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Identification and Antimicrobial Activity of Endophytic Fungi Associated with *Ocimum basilicum* (L.) from Sudan

Amna Mohamed El Obeid Sewaikit*

Faculty of Science, University of Khartoum Khartoum, Sudan

**Corresponding author*

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A B S T R A C T

Fungal endophytes are living inside plants without any harmful effects; the prospecting about them is increased day by day because they can produce bioactive compounds which can be used in different applications. Here in, the current study was aimed to isolate the endophytic fungi from the *Ociumum basilicum* (Al Rihan) plant as safe microorganisms and evaluate their antimicrobial activities. Altogether 150 segments of which 50 segments each from leaf, stem and flower tissues of the plant was screened for the enumeration of the endophytic fungi using the modified surface sterilization techniques. The fungal isolates were cultured in Potato Dextrose Agar (PDA) medium and the isolated fungi were identified on the basis of their colony characterization on PDA medium and morphological features. The cultures as well as the host plant parts were extracted by organic solvents (ethyl acetate and methanol). Antimicrobial activity investigation was carried out against Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria and two fungal species (*Candida albicans* and *Aspergillus niger*) using the disc diffusion method. Results showed that a total of 8 endophytic fungi were recorded and morphologically identified from the different plant parts; 3 strains from the leaves, 3 from the stems and 2 from the flowers. Among them 6 belong to Hyphomycetes and 2 belong to Coelomycetes. The most potent antibacterial activity was obtained from the ethyl acetate extract of *Chaetomium* sp. followed by the ethyl acetate extract of *Geotrichum* sp. The tested fungi were less susceptible to the endophytic fungi except the ethyl acetate extracts from *Alternaria* sp., *Geotrichum* sp. and *Curvularia* sp., which exerted moderate activity towards *A. niger* and *C. albicans*.

Introduction

Fungi are an important group of organisms that serve many vital functions in ecosystems including decomposition, nutrient recycling, symbiotic relationships with plants, biological control of other fungi as well as the causal agents of diseases in plants and animals. An endophyte is a bacterial (including actinomycete) or fungal microorganism, which spends the whole or part of its life cycle

colonizing inter- and /or intra- cellularly inside the healthy tissues of the host plant, typically causing no apparent symptoms of disease (Tan and Zou, 2001). Endophytic fungi have been recognized as a reservoir of novel secondary metabolites, which have antibiotic, antimycotic, anticancer and immunosuppressive activities (Premjanu and Jayanthy, 2012). Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites bearing antimicrobial activity. Endophytic fungi are now recognized as a new tool in the production of antimicrobial and pharmaceutical compounds. The antibiotics, therapeutic agents and agrochemicals can be synthesized from endophytic fungi (Tenguria *et al.,* 2011). These compounds are highly effective, with low toxicity and having minor environmental impact.

Antimicrobial metabolites (antibiotics) are low-molecular weight organic compounds that are active at low concentrations against microorganisms. Beside the toxic effect of higher doses of antibiotics, the extensive and inappropriate use of these antibiotics causes selective pressure on microbial populations so few resistant and more virulent mutants can flourish. Drug resistance could also be due to the wide genetic variability of bacteria which alters constant search for new antimicrobial compounds with novel modes of action. This has encouraged researchers to explore alternative bioactive molecules against pathogenic microbes (Raut and Karuppayil, 2014).

In Sudan although much research was done on medicinal plants but the field of endophyte remains poorly touched. The host plant in this study was *Ocimum basilicum* (L.). *Ocimum* (Basil) is the most important genus of the subfamily Nepetoideae under the family Lamiaceae. Basil is known locally in Sudan as "Rihan". The plant is aromatic and exists in Sudan as a wild plant, widely spread, as well as cultivated for ornamental purposes.

Materials and Methods

Source of Plant sample

Healthy plant samples of *Ocimum basilicum* were collected from Ahfad University, Khartoum State (Central Sudan). Plant samples were brought to the laboratory and processed immediately after collection following guidelines of Monnanda *et al.,* (2005). Fresh leaves, stems and flowers were separated for further isolation of endophytic fungi.

Surface- sterilization and isolation of endophytic fungi

The fresh and healthy plant samples were cleaned thoroughly in gentle running tap water in order to remove dirt and debris. The whole process of isolation of endophytic fungi was carried out under aseptic conditions (Sunitha *et al.,* 2013).

150 segments of which 50 segments each from leaf, stem and flower were obtained. Stem and flower samples were cut into 1.0×1.0 cm pieces; leaves were cut into small discs using sterile cork borer. The samples were immersed first in 70% ethanol for 1 min, followed by 1.0% sodium hypochlorite (NaOCL) for 1min and further cleaned by passing through two sets of sterile distilled water. Each sample was then dried under aseptic conditions.

The sterile samples were placed on plate containing Potato Dextrose Agar (PDA) medium supplemented with streptomycin (500mg/l) to suppress bacterial growth. Each Petri dish contained 3 segments of plant tissues on PDA media. The efficiency of the surface sterilization procedure was confirmed by plating 1ml of the final rinse water on PDA as controls where no plant tissues were inoculated.

The Petri dishes were then incubated at 27˚C, plates were checked on alternate days and pyphae of actively growing fungi were then sub cultured. The purified endophytic fungi isolates were transferred separately to PDA slants and stored at 4˚C, for the further studies.

Fungal morphological characterization

Sporulating fungi were identified based on colony morphology, conidiospores and conidiophores characteristics (Webster and Weber, 2007). The microscopic identification of the isolates was carried out by lactophenol staining technique.

Colonization frequency

The endophytic fungal isolates from the host plant tissue segment were analyzed based on the percentage of

density of colonization (colonization frequency) (Suryanarayanan *et al.,* 2000).

$$\text{Colonization frequency (CF \%)} = \frac{\text{Number of species isolated}}{\text{Number segments screened}} \times 100$$

Extraction of metabolites from endophytic fungi

Ethyl acetate and methanol were used for fungal metabolites extraction. According to Compos *et al.,* (2008) fungal isolates were cultured for large scale c Ethyl acetate and methanol were used for extraction of secondary metabolites from the host plant. 100g of powdered dried leaves, stems and flowers of the host plant materials were soaked, separately, in sufficient amount of ethyl acetate for 6 days and then filtered using filter papers. The residue of the plant tissue was re-extracted with methanol for another 6 days and then filtered. The two extracts were then evaporated to dryness and weighed and stored in well closed brown bottles at 4° C till used.

Extraction of metabolites from the host plant (*Ocimum basilicum)*

Ethyl acetate and methanol were used for extraction of secondary metabolites from the host plant. 100g of powdered dried leaves, stems and flowers of the host plant materials were soaked, separately, in sufficient amount of ethyl acetate for 6 days and then filtered using filter papers. The residue of the plant tissue was re-extracted with methanol for another 6 days and then filtered. The two extracts were then evaporated to dryness and weighed and stored in well closed brown bottles at 4° C till used.

Antimicrobial activity

The bacterial species used were the Gram – positive *Bacillus subtillus* (ATCC 19430) and *Satphylococcus aureus* (ATCC 25923) and Gram – negative *Escherichia coli* (ATCC25922) and *Pesudomonas aeruginosa* (ATCC 27853). Fungal species were; *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763).

Antibacterial assay

Antibacterial activity of endophytic fungi crude ethyl acetate and methanol extracts as well as plant part extracts was evaluated by the agar disc diffusion method (Mbavenge *et al.,* 2008). Sterilized filter paper discs with a diameter of 6 mm were impregnated with 1 ml of 20 mg of crude extracts dissolved in ml of 5% dimethyl sulfoxide (DMSO) and left to dry. After the plates were solidified the freshly prepared microbial broth culture suspension was spread over the MHA media using sterilized swaps under aseptic conditions.

After 5min, the discs were dispensed onto the surface of the inoculated agar plates. DMSO was used as a negative control, while Gentamicin (10 mg /disc) was used as a positive control. Three replicates were carried out for each extract against each of the test organisms. The Petri plates were incubated for18 – 24 hours at room temperature. After incubation, the diameters of clear zone of inhibition produced around the discs were measured in mm and the plates were photographed.

Antifungal assay

Antifungal activity was also evaluated by the disc diffusion method as described above for antibacterial activity but instead SDA medium was used (Mothana and Lindequist, 2005). Plates were incubated at room temperature for 24 hours for *C. albicans* and 48 hours for *A. niger*. DMSO was used as a negative control, while Nystatin (100 000IU/ml) was used as a positive control.

Results and Discussion

Isolation and identification of endophytic fungi

A total of eight isolates were obtained from leaves (3), stems (3) and flowers (2) of the plant samples as shown in Table1. The isolated fungi were identified on the basis of their colony characterization on PDA medium and morphological features.

Description of fungal isolates

Isolated fungal from leaves

L1. *Cladosporium* sp. (Hyphomycetes)

The strain had a rapid rate of growth, mature within seven days. Culture was dark green to black in colour and fluffy on PDA, reverse side of the colonies was dark black, septate mycelia with dark conidiohpores, the

hyphae support many oval one- celled conidia and aseptate (Plate1). Accordingly, the isolate was identified as *Cladosporium* sp.

L2. *Nigrospora* sp. (Hyphomycetes)

A rapid rate of growth, mature within seven days. Initially the colonies were white to light gray on PDA. The reverse side of the colonies was dark gray. The culture diffused over the surface of the agar with a well-developed fluffy aerial mycelium. The hyphae septate and brown in colour, globose single celled macroconidia and black in colour and asesptate (Plate 2).

L3. *Curvularia* sp. (Hyphomycetes)

Rate of growth: moderate, mature within ten days. Culture were deep brown to black in colour on PDA, reverse side of the colonies was black. Multi- celled conidia with septations mainly 4–celled, transversely, generally curved and brown in colour. Conidiophore short and septate (Plate 3).

Isolated fungal from stems

S1. *Chaetomium* sp. (Coelomycetes)

The strain had a slow rate of growth; mature within twenty days. On PDA, colony surface was cottony and white in colour initially and then becomes grayish black. The perithecia are superficial, barrel– shaped, clothed with projecting, dark, stiff hairs. Hairs were dark brown straight, wavy and curled. Inside the black mass is a definite cell wall enclosing many dark brown, ovoid spores (Plate 4).

S2. *Phoma* sp. (Coelomycetes)

The isolate had a moderate growth rate reaching sporulation within ten days. The colonies granular, spreading with areas of light gray on PDA, reverse surface was black. Microscopically: dematiaceous, septate hyphae, large asexual fruitin bodies (pycnidia) containing large numbers of small conidia. The conidia formed inside the pycnidia are rather oval, one celled hyaline, of released through a round ostiole (Plate5).

S3. *Aspergillus niger* van Tiegh. (Hyphomycetes)

The isolate had a fast growth rate reaching sporulation stage within five days. Colonies on PDA cultures were appeared dematiaceous due to the heavy production of black spores, reverse side of the colonies was pale yellow. Hphae were septate and hyaline. Conidial heads (phialosporous vesicle) were colourless, globose. Conidia chains were small oval to spherical borne from the tips of one or two rows of sterigmata arranged radially cover entire vesicle. The description of this fungus was closed to that of *Aspergillus niger* vanTiegh (Plate6).

Isolated fungal from flowers

F1. *Alternaria* sp. (Hyphomycetes)

Colonies growing restrictedly on PDA, surface was dark gray to black and reverse of the colonies was equally black. The culture matures within ten days. Microscopically: dematiaceous, slender, profusely branched, septate light brown hyphae. Conidia are club–shaped, multi–celled with septations arranged transversely and longitudinally (Plate7).

F2. *Geotrichum* sp. (Hyphomycetes)

The isolate had a moderate growth rate revealing sporulation within ten days. The surface of culture was dark gray in colour, fluffy and the reverse side was gray. These are yeast like fungi. The mycelium was septate and reproduction occurs by fragmentation of mycelium into arthrospores. The arthrospores appear more rectangular shape, bright, and aseptate in chain. The arthrospores of *Geotrichum* species characteristically germinate at one corner forming “hockey sticks” (Plate 8).

In summary, from 150 segments obtained from different parts of *O. basilicum* a total number of 8 endophytic fungi were isolated as shown in Table 1. The frequency of fungal colonization (CF %) differed among the fungal isolates (Table 2). The percentage of colonization frequency of *Cladosporium* sp. (46%), *Geotrichum* sp. (42%) and *Alternaria* sp. (38%), which belongs to Hyphomycetes group, had high colonization frequency. Shekhawat *et al.,* (2010) found that Hyphomycetes group largely occurs in all plants and protect them against pathogens. However, the majority of the endophytic fungi (6/8: 75 %) belong to Hyphomycetes and only two (2/8:25%) belong to Coelomycetes which are *Chaetomium* sp. and *Phoma* sp. This result is inconsistency with many researchers

who found endophytic fungi of the aerial plant parts generally consist of members of the Ascomycetes, and in their conidial (anamorphic) forms.

According to the morphological features, one endophytic fungus was identified to the species level namely; *Aspergillus niger* while other seven taxa were identified to the genus level which were; *Cladosporium* sp., *Nigrospora* sp., *Curvularia* sp., *Phoma* sp., *Chaetomium* sp., *Alternaria* sp. and *Geotrichum* sp. The species that belong to the genus *Aspergillus* was established as dominant endophytes in wide tropical hosts range (Nuramin *et al.,* 2014).

However, Chowdhary and Kaushik (2015) isolated several endophytic fungi from the leaf and stem of *O. sanctum* where four isolates namely; *A. niger, Alternaria* sp., *Chaetomium* sp. and *Curvularia* sp. were also obtained in the present study. Lakshman (2014) isolated seven endophytic fungi from *O. basilicum* where two isolates; *Alternaria* sp. and *Geotrichum* sp. were also isolated in this study. Thus, it was clear that the same species could host the same or different population of endophytic fungi. The endophytic flora, both numbers and types, differ in their host and depends on host geographical position (Gange *et al.,* 2007). The occurrence of endophytic fungi in host plants has been shown to be related to several factors, including the part of the plant (Sieber, 2002), environmental conditions (Griffin *et al.,* 2019) and the location of plant (Arnold, 2018). Seasonal variation plays a major role in endophyte harvesting where environmental conditions pave the way for symbiotic microbes to survive and explore. Precipitation was suggested to be one of the major factors that influences the infection by endophytes (Jena and Tayung, 2013).

Moreover, many of these endophytic fungi in the present study were also previously identified from other plant species. For example, *Aspergillus niger, Chaetomium* sp., *Alternaria* sp., *Curvularia* sp. and *Phoma* sp. were isolated by Khiralla (2015) from five plants; *Calotropis procera, Cantharanthus roseus, Euphorbia prostrata, Trigonella foenum – graecum* and *Vernonia amygdalina* endemic to Sudan. The same endophytic fungi were also isolated from *Acacia nilotica* by Kheir (2021). *Chaetomium* was found to resident many Orchid varieties (Sour *et al.,* 2015) and was isolated from *Actinidia macrospora* by Yin *et al.,* (2012). Furthermore, *Alternaria* sp. was known to be an endophytic species in many plants including *Ricinus communis* (Sandhu *et al.,* 2014) and Tunisian palm (Chobba, 2013). *Curvularia* sp. was also isolated from many plants like Cocoa trees (Song *et al.,* 2016), Tunisian palm trees (Chobba, 2013), Ricinus communis (Sandhu *et al.,* 2014) and Orchid varieties (Sour *et al.,* 2015)

Antimicrobial activity of endophytic fungi and host plant extracts

Antimicrobial activity of both ethyl acetate and methanol extracts of endophytic fungi and those from the leaf, stem and flower of the host plant was determined against four bacterial isolates; Gram positive (*Bacillus subtilis* and *Satphylococcus ureaus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as two fungal species (*Candida albicans* and *Aspergillus niger*) using the disc diffusion method (Mbavenge *et al.,* 2008). Antimicrobial activity of extracts was determined at concentration 20 mg /ml. Results are presented in Table 3 and Plates 9 and 10. Inhibition zone diameters (IZDs) obtained were classified into five categories; < 7 mm not active; 7–10. 9 mm weak activity; 11-16.9 mm moderate activity; 17–19.0 mm high activity and > 20 mm very high activity (Monks *et al.,* 2002).

Antibacterial activity against *Bacillus subtilis*

The ethyl acetate extract of *Chaetomium* sp., representing 6% (1/16) of the total number of extracts, displayed very high antibacterial activity against *B. subtilis* with inhibition zone value (29 mm) higher than those showed by the extracts of the host plant (< 17mm) and the standard antibiotic Gentamicin (10 mg) (24 mm). 31 % (5/16) of extracts revealed moderate activity while (38%; 6/16) of extracts were less sensitive towards the tested bacteria and 25% (4/16) were not active. The leaf methanolic extract of the host plant showed moderate activity while all other host plant extracts displayed weak activity.

Antibacterial activity against *Satphylococcus aureus*

About 13% (2/16) of fungal endophytic extracts exhibited moderate antibacterial activity against *S. aureus*, while the majority of extracts (56%; 9/16) showed weak activity. 31% (5/16) of the extracts did

not exert antimicrobial activity. Both leaf extracts of the host plant exerted moderate activity against *S. aureus* while the stem and flower extracts revealed weak activity.

Antibacterial activity against *Escherichia coli*

All the crude extracts of endophytic fungi possessed either weak or no activity against *E. coli*. With the exception of the ethyl acetate extract of the leaf (moderately active) all host plants exerted weak antimicrobial activity towards the same bacteria.

Antibacterial activity against *Pseudomonas aeruginosa*

The majority of the isolated endophytic fungi (56 %; 9/16) represented either weak or no activity against *P. aeruginosa*. 44% (7/16) of the extracts in addition to the methanolic extract of the flower gave moderate activity towards this bacterium.

Antifungal activity against *Aspergillus niger*

From the 13 endophytic extracts only one extract (6%;1/16), the ethyl acetate extract of *Alternaria* sp. revealed moderate activity while all the others of isolated endophytic fungi (94 %; 15/16) showed either weak or no activity against *A. niger*. All the host plants extracts displayed weak activity towards the fungus.

Antifungal activity against *Candida albicans*

About 25% (4/16) of the endophytic fungi extracts, *comprising both extracts of A. niger, Curvularia sp.* and *Geotrichum* sp., displayed moderate antifungal activity against *C. albicans*.

Other extracts gave either weak (50%) or no activity (25%). With the exception of the methanolic extract of the stem (highly active) all host plant extracts exerted either moderate or weak activity.

In the present study, crude metabolite extracts of fungal endophytes isolated from *O. basilicum* showed considerable activity against all test pathogens. The result showed that, the most potent antibacterial activity was obtained from the ethyl acetate extract of *Chaetomium* sp. (29 mm) followed by the ethyl acetate extract of *Geotrichum* sp. (16 mm). Also, it was observed that *B. subtilis* (+ve) and *P. aeruginosa* (-ve) were more susceptible to endophytic extracts than the other tested organisms (*S. aureus*, (+ve) and *E. coli*, (-ve) where the difference in the cell membrane structure of the two bacterial groups did not clearly affect the antimicrobial property of the endophytic fungi extracts.

Karunai and Balagengatharathilagam (2014) studied among the potent strains, crude metabolite of an endophytic fungus, *Cladosporium* sp. displayed significant inhibitory activity on *B. subtilis, S. aureus* and *E. coli*.

It was observed that the same species, in the present study possessed weak activity towards *B. subtilis* and *S. aureus* and was not effective against *E. coli*. Mahdi *et al.,* (2014) found that extracts of *Chaetomium* sp. isolated from *Prosopis chilennsis* exerted very high antibacterial activity against *S. aureus* and high antibacterial activity towards *E. coli*, however, in the present study this species had weak activity against these bacteria.

Ethyl acetate and methanolic extracts of *Phoma* sp., which was isolated from the stem of *A. nilotica* by Kheir (2021), were inactive towards *P. aeruginosa*. Interestingly, both extracts of the species in this study showed moderate antimicrobial activity against the same bacteria.

In addition, the ethyl acetate extract of *Curvularia* sp. isolated from *Datura stramonium* by Mahdi *et al.,* (2014), possessed no activity against *S. aureus, A. niger* and *C. albicans*, compared to this study, the same species showed weak activity against two first pathogens and moderate antifungal activity towards *C. albicans*.

Furthermore, the leaf methanolic extract of the host plant gave moderate activity towards *B. subtilis* and *S. aureus* and displayed poor activity against *E. coli*. These results correlated with the findings of Chareprasert *et al.,* (2006), they found that the leaves could inhibit the growth of Gram-positive bacteria such as *S. aureus* and *A. subtilis* to a greater degree than Gram negative bacteria (*E. coli*). The difference in endophytes, their metabolic profile and biological activity even if between the isolates of the same species, might be related to the chemical difference of host plants (Paulus *et al.,* 2006).

Table.1 Endophytic fungi isolated from different parts of *O. basilicum*.

Source	Number of Samples	Identification Remark	Number of fungi isolated
Leaf	50	*Cladosporium* sp. *Nigrospora* sp. *Curvularia* sp.	**23** **12** **10**
Stem	50	*Chaetomium* sp. *Phoma* sp. *Aspergillus niger*	**6** **9** **15**
Flower	50	*Alternaria* sp. *Geotrichum* sp.	**19** **21**
Total	**150**	**8**	**115**

Table.2 Colonization frequency (CF) of endophytes isolated from different parts of O. basilicum**.**

No.	Endophytic fungi	CF (%)		
		Leaf	Stem	**Flower**
1.	*Cladosporium* sp. (Hyphomycetes)	46	-	-
2.	*Nigrospora* sp. (Hyphomycetes)	24	-	-
3.	*Curvularia* sp. (Hyphomycetes)	20	-	-
4.	*Chaetomium* sp. (Coelomycetes)	-	12	-
5.	*Phoma* sp. (Coelomycetes)	-	18	-
6.	*Aspergillus niger* (Hyphomycetes)	-	30	-
7.	*Alternaria* sp. (Hyphomycetes)	-	-	**38**
8.	***Geotrichum* sp. (Hyphomycetes)**	-	-	**42**

Table.3 Antimicrobial activity of endophytic fungi and host plant (*Ocimum basilicum*) parts extracts.

Endophytic fungi/plant organ	Extract	Inhibition zones (mm)					
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	***C. albicans***
Cladosporium sp.	EtOAc	8.0 ±1.0	7.0 ± 0.0	N.A.	N. A.	N. A.	**9.3 ±7 .4**
	MeOH	10. 3 ±0.6	8 .0 ±1 .0	N. A.	N.A.	N. A.	**8.7 ±1.5**
***Nigrospora* sp.**	EtOAc	N.A.	N. A.	7.7 ± 0.6	7.3 ±0.6	N. A.	**N. A.**
	MeOH	9.3 ±1 .2	N. A.	7.0 ±0.0	11.0 ±1.0	N. A.	**N. A.**
***Curvularia* sp.**	EtOAc	N. A.	8.0 ±1 .0	N. A.	13.3 ±0.6	8 .0 ±0 .0	**11.7±0.6**
	MeOH	N. A.	N. A.	N. A.	13.7 ± 0.6	N.A.	**N. A.**
Chaetomium sp.	EtOAc	29.7 ±8.1	7.3 ± 0.6	8.7 ± 0.6	7.7± 0.6	10.0 ± 1.0	**8.7 ±2 .9**
	MeOH	**10. 0 ±1.0**	**8 .0 ±0 .0**	**7.7± 0.6**	**7.0 ±0.0**	**N. A.**	**N.A.**

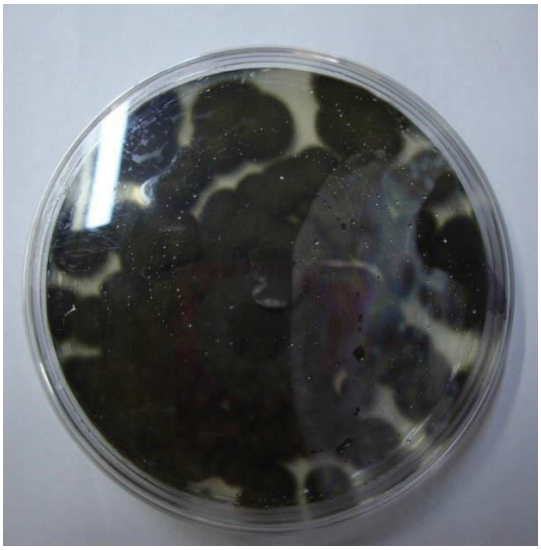
Endophytic fungi/plant organ	Extract	Inhibition zones (mm)					
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	***C. albicans***
***Phoma* sp.**	EtOAc	8.7±0.6	7.0 ±0.0	8.7 ± 0 .6	15 .0 ±1.0	N. A.	**10.0 ± 9.1**
	MeOH	N. A.	7.7 ± 1.2	N. A.	15.7 ±0.6	N. A.	**7.3 ± 0.6**
A. niger	EtOAc	13.7 ± 1.5	14.0 ±1 .0	9.7 ± 0.6	11.3 ±0 .6	9.7 ±0.6	**11.0 ±1.0**
	MeOH	11.7 ± 0.6	N. A.	N. A.	N. A.	7.7 ± 1.2	**11.0 ±1.0**
***Alternaria* sp.**	EtOAc	13.7 ± 0.6	8.0 ± 1.0	9.7 ± 0.6	11.3 ± 0.6	14.3 ± 1.5	**10.3 ±1.5**
	MeOH	11.0 ± 1.0	7.0 ± 0.0	7.3 ± 0.6	9 .3 ± 1.2	N. A.	**7.7 ±1.2**
Geotrichum sp.	EtOAc	13.0 ± 2.7	16.0 ± 4 .4	N. A.	7.3 ± 0.6	7.0 ± 0.0	**12.3 ±2.5**
	MeOH	9. 0 ±1.0	N. A.	7.3 ± 0.6	N. A.	N. A.	**9.0 ±1.0**
Flower	EtOAc	8.3 ± 0.6	7.3 ± 0.6	9.3 ± 0 .6	7.7 ± 0.6	8.7 ± 0.6	**13.3 ±3.2**
	MeOH	7.3 ±0.6	8.0 ± 0.0	7.0 ± 0 .0	10.7 ± 0 .6	7.7 ± 0.6	**10.0±1.0**

Endophytic fungi/plant organ	Extract	**Inhibition zones (mm)**					
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	***C. albicans***
Leaf	EtOAc	7.3 ± 0.6	11.7 ±0 .6	12 .7 ± 0.6	7.7 ±1 .2	7 .7 ± 1.2	**10.0±1.0**
	MeOH	12.0 ± 0 .0	11.0 ±0.0	9 .0 ± 0.0	9.0 ± 0.0	N. A.	**9.0 ± 1.0**
Stem	EtOAc	7.7 ± 0.6	7.3 ± 0.6	7.3 ± 0.6	8.0 ± 1.7	N. A.	**7.7±1.2**
	MeOH	7.0 ± 0.0	7.0 ± 0.0	7.7 ±0.6	7.7 ± 0.6	8.3 ± 0.6	**18.7 ±7.1**
Antibiotic	Gentamicin (10µg/disc)	24.0±1.0	17.3±0.6	16.7±0.6	12.0±1.0	-	**-**
	Nystatin (10^5IU/ml)	**-**	**-**	**-**	**-**	**21.3±1.5**	**29.3±1.2**

EtOAc, ethyl acetate; MeOH, methanol. <7 mm not active; 7 – 10.9 mm weak activity; 11-16.9 mm moderate activity; 17- 19.0 mm; high activity and > 20 mm very high activity (Monks *et al.,*2002).

Plate.1 *Cladosporium* **sp.**

Culture



Conidiophores and conidia

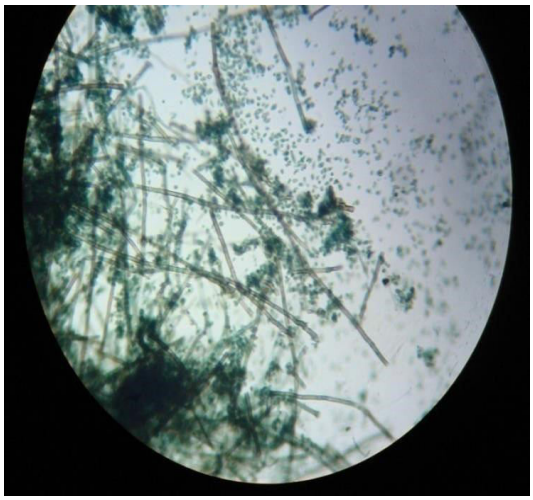


Plate.2 *Nigrospora* sp.

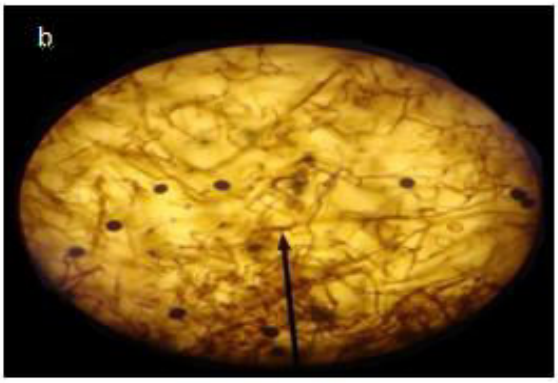
a: Culture	b**:** Conidiophores and conidia
	

Plate.3 *Curvularia* sp.

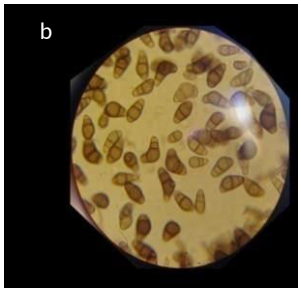
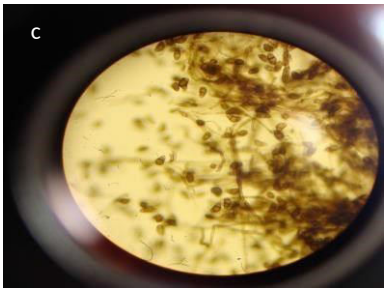
a: Culture	b**:** conidia	c**:** Conidiophores and conidia
		

Plate.4 *Chaetomium* sp.

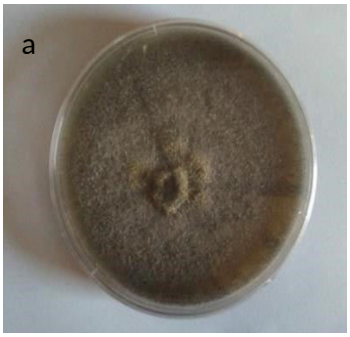
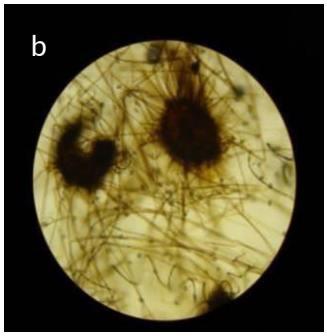
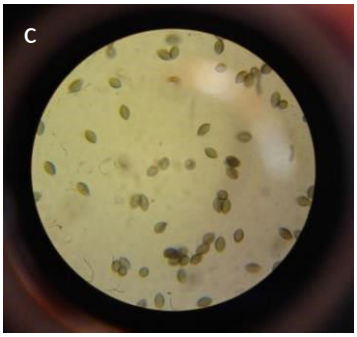
a: Culture	b: Pycnidia	c: Conidia
		

Plate.5 *Phoma* sp.

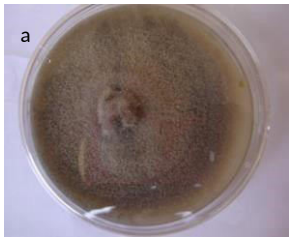
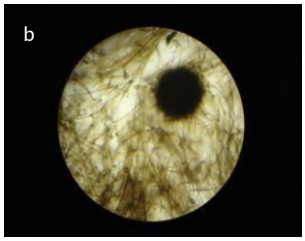
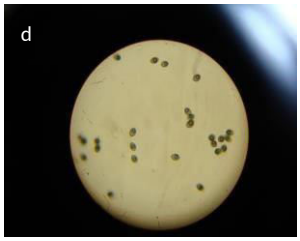
a: Culture	b and c: Pycnidia		d: Conidia
			

Plate.6 *Aspergillus niger*

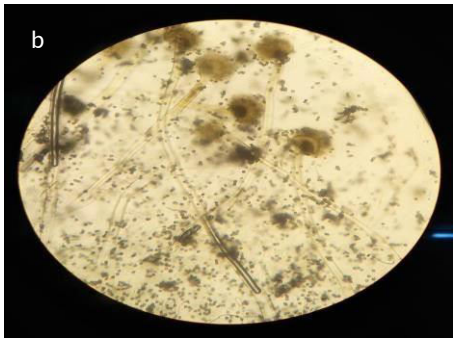
a: Culture	b: Conidiophores and conidia
	

Plate.7 *Alternaria* sp.

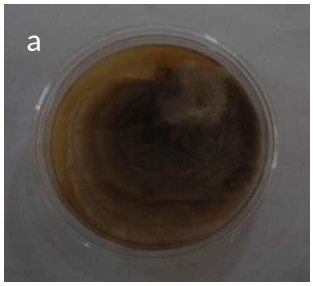
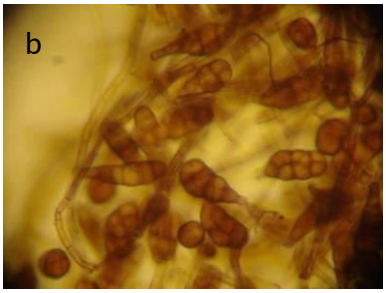
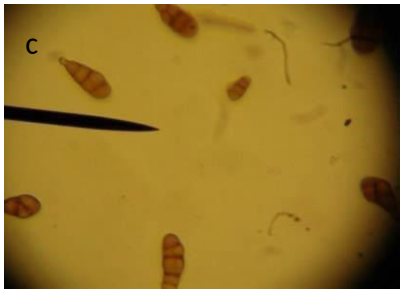
a: Culture	b: Hyhpae and conidia	c: Conidia
		

Plate.8 *Geotrichum* sp.

Culture

Hyhpae and arthrospores

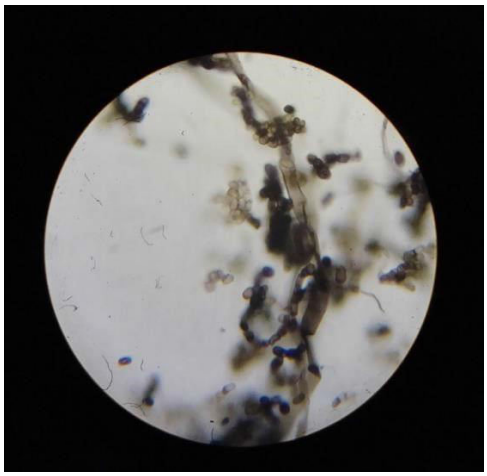


Plate.9 Antibacterial activity of endophytic extracts.

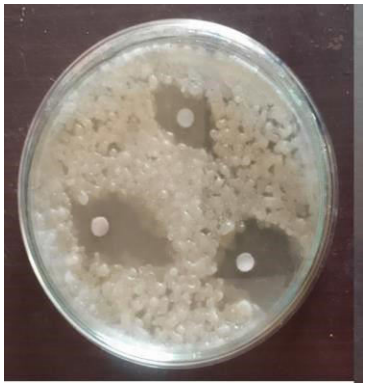
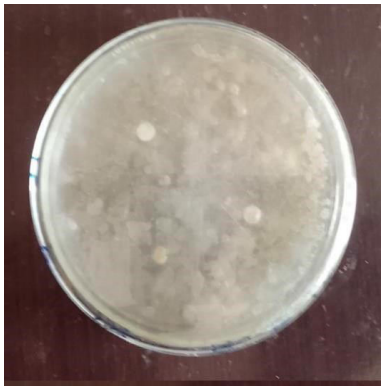
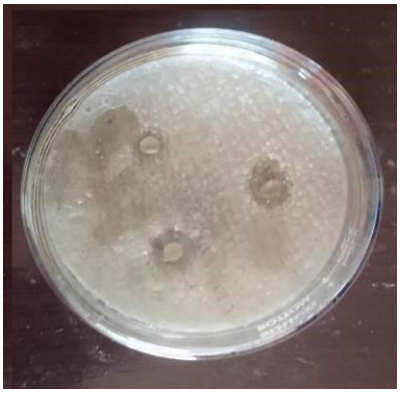
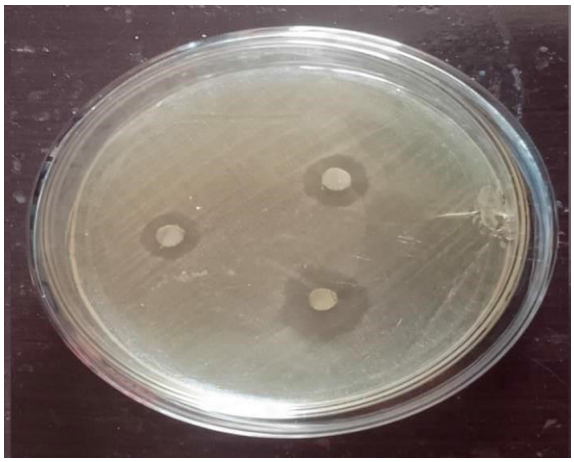
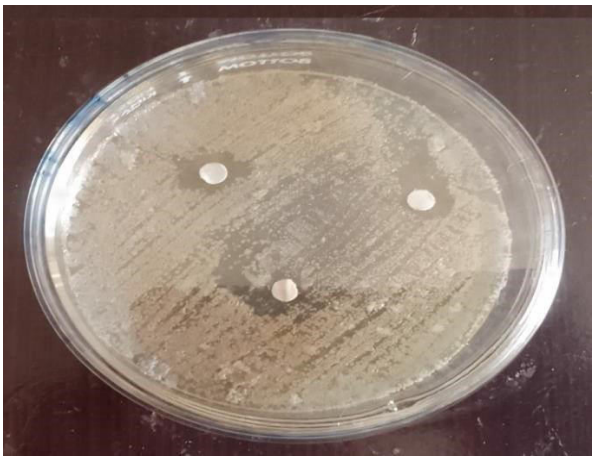
<table>
<tr><td colspan="3">EtOAc extracts against B. subtilis</td></tr>
<tr><td>Chaetomium sp.</td><td>A. niger</td><td>Geotrichum sp.</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="3">A. niger (MeOH extract against B, subtlis)</td></tr>
<tr><td colspan="3">  </td></tr>
<tr><td colspan="3">EtOAc extracts against S. aureus</td></tr>
<tr><td colspan="2">Geotrichumsp.</td><td>Alternaria sp.</td></tr>
<tr><td colspan="2">  </td><td>  </td></tr>
</table>

Plate.10 Antifungal activity of endophytic ethyl acetate extracts.

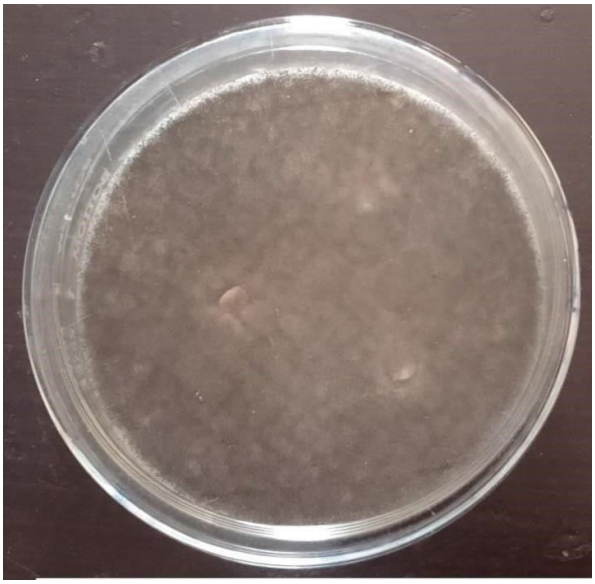
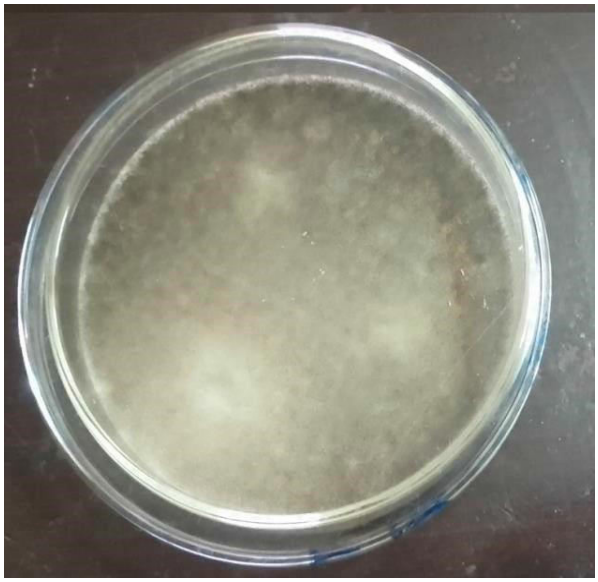
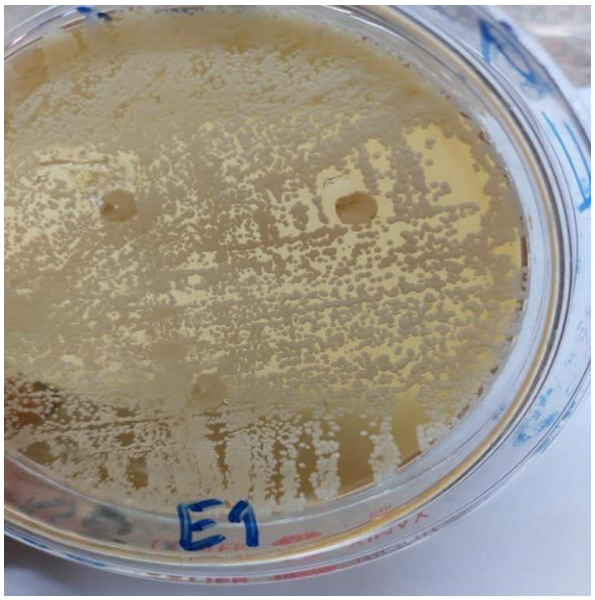
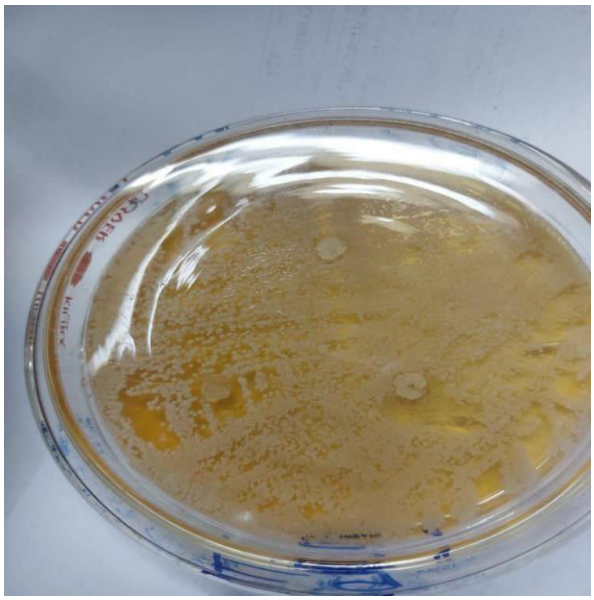
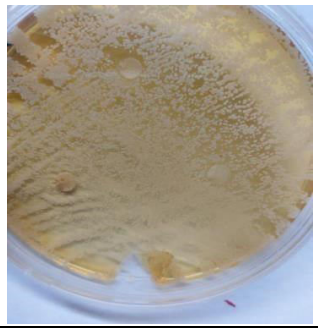
Against *A. niger*	
Chaetomium sp.	*Alternaria* sp.
	
Against *C. albicans*	
*Cladosporium s*p.	*Phoma* sp.
	

Plate.11 Antifungal activity of endophytic extracts and the +ve control Nystatin.

<table>
<tr><td colspan="3">Against C. albicans</td></tr>
<tr><td>Curvularia sp. (EtOAcextract)</td><td>Geotrichumsp. (EtOAcextract)</td><td>Cladosporium sp.(MeOH extract)</td></tr>
<tr><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="2">Against A. niger Against C. albicans</td><td>Nystatin (+ve control)</td></tr>
<tr><td colspan="2">  </td><td>  </td></tr>
</table>

In summary, it was clear that, ethyl acetate and methanolic extracts of endophytic fungi obtained in the present study showed a broad spectrum of antimicrobial activity. These differences in susceptibility could be due to the type of isolates and nature and level of the antimicrobial agents present in their extracts as well as their mode of action on different tested microorganisms. Also, the same endophytic fungi obtained from different plant parts of the same host plant showed different antimicrobial properties. This could be due to their variable ability to utilize nutritional substances in the host plant parts and consequently synthesize different types of secondary metabolites. The same was true for similar endophytic fungi species obtained from different host plants (Barbour *et al.,* 2004).

A total of 8 endophytic fungal isolates were isolated from the surface sterilized leaves, flowers and stems of *O. basilicum*. The fungal endophytes were identified based on morphological characters. The majority of the isolated endophytic fungi belonged to Hyphomycetes. Ethyl acetate and methanol extracts of the isolated fungi were rich in secondary metabolites and each species had its characteristic type of compounds. Crude metabolite extracts from these endophytes revealed variable antimicrobial activity. The extract by ethyl acetate of *Chaetomium* sp. showed very high antimicrobial activity against *B. subtilis* followed by ethyl acetate extract of *Geotrichum* sp. towards *S. aureus* and both extracts of *Phoma* sp. against *P. aeruginosa*. Thus, the antimicrobial activity exhibited by many crude extracts from these endophytic fungi suggested that further investigation of these extracts could lead to the identification of metabolites with the potential to treat some fungal and bacterial infections. Interestingly, some similarities were observed between the plant crude extracts and endophytic extracts, thus implying the existence of cooperative production of some secondary metabolites. The present study demonstrated that endophytes could be a key approach to search for bioactive molecules with interesting pharmaceutical applications.

Author Contributions

Amna Mohamed El Obeid Sewaikit: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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