 showed the highest percentages of alpha-amylase inhibition, with a percentage of 94%, followed by isolate NO. 1 with a percentage of 82%, compared to the synthetic standard drug (metformin 500 mg), which shows only 40% inhibition.

From all results of the previous tests, only seven endophytic fungal isolates exhibited antimicrobial activity, were able to produce indole acetic acid, and had antidiabetic activity (table 4). Two of them, isolate No. (1) and

isolate No. (38), were the most potent isolates, and hence they were selected for further experiments (identification and GC mass analysis).

Table 4. Endophytic fungal isolates exhibited antimicrobial activity, produced indole acetic, acid and had antidiabetic activity.

Isolated Endophytic Fungi	Antimicrobial activity Diameter of inhibition zone (mm)								IAA Production (µg/mL)	Antidiabetic Activity (Percent)
	Tested pathogens / Extracts									
	A. chrysogenum		*A.fumigtaus*		*B. subtilis*		*E.coli*			
	Et.	Pet.ether	Et.	Pet. ether	Et.	Pet. ether	Et.	Pet. ether		
Isolate No. (1)	- ve	- ve	23	10	29	12	31	10	5.7	82%
Isolate No. (38)	- ve	25	- ve	47	55	22.5	37.6	25.3	9.62	94%
Isolate No. (72)	- ve	-ve	-ve	-ve	40	- ve	29	- ve	11.08	39%
Isolate No. (42)	- ve	- ve	- ve	13.3	12	- ve	- ve	- ve	0.3	76%
Isolate No. (48)	- ve	- ve	- ve	- ve	20	- ve	13	- ve	1.57	31%
Isolate No. (35)	-ve	-ve	-ve	36	-ve	-ve	-ve	- ve	1.66	20%
Isolate No. (29)	- ve	- ve	- ve	- ve	35	10	24.8	- ve	3	54%

A: Aspergillus, Et: Ethyl acetate, Pet: Petroleum, B: Bacillus, E: Escherichia

Identification of the most potent endophytic fungal isolates

Fungal endophytes were identified based on their morphological and cultural characteristics. Fungal isolate NO.1, isolated from the leaves of *Ocimum basilicum*, was identified as *Alternaria alternata*. It was expanding quickly on MEA media, powdery or cottony, grey to olivaceous black, and dark grey to black in reverse (Fig. 4A). Microscopy examination of slide culture on PDA revealed the presence of simple or branched, straight or flexuous, curved, and occasionally geniculate conidiophores and one or more conidial scars. They are pale to mid-brown in color. The conidia are obclavate, ovoid, or elliptical, formed in chains, straight or branched, and typically have a short, cylindrical beak that is no longer than one-third the length of the conidium. They can be smooth or verruculose, with one or two longitudinal or oblique septa and one to eight transverse septa (Fig. 4B). Molecular identification verified its identity as *Alternaria alternata* AUMC16440 and deposited in "GeneBank" under accession number (PP564749) (Fig. 4C).

The second potent isolate (NO.38) was isolated from *Ammi visanga* stem; it was identified as *Curvularia pseudobrachyspora*; it appears as dark black colonies on PDA with complete floccose edges (Fig. 5A). Under microscopy, the conidia were straight or slightly curved, clavate, ellipsoid or fusiform, smooth, with an inconspicuous hilum. Conidia measure and are usually 2-4 septate (mainly 3). Conidiogenous cells have thicker and darker conidial scars that are integrated, terminal or intercalary, geniculate, and sympodial. Brown, macronematous conidiophores (fig. 5B). Molecular identification verified its identity as *Curvularia pseudobrachyspora* AUMC16441 and deposited in "GeneBank" under accession number (PP564982) (Fig. 5C).

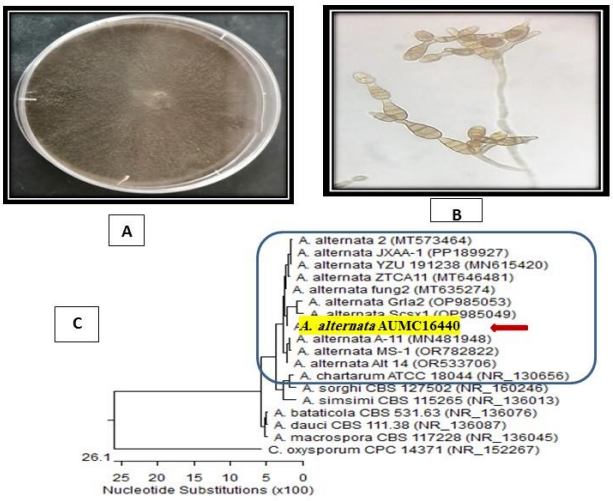

Fig. 4. Identification traits of *Alternaria alternata*. (A)Macroscopic morphology on MEA, (B) Microscopic morphology showing conidia and conidiophores ،(C) Phylogenetic tree of *Alternaria alternata* AUMC16440

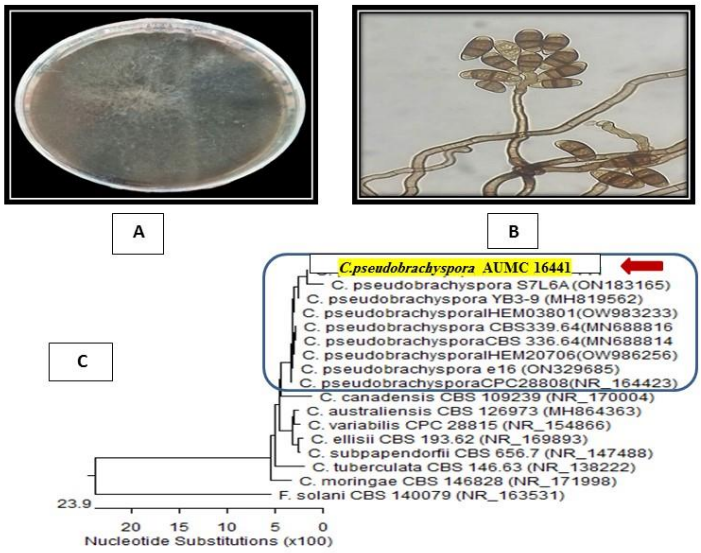

Fig. 5. Identification traits of *Curvularia pseudobrachyspora*. (A)Macroscopic morphology on MEA, (B) Microscopic morphology showing conidia and conidiophores, (C) Phylogenetic tree of *Curvularia pseudobrachyspora* AUMC16441

Diversity of endophytic fungal species among different plant segments.

The results revealed that twelve fungal species were isolated from 24 segments of the *Ammi visanga* plant, and two fungal species were isolated from 16 segments of *Ocimum basilicum*. The root of *Ammi visanga* was the most colonized tissue. On the other hand, the leaves of *Ocimum basilicum* were the most colonized tissue (table 5). As shown in Table 6, *Alternaria alternata* was the most frequently isolated fungal species from *Ammi visanga*as; it was found in the root, leaf, and stem tissues. While, *Fusarium oxysporium* was isolated from leaf and stem tissues, *Curvularia pdeudobrachyspora*, *Ulocladium botrytis***,** and *Nigrospora sphaerica* were only isolated from stem, leaf, and root tissues, respectively. Regarding *Ocimum basilicum*, *Alternaria alternata* and *Alternaria tennnuisima* were the most frequent isolated species, followed by *Nigrospora sphaerica*.

Table 5. The isolation rate (IR%) and colonization rate (CR%) of endopytic fungal species from different *Ocimum basilicum* and *Ammi visanga* tissues.

Plants	***Ammi visanga***					***Ocimum basilicum***		
Parameter	Stem	Leave	Root	Total		Stem	Leave	Total
Total number of segments	8	8	8	24		8	8	16
No. of segments colonized by fungi	4	5	8	17		1	6	7
No of fungal isolates	3	3	6	12		1	2	3
Colonization rate (CR%)	50%	63%	100%	71%		12.5%	75%	44%
Isolation rate (IR%)	0.38	0.38	0.75	0.5		0.13	0.25	0.19

Table 6. Endophytic fungal species recovered from different parts of medicinal plants.

Endophytic fungal species	***Ammi visanga***					
	Leaf	**Colonization frequency %**	**Stem**	**Colonization frequency%**	**Root**	**Colonization frequency%**
Alternaria alternata	**2**	**25**	**1**	**12.5**	**1**	**12.5**
Ulocladium botrytis	**1**	**12.5**	**_**	**_**	**_**	**_**
Nigrospora sphaerica	**_**	**_**	**_**	**_**	**5**	**62.5**
Fusarium oxysorium	**_**	**_**	**1**	**12.5**	**2**	**25**
Curvularia pdeudobrachyspora	**_**	**_**	**1**	**12.5**	**_**	**_**
	Ocimum basilicum					
Endophytic fungal species	**Leaf**	**Colonization frequency %**	**Stem**	**Colonization frequency%**		
Alternaria alternata	**3**	**37.5**	**_**	**_**		
Alternaria tennnuisima	**3**	**37.5**	**_**	**_**		
***Nigrospora sphaerica*.**	**_**	**_**	**1**	**12.5**		

Analysis of fungal and plant extracts by (GC-MS) analysis

The GC-MS analysis of the petroleum ether extract of *Curvularia pseudobrachyspora* AUMC16441 showed sixty-six compounds, and the main organic components were hexane,2,4-dimethyl (16.79%), hexadecanoic acid (1.17%), and pentadecane (0.93%) (Table 7, Fig. 6B).

The ethyl acetate extract of *Curvularia pseudobrachyspora* AUMC16441 contains forty-four compounds. The major components were phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl- (0.69%) and 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.25%) (Table 8, Fig. 7B).

The chemical constituents obtained from the petroleum ether extract of *Alternaria alternata* AUMC16440 consists of fifty-seven compounds; of them, nonane,3,7-dimethyl- (18.33%), 13-docosenamide,(z)- (4.40%), tetradecane (1.26%), and ethyl iso-allocholate (1.01) were the most predominant (Table 9, Fig. 6A).

On the other hand, the chemical constituents obtained from the ethyl acetate extract of *Alternaria alternata* AUMC16440 consist of fifty-two compounds (Table 10, Fig. 7A). The main compounds were 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (0.32%), cyclononasiloxane octadecamethyl (0.09%), and cyclooctasiloxane hexadecamethyl (0.06%).

The petroleum ether extract and ethyl acetate extract of *Alternaria alternata* AUMC16440 have components similar to those present in the extract of the *Ocimum basilicum* leaves; some of these compounds are (Z)-1-Chloro-2-(methylsulfonyl)ethylene, Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo-, methyl ester,(16à)-, desulphosinigrin, and malonic acid,6-heptynyl (table 13). In addition, the petroleum ether extract and ethyl acetate extract of *Curvularia pseudobrachyspora* AUMC16441 have components similar to those present in the methanol extract of *Ammi visanga* stem, such as **3**,6-Dimethoxy-2,5-dinitrobenzaldehydeoxime, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, and 1H-purin-6-amine,[(2-fluorophenyl)methyl]- (table 14).

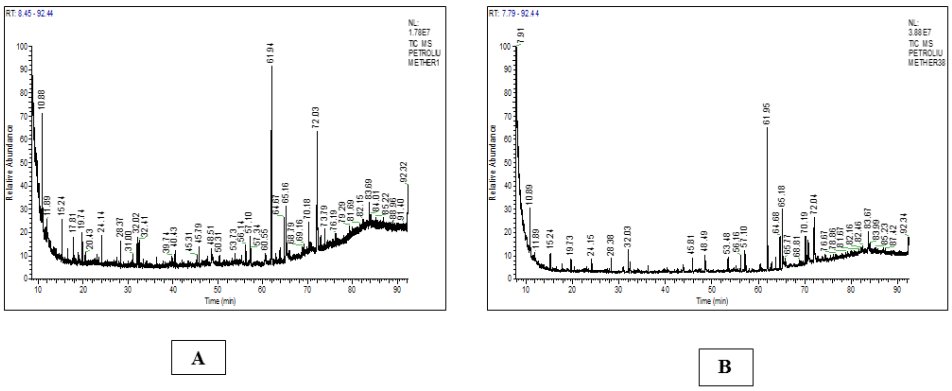

Fig. 6. GC-MS chromatogram of petroleum ether extract of A: *Alternaria alternata* AUMC16440, B: *Curvularia pseudobrachyspora AUMC16441*

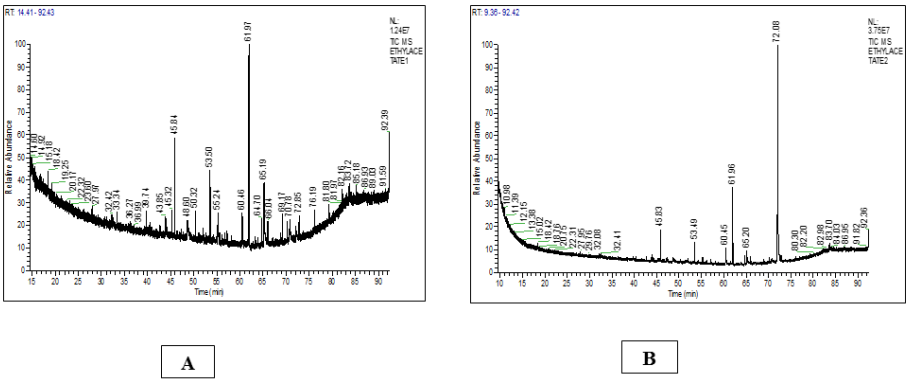

Fig. 7. GC-MS chromatogram of Ethyl acetate extract of A: *Alternaria alternata* AUMC16440, B: *Curvularia pseudobrachyspora AUMC16441*

Table 7. Phytocomponents identified from the petroleum ether extract of *Curvularia pseudobrachyspora* by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
Bicyclo[3.2.1]octan-2-one,1-(1-propenyl)-	7.28	4.38
Hexane,2,4-dimethyl	7.38	16.79
Nonane,3,7-dimethyl	7.92	2.95
1-Decanol, 2-ethyl-	9.69	0.59
1-Octanol,3,7-dimethyl	10.10	0.55
1-Dodecanol,3,7,11-trimethyl	10.20	0.38
1-dodecene antibacterial (pdf decene)	10.50	0.28
Decane	10.89	2.81
1-hexadecanol,2-methyl(in 11.48)	11.62	0.40
3-hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	11.89	0.75
2-Decen-1-ol, (E)-	12.67	0.19
1-tetradecanol	13.36	0.20
Undecane	15.24	1.00
Ethanol,2-(octadecyloxy)-	16.51	0.36
Levomenthol	17.80	0.55
methyl-2,3,4-tri-o-trideuteromethyl-á-d-arabopyranoside	18.90	0.18
Dodecane	19.74	0.68
Decane,1,1'-oxybis	22.59	0.24
Undec-10-ynoic acid,dodecyl ester	23.05	0.26
Hexadecane	24.14	0.65
4-Nonene, 5-butyl-	27.93	0.27
Tetradecane	28.38	0.76
4,6,6-trimethyl-2-(3-methyl-buta-1,3-dienyl)-3-oxa-tricyclo[5.1.0.0 2,4]octane	30.76	0.22
Methyl4,4,7-trimethyl-4,7-dihydroindan-6-carboxylate	32.03	1.23
Pentadecane	32.41	0.93
Docosane	36.27	0.35
Phenol,3,5-bis(1,1-dimethylethyl)-4-nitro	38.66	0.20
Pregnane-3,11,20,21-tetrol,cyclic 20,21 – (butylboronate),(3à,5á,11á,20R)-	39.31	0.16
Sarreroside	39.74	0.18
Ethanol,2-(hexadecyloxy)	40.43	0.26
Methyl 3-(2,'4-'dihydroxyphenyl)prop-2-enoate	42.68	0.24
2(3H)-Furanone,3-(2-bromoethyl)dihydro-	43.80	0.46
8,8-dicyano-9-n-butyl-isoquinolino[1,2a]pyrrole	43.92	0.33
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.81	0.84
Phthalic acid,2-cyclohexylethylisobutyl ester	47.25	0.16
Dotriacontane	47.66	0.20
n-Hexadecanoic acid	48.50	1.17
2,8,9-Trioxa-5-aza-1-silabicyclo[3.3.3]undecane, 1-ethyl	53.49	1.01
Palmitic acid,(2-tetradecyloxy)ethyl ester	54.70	0.22
Glycine,N-(N-glycyl-L-leucyl)-	55.26	0.39
2,4-dimethyl-6-(phenylamino)-1H,2H-phthalazin O[2',1':3,4]pyrimido[4,5-D]pyrimidine-1,3-(2H,4H)-dione	55.60	0.22
Acetic acid n-octadecyl ester	56.15	1.01
N,N-dimetylpalmitamide	57.10	1.45
Methyl2-[2-(2-ethyl-1,3-dioxolan-2-yl methyl)-1-hydroxypent-4-enyl]-4-methylfuran-3-caboxylate	57.34	0.38
4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	60.44	0.49
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	60.53	0.19
Oleic Acid	60.76	0.29
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl	61.96	9.63
6-isopropenyl-4,8a-dimethyldecahydro-1-naphthalenol#	62.51	0.26
17-Pentatriacontene	62.88	0.35
2-Nonadecanone,O-methyloxime	63.82	0.79
3-Hydroxypropylpalmitate, TMS derivative	64.68	2.06
Hexadecanoicacid,[(3-trimethylsilyl)oxy]propylester	65.18	3.78
8-Dodecen-1-ol,acetate, (Z)-	65.77	0..37

4h-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	66.03	0.30
octadecanoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester	70.19	1.56
Undec-10-ynoic acid,pentadecyl este	70.50	0.36
Octadecanoic acid,2,3-dihydroxypropyl ester	70.73	1.44
13-Docosenamide,(Z)-	72.04	2.82
03027205002 flavone 4'-OH,5-OH,7-DI-OGLUCOSIDE	73.72	0.40
9-Octadecenoic acid (Z)	78.08	0.17
Hahnfett	78.82	0.52
2-Nonadecanone 2,4-dinitrophenylhydrazine	80.70	0.34
á-Sitosterol	83.67	1.28
Spirost-8-en-11-one,3-hydroxy-,(3á,5à,14á,20á,22á,25R)-	83.99	0.48
Ethyl iso-allocholate	86.93	0.17
5,11,17,23-tetrakis(1,1-dimethylethyl)-28-methoxypentacyclo[19.3.1.1(3,7).(9,13).1(15,19)19] octacosa-1(25),3,5,7(28),9,11,1,3(27),15,17,19(26),2,1,23-dodecene 25,26,27-triol	92.34	0.93

Table 8. Phytocomponents identified from the ethyl acetate extract of *Curvularia pseudobrachyspora* by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
Dimethylsulfoxonium formylmethylide	32.08	0.03
3,4-Dihydro-2h-1,5-(3"-t-butyl) benzodioxepine	32.53	0.02
(1-Bromo-ethanesulfinyl)-ethane	34.15	0.02
Acetaldehyde,(2,4-dinitrophenyl) hydrazine	38.71	0.01
Malonic Acid,6-Heptynyl	41.93	0.01
2-(3-carboxy-propionylamino-(7-oxo-4,5,6,7-tetrahydro-benzo [b]thiophene-3-carboxylic acid ethyl ester	42.69	0.03
2(3H)-Furanone,3-butyldihydro	43.84	0.03
cis-Trismethoxyresveratrol	43.93	0.06
3,6-Dimethoxy-2,5-dinitrobenzaldehydeoxime	44.27	0.02
1H-purin-6-amine,[(2-fluorophenyl) methyl]-	45.32	0.01
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.83	0.25
Phthalic acid, butylundecyl ester	47.26	0.04
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo,-methyl ester,(16à)-	48.63	003
2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole	48.70	0.01
Hexadecanoic acid (fatty acid)	48.79	0.01
Glycine,N-[(3à,5á)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl,-[methyl ester	50.33	0.02
ethanol,(2-hexadecyloxy)-	52.00	0.03
2,8,9-Trioxa-5-aza-1-silabicyclo[3.3.3]undecane, 1-ethyl	53.50	0.15
08217205002 flavonol3',4',5,7 -OH,3-O-araglucoside	55.27	0.06
Palmitic acid,2-(tetradecyloxy)ethyl ester	56.17	0.03
2-Nonanone,O-methyloxime	57.13	0.05
N-[4,5-Dimethoxy-2-(morpholin-4-yl)phenyl]pyridine-3-carboxamide	58.11	0.02
Pentylidenetriphenylphosphorane-boran	60.45	0.13
Octadecane,3-ethyl-5-(2-ethylbutyl)-	61.46	0.02
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl-	61.96	0.69
Octadecanoic acid,17-hydroxy,-methyl ester	62.87	0.02
7-[(4'-nitrophenyl)ethynyl]-2-[-(4-'methoxyphenyl)ethynyl]bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	63.26	0.01
2-nonenoic acid,9-(dimethylamino)-7-hydroxy-2-methyl-9-oxo-, methyl ester,(E)-	63.84	0.02
Hexadecanoic acid,3-[(trimethylsilyl)oxy]propylester	64.68	0.07
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	65.19	0.15
1,2-benzenedicarboxylic acid	66.05	0.07
s-(tert-butyl)[2-(5-hydroxy-2-pentynyl)-3-oxocyclopentyl] ethanethioate	67.08	0.01
(22S)-21-Acetoxy-6à,11á-dihydroxy-16à ,17à-propylmethylenedioxypregna-1,4-diene-3,20-dione	68.80	0.03
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	69.18	0.02

4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	70.19	0.04
Ethyl iso-allocholate	70.78	0.03
13-Docosenamide,(Z)	72.08	1.77
4H-1-benzopyran-4-one,2-(3,4- dihydroxyphenyl) -3,5-dihydroxy-7-methoxy	72.81	0.05
9,12,15-octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester,(Z,Z,Z)-	76.19	0.02
Spirost-8-en-11-one,3-hydroxy-,(3á,5à,14á,20á,22á,25R)-	79.29	0.02
4H-1-benzopyran-4-one,2-(3,4- dihydroxyphenyl) -6,8-di-á-d-glucopyranosyl-5,7-dihydroxy	82.43	0.02
9,12-octadecadienoic acid(z,z,)-,2,3-bis[(trimethylsilyl)oxy]propyl ester	82.96	0.02
á-Sitosterol	83.70	0.07
9-octadecenoic acid (z),-2-[(trimethylsilyl)oxy]-1- [(trimethylsilyl)oxy] methyl] ethyl ester	89.04	0.01
5,11,17,23-tetrakis-(1,1-dimethylethyl)-25,27-dihydroxy-26,28-bis-[2-(methylsulfanyl)-ethoxy]-calix-[4]-arene	92.36	0.09

Table 9. Phytocomponents identified from the petroleum ether extract of *Alternaria alternata* by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
Nonane,3,7-dimethyl-	7.34	18.33
Hexane, 3-methyl-	8.87	0.30
1-octanol,2,7-dimethyl-	9.62	0.24
Silane,trichlorodocosyl	9.71	0.62
1-hexadecanol,2-methyl-	10.09	1.32
1-decene	10.50	0.48
Decane	10.88	3.41
Hexadecane,1,1-bis(dodecyloxy)-	11.49	0.26
Octadecane,1-(ethenyloxy)-	11.60	0.49
3-Trifluoroacetoxypentadecane	12.16	0.28
2-tetradecyloxirane	12.67	0.29
Tetradecane	15.24	1.26
7-Hexadecenal, (Z)-	16.50	0.49
Levomenthol	17.80	1.01
Chloroethyl 2-hexylether	18.88	0.37
Dodecane	19.74	0.97
Octadecane,1-chloro	20.42	0.29
2-myristynoylpantetheine	23.05	0.31
Tridecane	24.14	0.83
Tetrahydroisovelleral	30.75	0.20
Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-	32.03	1.10
Docosane	32.41	0.98
Ethanol,2-(hexadecyloxy)-	34.14	0.25
Cycloheptasiloxane,tetradecamethyl-	39.74	0.26
Pentacosane	40.43	0.49
3-oxo-20-methyl-11-à-hydroxyconanine-1,4-diene	43.92	0.20
Cyclononasiloxane,octadecamethyl	45.31	0.25
7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.81	0.65
4-methoxycarbonylmethylundec-3-enedioic acid, dimethylester	47.25	0.19
Dotriacontane	47.64	0.35
Hexadecanoicacid,2,3-dihydroxypropyl ester	48.51	0.69
1H-purin-6-amine, [(2-fluorophenyl)methyl]-	50.31	0.33
9-octadecenoicacid (z)-	54.74	0.24
1-Hexadecanol,acetate	56.15	0.74
N,N-dimetylpalmitamide	57.10	1.07
Indazolo[2,3-a]quinoline,2,3,8,9-tetramethoxy-	57.34	0.53
1H-purin-6-amine, [(2-fluorophenyl)methyl]-	60.54	0.50
Oxiraneundecanoicacid, 3-pentyl,-methyl ester, trans-	60.76	0.22
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl-	61.94	7.51
17-pentatriacontene	62.88	0.36
2-Nonadecanone,O-methyloxime	63.82	0.56
Hexadecanoicacid,[(3-trimethylsilyl)oxy]propylester	64.67	1.58

Hexadecanoicacid,[(2-trimethylsilyl)oxy]-1,3-propanediyl ester	65.16	2.19
4H-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	65.77	0.28
4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-Triene	70.18	1.03
13-docosenamide,(z)-	72.03	4.40
4h-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	72.84	0.48
11,14-Eicosadienoicacid, methyl ester	73.79	0.64
03027205002 flavone 4-'oh,5-oh,7-di-o-glucoside	78.06	0.16
Hahnfett	79.90	0.24
Ethyl iso-allocholate	83.67	1.01
Spirost-8-en-11-one,3-hydroxy-,(3á,5à,14á,20á,22á,25r)-	84.00	0..39
9,12-octadecadienoic acid(z,z)-,2,3-bis[(trimethylsilyl)oxy]propyl ester	85.19	0.33
Tris(2,4-di-tert-butylphenyl) phosphate	92.32	1.51
Nonane,3,7-dimethyl-	7.34	18.33

Table 10. Phytocomponents identified from the ethyl acetate extract of *Alternaria alternata* by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl	32.09	0.03
(Z)-1-Chloro-2-(methylsulfonyl)ethylene	32.55	0.03
Cycloheptasiloxane,tetradecamethyl	33.33	0.04
Cyclooctasiloxane,hexadecamethyl-	39.74	0.06
Desulphosinigrin	40.45	0.02
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo,-methyl ester,(16à)-	40.72	0.02
2-(3-carboxy-propionylamino)-7-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylicacid ethyl ester	42.70	0.02
Diethyl4-hydroxypyridin-2,6-dicarboxylate	43.83	0.05
2,6-dimethylthieno[2,3-d]pyrimidine-4-carbaldehyde(2,4-dinitrophenyl)hydrazine	43.93	0.06
2,7-diphenyl-1,6-dioxopyridazino[4,5:2,' 3'] pyrrolo[4',5'd]pyridazine	44.28	0.02
Cyclononasiloxane,octadecamethyl	45.32	0.09
7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.84	0.32
Cyclopropanepropionic acid,2-[(2-decylcyclopropyl) methyl]-, methyl ester	47.27	0.02
Ethanol,(2-hexadecyloxy)-	47.65	0.02
Hexadecanoic acid,2,3-dihydroxypropyl ester	48.60	0.09
D-Fructose, diethylmercaptal,pentaacetate	48.79	0.03
Methanone,[4-[[4-methyl-5-(phenylmethyl)-4h-1,2,4-triazol-3-yl]thio]-3-nitrophenyl]2-pyridinyl	51.07	0.03
2-nonadecanone2,4-dinitrophenylhydrazine	52.01	0.04
2,8,9-trioxa-5-aza-1-silabicyclo[3.3.3]undecane, 1-ethyl	53.50	0.25
Oxiraneundecanoic acid,3-pentyl,-methyl ester,cis-	54.20	0.03
1h-purin-6-amine,[(2-fluorophenyl)methyl]-	55.24	0.13
Propane,1,3-bis(dicyclohexylphosphinyl)-	55.61	0.03
2,2-dideuterooctadecanal	56.16	0.02
Malonic acid,6-heptynyl-	56.66	0.03
Tridecanedioic acid,2-hexyl-, dimethylester	57.14	0.05
4,2-Cresotic acid,6-methoxy-, bimol.ester, methyl ester,4,6-dimethoxy-o-toluate	60.45	0.10
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl-	61.98	0.75
7-[(4'-nitrophenyl)ethynyl]-2-[-(4'-methoxyphenyl)ethynyl]bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	63.26	0.03
2-heptadecanol,acetate	63.84	0.02
Hexadecanoicacid,3-[(trimethylsilyl)oxy]propylester	64.70	0.09
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	65.19	0.26
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	66.05	0.08
2-Nonadecanone 2,4-dinitrophenylhydrazine	68.82	0.04
4-hexyl-1-(7-methoxycarbonylheptyl)bicyclo[4.4.0]deca-2,5,7-triene	70.21	0.06
Octadecanoic acid,2,3-dihydroxypropyl ester	70.78	0.09
9-octadecenamide	72.07	0.04
4H-1-BENZOPYRAN-4-ONE,2-(3,4-DIMETHOXYPHENYL)-3,5-DIHYDROXY-7-METHOXY-	73.72	0.02
Cyclodecasiloxane,eicosamethyl-	76.20	0.07

9,12-octadecadienoic acid(z,z)-,2,3-bis[(trimeth ylsilyl)oxy]propyl ester	82.16	0.05
4h-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	83.06	0.03
Ethyl iso-allocholate	83.70	0.07
5,11,17,23-tetrakis(1,1-dimethylethyl)-28-methoxypentacyclo[19.3.1.(3,7).1(9,13).1(15,19)]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecene-25,26,27-triol	92.37	0.07

Table 11. Phytocomponents identified from the ethanolic extract of *Ammi visanga* stem by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
Bis(methylsulfonyl) methane	9.45	0.28
Malonic acid,6-heptynyl-	28.00	0.02
Nitrazepam	32.56	0.02
21h,23h-porphine-5,10,15,20-d4,2,7,12,18-tetra(ethyl-d5)-3,8,13,17tetra(methyl-d3)-	33.33	0.03
8,11-Octadecadiynoic acid, methyl ester	37.69	0.01
Cyclohexanone,2-(2-furanylmethylene)-6-methyl-	39.18	0.06
1,3-bis(trimethylsilyloxy)-2-trimethylsilyla mino-3-[3',4'-bis(trimethylsilyloxy)phenyl]-propanone	39.75	0.03
2-(3-carboxy-propionylamino)-7-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethylester	42.71	0.02
2,6-dimethylthieno[2,3-d]pyrimidine-4-carbaldehyde(2,4-dinitrophenyl)hydrazine	43.94	0.04
(5à,6à)-4,5-epoxy-6-methoxy-17-propyl-3á-phthalimidomorphinan	45.33	0.04
5-isopropenyl-3,6-dimethyl-6-vinyl-4,5,6,7-tetrahydro-1-benzofuran#	45.65	0.04
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.85	0.12
3,6-Dimethoxy-2,5-dinitrobenzaldehydeoxime	46.83	0.01
Tricyclo[4.3.1.1(3,8)]undecane-3-carboxylic acid	47.27	0.02
n-Hexadecanoic acid	48.54	0.21
Hexadecanoic acid,2,3-dihydroxypropyl ester	48.80	0.01
Visnagin	48.98	0.46
Methoxsalen	49.68	0.10
Octanal,2-(phenylmethylene)-	50.02	0.05
1h-purin-6-amine,[(2-fluorophenyl)methyl]-	50.34	0.03
Phenol,4 -(1,1-dimethylethyl)-2,6-dinitro-	53.19	0.01
4-chloro-2,5-dimethoxyphenethylamine,n,n-bis(trimethylsilyl)-	53.51	0.09
2H-Pyran,2 -(2-heptadecynyloxy)tetrahydro	54.33	0.01
5,8A-dimethyl-3-methylene-3a,7,8,8a,9,9a-hexahydro-3h-naphtho[2,3-b]furan-2-one	55.03	0.08
9-Octadecenoic acid,(2-phenyl-1,3-dioxolan-4-yl)methyl ester,cis	55.26	0.01
5-amino-2-(p-methoxymethyl)-2-methyl-2h-[1,2,4]triazolo[1,5-a][1,3,5]triazine	55.45	0.05
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo,-methyl ester, (16à)-	58.13	0.02
6-Methyl-11-propenyl-5-(toluene-4-sulfonyloxy)-12,13-dioxatricyclo[7.3.1.0(1,6)]tridecane-8-carboxylic acid, methyl ester	59.01	0.02
4,2-Cresotic acid,6-methoxy-, bimol.ester, methyl ester,4,6-dimethoxy-o-toluate	60.46	0.05
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	60.56	0.02
2á,9à-dihydroxyverrucosane	61.36	0.03
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl	62.00	0.08
N,N-bis(trimethylsilyl)-2-phenyl-7-(trifluoromethyl)quinolon-4-amine	63.26	0.02
3,17-dioxo-11-à-hydroxyandrostane-1,4-diene	63.75	0.03
3-methyl-but-2-enoic acid2,2-dimethyl-8-oxo-3,4-dihydro-2h,8h-pyrano[3,2-g]chromen-3-yl ester	64.71	0.11
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	65.20	0.11
4H-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy	66.06	0.03
Benzo[e](1h)indene,1,2,3a,4,5,9b-hexahydro-7-methoxy-3-oxo-3a,9b-dimethyl-	66.48	0.11
9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	67.70	0.02
8-methyl-2-oxo-2h,8h-benzo[1,2-b:3,4-b']dipyran-9,10-diyl-10-acetate-9-(2-methylbutyrate)	69.16	2.76

(Z)-Cnidimine	69.67	0.05
6á,19-Cyclo-5à-androstane-3,17-dione	70.85	0.08
9,12,15-octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester,(z,z,z)-	72.09	0.04
9,12-octadecadienoic acid(z,z)-,2,3-bis[(trimethylsilyl)oxy]propyl ester	72.85	0.03
1,25-Dihydroxyvitamin D3, TMS derivative	78.74	0.02
9-octadecenoic acid (z),-[(2-trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	82.17	0.02
Stigmasterol	82.46	0.14
Ethyl iso-allocholate	83.71	0.08
4H-1-benzopyran-4-one,2- (3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	85.19	0.02
5,11,17,23-tetrakis(1,1-dimethylethyl)-28-methoxypentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)] Octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26)2,1,23-dodecene-25,26,27-triol	92.40	0.02

Table 12. Phytocomponents identified from the ethanolic extract of *Ocimum basilicum* leaves by GC-MS analysis.

Phytochemical Compound	Retention Time (min)	Area %
3Phosphonoalanine	8.74	0.28
(Z)-1-Chloro-2-(methylsulfonyl)ethylene	14.82	0.07
N,N'-bis-(3-methyl-but-2-enylidene)-1,2-di-2-hydroxyphenyl-ethane-1,2-diamine	15.58	0.07
DL-Leucine,N-[2-(chloroimino-(1-oxopropyl]-	17.69	0.05
8-azabicyclo[3.2.1] octane-2-carboxylic acid,3-(benzoyloxy)-8-methyl-,methyl ester,[1r-(exo,exo)]-	18.96	0.05
3-Cyclohexene-1-propanal	21.06	0.03
1 ,4,7-triazaheptane, 1,7-bis(1-methyl-1-phosphonato)ethyl-	21.84	0.03
3,5-Heptadienal,2-ethylidene-6-methyl-	23.80	0.06
2-Methoxy-5-nitrophenol,chlorodifluoroacetate	24.98	0.03
Eugenol	25.60	0.17
2-Propenoic acid,3-phenyl-, methylester	26.40	0.47
Methyleugenol	27.47	15.27
Desulphosinigrin	29.96	0.04
2-aminoethanethiol hydrogensulfate (ester)	30.06	0.03
Benzeneethanamine,2,5-difluoro-á,3,4-trihydroxy-N-methyl-	30.64	0.02
Bergenin	31.00	0.07
2,6-Dihydroxybenzaldehyde,carbamoylhydrazone	31.10	0.06
3-buten-2-one,4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-	31.54	0.03
9,10-secochola-5,7,10(19)-triene-3,24-diol,(3á,5z,7e)-	32.60	0.04
2-Butanone,(2,4-dinitrophenyl)hydrazine	33.34	0.02
4,5-Dimethoxy-6-[2-nitroethenyl]-2H-1,3-benzodioxole	33.69	0.02
Methyl 2,4-tridecadiynoate	34.22	0.02
Cyclopenta[1,3]cyclopropa[1,2]cyclohepten-3(3aH)-one,1,2,3b,6,7,8-hexahydro-6,6-dimethyl-	35.88	0.06
.tau.-Cadinol	36.99	0.11
1-(4-isopropylphenyl)-2-methylpropylacetate	37.68	0.04
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo,-methyl ester,(16à)-	38.12	0.02
1-(3-Methyl-2-butenyl)-3,6-diazahomoadamantan-9-ol	38.69	0.02
Sarreroside	39.76	0.03
Malonic acid,6-heptynyl-	40.25	0.05
Methyl 2,4-tridecadiynoate	41.03	0.02
8,11-Octadecadiynoic acid, methyl ester	41.43	0.04
Acetamide,N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-Butynyl]-	41.78	0.03
7-isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-2(3h)-naphthalenone	24.34	0.04
Panaxydol, TMS	43.96	0.04
[1,1'-bicyclopropyl]-2-octanoic acid,2-'hexyl,-methyl ester	44.63	0.02
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	45.85	0.12
9,12,15-octadecatrienoic acid,2-(phenyl-1,3-dioxolan-4-yl)methyl ester	47.27	0.02
Hexadecanoicacid	48.54	0.27
Hexadecanoicacid,2,3-dihydroxypropyl ester	48.82	0.02
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	50.33	0.02

3,4-dimethoxyphenethyl-n,n-bis (trimethylsilylamine)	53.51	0.08
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	53.61	0.03
Aminoethanethiol hydrogensulfate (ester)	54.73	0.04
08217205002 FLAVONOL 3',4',5,7-OH,3-O-ARAGLUCOSIDE	55.30	0.02
Alanine,(3-benzyloxy-),L-	57.72	0.02
Dimethyl*u-truxinate	58.70	0.14
3,20-dioxo-11-à-hydroxyconanine-1,4-diene	59.14	0.02
4,7-Octadecadiynoicacid, methyl ester	59.58	0.04
Propanedioicacid,amino[[4-(1-hydroxy-3-methyl-2-butenyl)-1h-indol-3-yl]methyl]-, dimethylester ,(.-+.)-	60.47	0.06
Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl-	62.01	0.05
Hexadecanoicacid,[(2-trimethylsilyl)oxy]-1,3-propanediyl ester	64.40	0.03
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	65.20	0.15
4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	67.62	0.05
9,12-octadecadienoic acid(z,z,)-2,3 bis[(trimethylsilyl)oxy]propyl ester	69.18	0.03
Pregnane-3,11,20,21-tetrol,cyclic20,21 (-butylboronate,(3à,5á,11á,20r)-	70.20	0.02
Ethyl iso-allocholate	72.09	0.04
4h-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	79.28	0.03
á-sitosterol	83.71	0.07

Table 13. Common compounds in extracts of *Alternaria alternata* and *Ocimum basilicum* leaves.

Compounds	***Alternaria alternata***	***Ocimum basilicum* leaves**
	Percentage (%)	
(Z)-1-Chloro-2-(methylsulfonyl)ethylene	0.03	0.07
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo-,methyl ester,(16à)-	0.02	0.02
Desulphosinigrin	0.02	0.04
Malonic acid,6-heptynyl-	0.03	0.05
4h-1-benzopyran-4-one,2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-	0.28	0.02
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.65	0.12
hexadecanoicacid,2,3-dihydroxypropyl ester	0.65	0.02
Phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	7.51	0.05
Hexadecanoicacid,[(2-trimethylsilyl)oxy]-1,3-propanediyl ester	2.19	0.03
hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	0.26	0.15
Ethyl iso-allocholate	1.01	0.04
4h-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy-	0.48	0.02
9,12-octadecadienoic acid(z,z-(,2,3 bis[(trimethylsilyl)oxy]propyl ester	0.33	0.02

Table 14. Common compounds in extracts of *Curvularia pseudobrachyspora* and *Ammi visanga* stems.

Compounds	***Curvularia pseudobrachyspora***	***Ammi visanga* stems**
	Percentage (%)	
3,6-Dimethoxy-2,5-dinitrobenzaldehydeoxime	0.02	0.01
N-Hexadecanoic acid	1.17	0.21
Yohimban-16-carboxylic acid,19,20-didehydro-17-oxo,-methyl ester,(16à)-	0.03	0.02
2-(3-carboxy-propionylamino)-7-oxo-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethylester	0.03	0.02
9,12,15-octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester,(z,z,z)-	0.02	0.04
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	0.84	0.12
1H-purin-6-amine,[(2-fluorophenyl)methyl]-	0.01	0.03
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	0.19	0.02

Phenol,2,2-'methylenebis[6-(1,1-dimethylethyl)-4-methyl	9.63	0.08
Hexadecanoic acid,1-(hydroxymethyl)-1,2-ethanediyl ester	0.15	0.11
9,12-octadecadienoic acid(z,z)-,2,3-bis[(trimethylsilyl)oxy]propyl ester	0.02	0.03
4H-1-benzopyran-4-one,2-(3,4-dihydroxyphenyl)-6,8-di-á-d-glucopyranosyl-5,7-dihydroxy	0.30	0.03
Ethyl iso-allocholate	0.17	0.08
9-octadecenoic acid (z),-[(2-trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester	0.01	0.02
4H-1-benzopyran-4-one,2- (3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy	0.49	0.02
5,11,17,23-tetrakis(1,1-dimethylethyl)-28-methoxypentacyclo[19.3.1.1(3,7).1(9,13).1(15,19)] Octacosa 1(25),3,5,7(28),9,11,13(27),15,17,19(26)2,1,23-dodecene-25,26,27-triol	0.93	0.02

Discussion

In the present work, the endophytic fungi were isolated from ten different medicinal plants, which were collected from MEPACO-MEDIFOOD (Arab Company for Pharmaceutical and Medicinal Plants), Inshas Al-Raml, Belbeis Center, Sharkia governorate, Egypt.

In this study, two fungal species were isolated from *Ocimum basilicum*. In previous research, **Jayshree, 2014**, isolated seven endophytic fungal species from *Ocimum basilicum L*., and **Karthika and Rasmi, 2022**, isolated seventy-one fungal species from *Ocimum basilicum*, thirty-nine fungal species from *Ocimum gratissimum*, and *Ocimum tenuiflorum*. For the first time in this work, twelve fungal species were isolated from the stem, leaves, and roots of *Ammi visanga*.

In the present research, *Alternaria alternata* AUMC16440 was isolated from the leaves of *Ocimum basilicum*, and *Nigrospora* sphaerica was isolated from the stem of *Ocimum basilicum*. In a previous study by **Al-Harthi *et al*., 2023,** endophytic fungi were isolated from *Jasminum sambac*, *Camellia sinensis*, and *Ocimum basilicum* and were identified as species of *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Phoma*, and *Trichoderma*. However, ***Mohamed Mohram et al*., 2024** isolated three other endophytic fungi from *Ocimum basilicum* leaves and identified them as *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus nidulans*.

For the first time in this work, *Curvularia pseudobrachyspora* AUMC16441 was isolated from the stem of *Ammi visanga*, *Alternaria tennuisima*, *Phoma herbarum*, and *Fusarium oxysporium* from roots, and *Alternaria infectoria* from leaves of *Ammi visanga*. Only one previous study reported the isolation of only one endophytic fungus from *Ammi majus* fruits and identified it as *Aspergillus amstelodami* **(Salama *et al*., 2019)**.

It is known that a variety of compounds obtained from endophytic fungi have a broad spectrum of bioactivities, including immunomodulatory, antiviral, antidiabetic, antioxidant, anticancer, and antibacterial properties, and the discovery of new active substances that have antimicrobial activity from endophytic fungi has received attention **(Strobel and Daisy, 2003; Kaul *et al*., 2012; and Kaur *et al*., 2020)**.

In this study, the crudes extract of *Alternaria alternata* AUMC16440 isolated from the leaves of *Ocimum basilicum* showed a broad antimicrobial spectrum against tested pathogenic bacteria *Escherichia coli* and *Bacillus subtilis*. There is evidence from a number of earlier investigations that the endophytic fungus *Alternaria alternata* produces bioactive substances with strong antibacterial activity **(Katoch *et al*., 2017)**.

The results were in close agreement with those of past research, **Chandra *et al*., 2021**, reported that there is antibacterial activity in the extract of *Alternaria alternata* that was isolated from *Picrorhiza kurroa* against *Staphylococcus aureus* and *Bacillus subtilis*. In a previous study, the ethyl acetate crude extract of *Alternaria alternata* showed promising antimicrobial activity against unicellular fungi (*Candida albicans* ATCC 90028), Gram positive bacteria (*Bacillus subtilis* RCMB 015), and Gram-negative bacteria (*Escherichia coli* ATCC 11229) **(Elghaffar *et al*., 2022)**. Also, **Khalil *et al*., 2021** isolated *Alternaria alternata* EP-12 from the medicinal plant *Ephedra pachyclada*, which exhibited antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*, but, contrary to our results, it has no inhibitory activity against *Escherichia coli*.

In this study, the crude extracts of *Alternaria alternata* AUMC16440 also exhibited antifungal activities against *Aspergillus fumigatus*. Similarly, **Erfandoust *et al*., 2020** reported that a methanol extract of *Alternaria alternata* (CSE92) isolated from *Cupressus arizonica* had antifungal activity against *Aspergillus* fumigatus and *Aspergillus* niger. **Al Mousa *et al*., 2021**, also reported that the ethyl acetate crude extract from *Alternaria tenuissima* AUMC14342 exhibited the strongest antimicrobial activity against *Fusarium solani* and *Aspergillus niger*.

In this study, for the first time, *Curvularia pseudobrachyspora* AUMC16441 was isolated from the stem of *Ammi visanga* and exhibited antimicrobial activities against tested pathogenic bacteria, *Bacillus subtilis* and *E. coli*, and tested pathogenic fungi, *Aspergillus chrysogenum* and *Aspergillus fumigatus*.

Nonetheless, a number of studies demonstrated the potentially effective antibacterial properties of crude extracts from *Curvularia* sp. **Avinash *et al*., 2015** reported that *Curvularia lunata* extract exhibited antibacterial properties against *Staphylococcus aureus* and *Salmonella typhi*. **Khiralla *et al*., 2020**, reported that *Curvularia tuberculata's* ethyl acetate crude extract has demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Moreover, the extract of endophytic fungus *Curvularia* sp. T12 isolated from *Rauwolfia macrophylla* had antibacterial activity against *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas agarici*, and *Staphylococcus warneri* **(Kaaniche *et al*., 2019)**. The ethyl acetate extract of *Curvularia eragrostidis* isolated from *Helicteres isora* showed antifungal activity against *Aspergillus fumigatus* **(Santra and Banerjee, 2022)**. But the result doesn't agree with the result of **Nwobodo *et al*., 2022,** reported that the crude extract of *Curvularia lunata*, which was separated from *Elaeis guineensis*, showed no antibacterial activity against *B. subtilis* or *E. coli*.

The antimicrobial activities of *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441 may be due to the presence of secondary metabolites, that are responsible for the deformation of cell walls, depolarization of the cell membrane, suppression of protein synthesis, inhibition of nucleic acid synthesis, and inhibition of metabolic pathways in bacteria **(Ebrahimi *et al*., 2010; and Reygaert, 2018)**. The mode of action of each compound will be discussed laterally.

In the current research, *Curvularia pseudobrachyspora* AUMC16441 and *Alternaria alternata* AUMC16440 produced IAA in concentrations (5 mg/mL) of tryptophan with values of 9.62 µg/mL and 5.7 µg/mL, respectively. Similarly, **Munir *et al*., 2021** reported that three endophytic fungi, *Curvularia lunata*, *Schizophyllum commune*, and *Trichoderma atroviride*, which were isolated from the rhizome of *Elettaria*, may produce IAA at concentrations of 45.17, 11.7, and 5.27 µg/mL, respectively. **Fouda *et al*., 2020**, also stated that *Alternaria alternata* Aa_27, *Penicillium chrysogenum Pc_25*, and Sterile hyphae Sh_26 were isolated from medicinal plant *Asclepias sinaica* and were able to produce IAA. **Khalil *et al*., 2021**, reported that *Alternaria tennuisima* EP-13 isolated from leaves of *Ephedra pachyclada* is able to produce IAA in concentration (5 mg/mL) of tryptophan with a value of 176.9 ± 15 µg/mL.

In this study, ethyl acetate extracts of *Curvularia pseudobrachyspora* AUMC16441 and *Alternaria alternata* AUMC16440 showed maximum alpha-amylase inhibition of 94% and 82%, respectively. Previous research has indicated that the ethyl acetate extract of *Alternaria tenuissima* isolated from *Ocimum sanctum* showed the greatest inhibition of the pancreatic α-amylase enzyme in *vitro*, with a value of 27.34 µg/mL **(Pavithra *et al*., 2014)**. Alike, **Jayant and Vijayakumar, 2021** reported that the ethyl acetate extract of *Curvularia lunata*, which was isolated from *Ficus religiosa*, showed anti-diabetic potential.

In the current research, GC-MS examination of the petroleum ether and ethyl acetate extracts of the isolated endophytic fungal species showed the presence of bioactive compounds, which are mainly hydrocarbons, fatty acids, flavonoids, terpenoids, phenolic compounds, and alcohols. It was discovered that the majority of these compounds have antimicrobial properties.

Flavonoids are one of the common compounds found in extracts of isolated endophytic fungi and have antimicrobial activities. Fungal growth is frequently inhibited by flavonoids through a variety of methods, including disruption of the plasma membrane and inhibition of cell wall production, cell division, and RNA and protein synthesis **(Saleh *et al*., 2020)**.

The result of the present study showed that**, 7**,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione belongs to flavonoid compounds found in the ethyl acetate extract of *Curvularia pseudobrachyspora* AUMC16441 and *Alternaria alternata* AUMC16440. It was reported that **7**,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione was extracted from many fungal isolates and some marine algae with and had antimicrobial activities **(Yasa, 2009** and **Ahmed *et al*., 2021)**.

Our research revealed the existence of aliphatic hydrocarbons such as tetradecane (long-chain alkane) found in petroleum ether extracts of *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441. Previous research had indicated that tetradecane had antibacterial activity against: *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Lactobacillus acidophilus*, and antifungal activity against *Candida albicans* **(Girija *et al*., 2014)**.

The results showed that, phenol 2, 2'-methylenebis [6-(1, 1-dimethylethyl)-4-methyl-] is the main phenolic compound, and ethyl iso-allocholate belongs to alcohol groups present in ethyl acetate and petroleum ether extracts of *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441. Previous studies have reported that they have antimicrobial activities **(Celis *et al*., 2011; Muthulakshmi *et al*., 2012 and Dias *et al*., 2022)**.

Some compounds belonging to organosiloxane were found in the ethyl acetate extract and petroleum ether extract of *Alternaria alternata* AUMC16440, including cyclooctasiloxane hexadecamethyl, cyclodecasiloxane, eicosamethyl, cycloheptasiloxane tetradecamethyl, cyclohexasiloxane dodecamethyl, and cycloconasiloxane octadecamethyl. Previous studies reported that these compounds had antimicrobial activities. For instance, **Bratty *et al*., 2020** found that cyclooctasiloxane hexadecamethyl, cyclodecasiloxane eicosamethyl, and cycloheptasiloxane tetradecamethyl present in the ethanol extract of the *Salvadora persica* fruits had

antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*. Cycloheptasiloxane tetradecamethyl was detected in the ethyl acetate and hexane extracts of *Streptomyces celluloflavus* that had antifungal activity **(Nguyen and Cao, 2022)**.

In this study, hexadecanoic acid is a long-chain fatty acid identified in petroleum ether and ethyl acetate extracts of *Curvularia pseudobrachyspora* AUMC16441. Previous studies indicated that hexadecanoic acid was found in the methanol extract of hydroid *Aglaophenia cupressina Lamoureoux*, inhibited *Fusarium oxysporum*, and had antibacterial activity against *Staphylococcus aureus, Bacillus subtilus*, *Escherichia coli*, *Klebsiella pneumoniae, and Pseudomonas aeruginosa* (**Mickymaray *et al*., 2016; and Idris *et al*., 2018).** Antibacterial activity of fatty acid derivatives may be because they prevent the disruption of cell walls, transcription, and translation **(Yinusa *et al.*, 2014)**. Fatty acids with antifungal properties naturally break the lipid bilayer that makes up fungal membranes. The pathogenic fungus's cell wall or spore wall may be disrupted or broken down by the fatty acid derivatives **(Karthikeyan *et al*., 2014)**.

The results of the present study indicated that the ethyl acetate extract was able to extract some components, including Desulphosinigrin, 2-benzenedicarboxylic acid, Trismethoxy resveratrol, D-Fructose diethylmercaptal pentaacetate, Cyclooctasiloxane hexadecamethyl-, Cyclodecasiloxane eicosamethyl, Cycloheptasiloxane tetradecamethyl, Cyclononasiloxane octadecamethyl, and flavonol 3',4',5,7-OH,3-O-araglucoside with powerful antimicrobial activity against tested pathogenic microorganisms that are not found in petroleum ether extract. Similarly, **Khalil *et al*., 2021; and Elghaffar *et al*., 2022** reported that ethyl acetate extracts of endophytic fungal isolates had strong antimicrobial action against a variety of pathogens.

In addition, the GC-MS results revealed that many compounds are common between endophytic fungal isolates and their host, many compounds are common between the medicinal plant *Ammi visanga* and *Curvularia pseudobrachyspora* AUMC16441 and the most dominant compounds are hexadecanoic acids and 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione as well as between the medicinal plant *Ocimum basilicum* and the extracts of *Alternaria alternata* AUMC16440 and the most dominant compounds are ethyl iso-allocholate and phenol,2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl, this indicates the presence of relation between endophytic fungi and their medicinal plant, endophytic fungi produce secondary metabolites as the same produced by medicinal plant or medicinal plant affecting on endophytic fungi **(Jia *et al*., 2016)**.

Despite the fact that endophytic fungal metabolites may serve unique purposes in plant defense, **Yang, 2002** has linked the endophytic fungus *Acremonium zeae's* production of two macrocyclic alkaloids, pyrrocidines A (106a) and B (106b), with antibiotic activity, to maize's defense against the pathogenic and mycotoxin-producing fungi *Aspergillus flavus* and *Fusarium verticillioides*. Similarly, the tropical plant *Huperzia serrata* is used medicinally and can provide Huperzine-A chemicals that are thought to be induced by the endophytic fungus *Shiraia* sp. and *Acremonium* sp. **(Wang *et al*., 2009; Zhou *et al*., 2009; and Wang *et al*., 2011)**. It was also that the antimicrobial activities of the fruits of *Piper longum* and *Piper nigrum* are due to piperine, which was found in culture extracts of endophytic *Periconia* strains isolated from *P. longum* leaves **(Verma *et al*., 2011)** and *Colletotrichum gloeosporioides* from *P. nigrum* stems **(Chithra *et al*., 2014)**.

Conclusion

In conclusion**,** there was successful isolation and identification of two endophytic fungi as *Alternaria alternata* AUMC16440 and *Curvularia pseudobrachyspora* AUMC16441 from two medicinal plants, *Ocimum basilicum* and *Ammi visnaga. Curvularia pseudobrachyspora* AUMC16441 and *Alternaria alternata* AUMC16440 were able to produce indole acetic acid and had antimicrobial and antidiabetic activities. Analysis using gas chromatography-mass spectrometry (GC-MS) revealed a number of bioactive substances that possess several beneficial biological properties. The endophytic fungi had a role in protecting their host and vice versa; endophytic fungi and their host shared numerous chemicals.

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