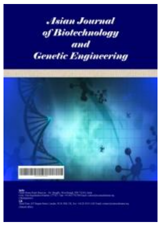

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Antimicrobial Activities and GC-MS Analysis of Endophytic Fungi Isolated from *Pluchea dioscoridis* and *Withania somnifera* Medicinal Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AHMES and YMS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MAH and EGED managed the analyses of the study. Author EGED carried out practical experiments, performed the statistical analysis managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Twenty three genera (46 species and 2 species variety) of endophytic fungi were isolated and identified from the leaves of *Pluchea dioscoridis* and *Withania somnifera* on GPY agar medium at 28°C. *Alternaria*, *Aspergillus*, *Cladosporium* and *Setosphaeria* were the most common genera isolated from *P. dioscoridis* and *W. somnifera*. The extracts of isolated endophytic fungi were tested for antimicrobial activities against nine strains of pathogenic bacteria and seven isolates of phytopathogenic fungi by disc diffusion method. Extracts of endophytic fungal showed antibacterial activity by different degrees ranged between highly, moderate, narrow, weak and non-active but, didn't had effect on tested fungal isolates. *Alternaria alternata* and *Microascus trigonosporus* were chosen from the most potent antibacterial fungi to determine the antibacterial ingredients by gas chromatography-mass spectrometry (GC-MS) analysis from which seventeen and twenty nine compounds were identified, respectively.

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Keywords: Pluchea dioscoridis; Withania somnifera; endophytic fungi; antibacterial activity; GC-MS analysis.

1. INTRODUCTION

Endophytes are microorganisms that infect living plant tissues without causing any visible disease symptoms, and live in symbiotic association with plants for at least a part of their life cycle [1,2]. The medicinal plants are known to be a resvoir for endophytic fungi which have the capability to produce the antimicrobial compounds, which can be used for pharmaceutical application [3,4] (*Pluchea dioscoridis* has a good reputation in folk medicine, used for rheumatic pains [5]. More than 91 pharmaceutical products are produced from *Withania somnifera* [6]. Wide range of activity including anticancer, antistress, anti-inflammatory, antitumor, antibiotic, anticonvulsant, CNS depressant, hepatoprotec-tive, immunomodulatory, insect antifeedant properties are reported [7,8].

Previous studies revealed that, diseases of fungal, bacterial, viral origin and in some instances even damage caused by insects and nematodes can be reduced following by the inoculation with endophytes [9,10]. Moreover, the endophytes exhibited antimicrobial sensitivity against bacteria and fungi [11], *Plasmodium* [12] and virus [13]. Numerous investigations have been carried out on the antimicrobial activity of endophytic fungi associated with various types of medicinal plants by several researches [14,15,16]. All the isolates of endophytic fungi which isolated from two medicinal plants showed varying degree of antimicrobial activity against the test pathogens [17]. Endophytes are a rich and reliable source of bioactive and chemically novel compounds with huge medicinal and agricultural potential. Hence, GC-MS technique used in identify these compounds [18,19,20].

This study aimed to isolate endophytic fungi associated with *P. dioscoridis* and *W. somnifera* plants, identify the fungal communities, detect their antimicrobial activity and identify its components.

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

A total of forty samples of medicinal plants from *P. dioscoridis* and *W. somnifera* (twenty samples from each plant) were chosen to isolate endophytic fungi, which collected from River Nile habitat in Qena. Each sample was put in a sterile polyethylene bag, sealed and kept in another bag which was also sealed. Prolonged transport in sealed plastic bags, perforated bags designed for vegetable storage work well for transport and temporary storage of most types of plant tissues [21]. Samples were transported in the same day to laboratory and were kept at (5°C) for mycological analysis.

2.2 Determination of Endophytic Fungi

Isolation of endophytic fungi from plant parts was done according to the method described by Rossman et al. [22]. First the plant leaves were rinsed gently in running water to remove dust and debris. After proper washing, leaves were cut into 1 cm in diameter and also 1 cm in length with mid rib. The surface sterilization was done by sequel immersion in 75% ethanol for 1 min followed by sodium hypochlorite (5% available chlorine) for 2 min and treated with 75% ethanol for 1 min. Later the segments were rinsed three times with sterile distilled water and dried between sterile filter paper. After proper drying four segments were inoculated on GPY plate amended with chloramphenicol. The plates incubated at 28±2ºC for 2-3 weeks then the developing fungi were counted and identified according to [23,24,25,26,27].

2.3 Crude Extracts from Fungi

Firstly, the endophytic fungi strains were grown in GPY medium at 28±2°C for 3-5 days. After that, 6 mm discs of the growth culture were introduced into 250 ml Erlenmeyer flasks containing 50 ml of GPY broth and incubated at 28±2°C on a rotary shaker at 160 rpm with normal daily light and dark periods for 10 days. At the end of incubation, the culture broth was separated from the mycelium by filtration through Whatman filter paper and the filtrate was extracted with chloroform (1:1, v/v) under constant shaking. The organic phase was concentrated under reduced pressure using a rotary evaporator at ±45°C and, finally, the concentrated extract was stored in a vacuum desiccator until constant weight [14].

2.4 Antimicrobial Assay

The antimicrobial activity test was carried out by disk diffusion method [28] against the following bacteria (*Enterobacter aerogenes*, *Enterococcus faecolli*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseadomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Shigella flexneri* and *Staphylococcus aureus*) and fungi (*Aspergillus niger, A. flavus*, *Alternaria alternata*, *A. citri*, *Cochliobolus spicifer*, *Ulocladium botrytis* and *Stemphylium vesicarium*). The crude extracts of endophytic fungi (0.001 g) dissolved with 1000 µl of dimethylsulfoxide (DMSO) and sterile paper disks (7 mm) were impregnated with 10µl of these extracts and placed on the petri dishes surface containing Luria Bertani agar medium (g/L; tryptone 10.0, yeast extract 5.0, NaCl 5.0, agar-agar 15.0) [29] previously spread with bacterial suspension. Subsequently, the petri were incubated at 37±2°C and the diameter of the inhibition zones was measured after 24 hr. For antifungal test, the fungal species were employed with GPY agar medium and the plates were incubated at 28±2°C up to 5-7 days [30]. Chloramphenicol and nystatin used as positive control for the bacterial and fungal strains, respectively.

2.5 Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Based on antibacterial results extracts of *Alternaria alternata* and *Microascus trigonosporus* were chosen randomly from highly active endophytic fungi to analyzed using the Thermo Scientific TRACE GC UltraTM gas chromatograph. It was fitted with a split-splitless injector and connected to an MS Polaris Q-Quadrupole Ion Trap (Thermo Electron) fused silica column VB5 (5% phenyl, 95% methylpolyxiloxane, 30 m with 0.25 mm i.d. film thickness 0.25 µm) (J & W Scientific Fisons, Folsom, CA). The injector and interface were operated at 250 and 300°C, respectively. The oven temperature was programmed as follows: 50°C raised to 250°C (4ºC/min) and held for 3 min. Helium was the carrier gas at 1 ml/min. The sample (1 µl) was injected in the split mode (1:20). MS conditions were as follows: ionization voltage EI of 70 eV, mass range 10 - 350 amu. The extracts of endophytic fungi components were identified by comparing their relative retention times and mass spectra with those of authentic samples (analytical standards from data base) [31].

3. RESULTS AND DISCUSSION

3.1 Mycobiota of *Pluchea dioscoridis* and *Withania somnifera* Plant

Forty six species and 2 varieties belonging to 23 genera were collected from 40 plant samples. These endophytic fungi recovered from *Pluchea dioscoridis* (9 genera and 15 species + 1 var.), and *Withania somnifera* (21 genera and 38 species +2 var.) on GPY agar medium at 28°C (Table, 1). The most common genera were *Alternaria* (5 species), *Aspergillus* (4 species and 1 variety), *Cladosporium* (4 species) and *Setosphaeria* (1 species). From the above genera, the most prevalent species were: *A. alternata*, *A. brassicola*. *A. citri*, *A. raphani*, *A. fumigatus*, *A. niger*, *C. uredinicola* and *Setosphaeria rostrata*. These species were isolated with different numbers and frequencies from various plants in many places of the world by several work [32,33,34,35,36]. Ding et al. [37] isolated from *Camptotheca acuminata* plant 26 endophytic fungi belonging to nine taxa including *Alternaria* which represented by 5 species from which, *A. alternata*, *A. brassicicola*, *A. citri* and *A. raphani* were the dominant species. Bharathidasan and Panneerselvam [38] isolated *A. niger* from *Avicennia marina* which occupied the second place in the frequency of colonization. Ramesha and Srinivas [39] Isolated endophytic fungi from different parts of *Plumeria acuminata* and *Plumeria obtusifolia* and identified them morphologically from which *Alternaria* sp., *Aspergillus* sp., *Chaetomium* sp., *Cladosporium* sp., *Cochliobolus* sp., *Curvularia* sp., mycelia sterilia, *Fusarium* sp. and *Penicillium* sp.

3.2 Antimicrobial Activities of Endophytic Fungi Isolated from *Pluchea dioscoridis* and *Withania somnifera*

The endophytic fungal extracts exhibited antibacterial activities with different degrees, while all tested isolates of endophytic fungi did not have any effect on phytopathogenic fungal species. Endophytic fungi had been reported as potential sources of various bioactive metabolites having therapeutic values [40,41,42].

3.3 Antibacterial Effect of Fungal Extract from *P. dioscoridis*

Endophytic fungal isolates which isolated from *P. dioscoridis* plant appeared inhibition effects on tested bacterial species by different degrees ranged from highly to moderate antibacterial activity (Table 2). Fourteen isolates of endophytic fungi showed highly antibacterial activity against 7 – 9 of tested bacterial species and these were *Alternaria alternata, A. brassicicola, Aspergillus niger*, *A. terreus* var. *auraus*, *Cladosporium cucumerinum*, *Microascus trigonosporus*, *Myrothecium* state of *Nectria bactridioides*, *Nigrospora sphaerica*, *Penicillium aurantiogriseum*, *P. chrysogenum*, *P. rubrum*, *Phoma glomerata*, *P. pomorum* and sterile mycelium white, with inhibition zone ranged from 8-14 mm. the remaining endophytic fungal extracts showed moderate antibacterial activity on tested bacterial species (5-7 speies) with inhibition zone ranging from 8 to 13 mm.

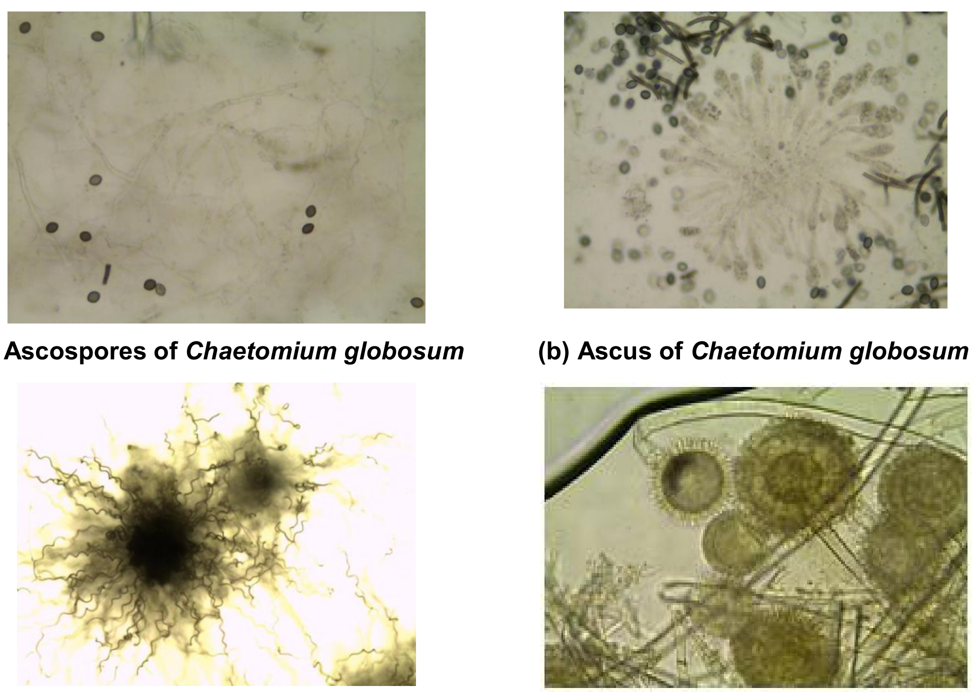
Table 1. Total counts (Calculated per 240 leaf segments), percentage of fungal counts (%TC, calculated per total fungi) and frequency of fungal species (% F, calculated per 20 samples) of various fungal genera and species recovered from leaves of *Pluchea dioscoridis* and *Withania somnifera*

Genera and species	*Pluchea dioscoridis*			*Withania somnifera*		
	Tc	%TC	%F	Tc	%TC	%F
Acremonium	0	0	0	10	4.50	20
A. butyri	0	0	0	1	0.45	5
A. cerealis	0	0	0	1	0.45	5
A. furcatum	0	0	0	1	0.45	5
A. kiliense	0	0	0	2	0.90	5
A. rutilum	0	0	0	5	2.25	5
Alternaria	4	9.10	10	112	50.45	85
A. alternata	3	6.81	10	49	22.07	70
A. brassicicola	1	2.27	5	22	9.90	50
A. citri	0	0	0	13	5.85	25
A. dianthi	0	0	0	1	0.45	5
A. raphani	0	0	0	27	12.16	45
Aspergillus	17	38.63	45	20	9.00	55
A. fumigatus	11	25.00	30	0	0	0
A. flavo-furcatis	0	0	0	1	0.45	5
A. niger	5	11.36	10	15	6.75	45
A. ohraceous	0	0	0	1	0.45	5
A. terreus var. auraus	1	2.27	5	3	1.35	15
Cadophora meleini	0	0	0	1	0.45	5
Chaetomium globosum	0	0	0	1	0.45	5
Cladosporium	2	4.54	10	10	4.50	25
C. cladosporioides	0	0	0	2	0.90	5
C. cucumerinum	2	4.54	10	0	0	0
C. sphaerospermum	0	0	0	1	0.45	5
C. spongiosum	0	0	0	1	0.45	5
C. uredinicola	0	0	0	6	2.70	25
Cochliobolus	0	0	0	8	3.60	20
C. bicolor	0	0	0	5	2.25	15
C. spicifer	0	0	0	3	1.35	10
Curvularia ovoidea	0	0	0	1	0.45	5
Emericella nidulans var. lata lata	0	0	0	1	0.45	5
Epicoccum purpurascens	0	0	0	3	1.35	10
Eurotium chevalieri	2	4.54	10	0	0	0
Memnoniella levispora	0	0	0	1	0.45	5
Microascus trigonosporus	1	2.27	5	0	0	0
Mucor hiemalis	0	0	0	2	0.90	10
Myrothecium	2	4.54	10	1	0.45	5
M. state of Nectria bactridioides	1	2.27	5	1	0.45	5
M. verrucaria	1	2.27	5	0	0	0
Nigrospora sphaerica	3	6.81	15	1	0.45	5
Penicillium	5	11.36	15	3	1.35	15
P. aurantiogriseum	3	6.81	5	0	0	0
P. chrysogenum	1	2.27	5	0	0	0
P. duclauxii	0	0	0	1	0.45	5

Continued…

Genera and species	*Pluchea dioscoridis*			*Withania somnifera*		
	Tc	%TC	%F	Tc	%TC	%F
P. erythromellis	0	0	0	1	0.45	5
P. funicolusum	0	0	0	1	0.45	5
P. rubrum	1	2.27	5	0	0	0
Phoma	4	9.10	15	1	0.45	5
P. glomerata	1	2.27	5	0	0	0
P. leveillei	0	0	0	1	0.45	5
P. pomorum	3	6.81	10	0	0	0
Quambalaria cyanbstens	0	0	0	1	0.45	5
Scopulariopsis brevicaulis	0	0	0	1	0.45	5
Setosphaeria rostrata	0	0	0	28	12.61	35
Stemphyllium	0	0	0	12	5.40	20
S. sarciniforme	0	0	0	2	0.90	5
S. vesicarium	0	0	0	10	4.50	20
Sterile mycelia	4	9.10	20	2	0.90	5
Sterile mycelium black	1	2.27	5	2	0.90	5
Sterile mycelium white	2	4.54	10	0	0	0
Sterile mycelium yellow	1	2.27	5	0	0	0
Ulocladium	0	0	0	2	0.90	10
U. botrytis	0	0	0	1	0.45	5
U. tuberculatum	0	0	0	1	0.45	5
Total account	44			222		
No. of genera	9			21		
No. of species	15+1var			38+2 var		

Occurrence Remarks: OR (out of 20 samples), H = high occurrence from 10-20 cases, M= moderate occurrence from 5-9 cases, L= low occurrence from 2-4 cases and R= rare occurrence 1 case



(a) Ascospores of *Chaetomium globosum* **(b) Ascus of *Chaetomium globosum***

(c) Ascomata *Chaetomium globosum* **(d) *Aspergillus niger***

Fig. 1 (a-c). Photomicrograph showing different stages of *Chaetomium globosum* and (d) *Aspergillus niger*

Table 2. Antibacterial effects of some species of endophytic fungi isolated from *Pluchea dioscoridis*

Endophytic species	Antibacterial activity								
	Enterobacter aerogenes	***Enterococcus faecolli***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Salmonella typhimurium***	***Shigella flexneri***	***Staphylococcus aureus***
Alternaria alternata[(a)]	9	9	8.5	10	8	9	10	10.5	8
A. brassicicola[(a)]	10	10	11	9	9	9	9	8	9
Aspergillus fumigatus[(b)]	8	N.I.	N.I.	10	8	9.5	8	9	N.I.
A. niger[(a)]	9	9	N.I.	9	8	10.5	8	8.5	N.I.
A. terreus var. *auraus*[(a)]	8	8	10.5	14	8	10	11.5	8	N.I.
Cladosporium cucumerinum[(a)]	9	9.5	11	9	8	9	9	8	8.5
Eurotium chevalieri[(b)]	10	9	10.5	10.5	N.I.	N.I.	N.I.	N.I.	8
Microascus trigonosporus[(a)]	10	8	8	11	8	8	9	8	11
Myrothecium state of *Nectria bactridioides*[(a)]	11.5	N.I.	8	11.5	10	N.I.	12.5	12	11.5
M. verrucaria[(b)]	10	N.I.	N.I.	11	8	N.I.	13	8	11
Nigrospora sphaerica[(a)]	9	8	N.I.	9	8	9	9	8	8
Penicillium aurantiogriseum[(a)]	11	8	8.5	9	8	10	8	10	8
P. chrysogenum[(a)]	10	10.5	11	10.5	9	9.5	9	8	8
P. rubrum[(a)]	10.5	9	N.I.	9	8	9.5	9	11	N.I.
Phoma glomerata[(a)]	10	10	11	9	9	9	9	8	8
P. pomorum[(a)]	9	9	11	10	10	9.5	9	9	8.5
Sterile Mycelium black[(b)]	10	N.I.	N.I.	11	9	N.I.	8	8	11
Sterile Mycelium white[(a)]	9	10	10.5	10	8	9	N.I.	N.I.	10
Chloramphenicol	37	35	12.5	32	20	32	32	34	32

N.I. = no inhibition; (a) highly antibacterial activity: Endophytic fungal extract inhibited growth of 7-9 of bacterial species tested. (b) Moderate antibacterial activity: Endophytic fungal extract inhibited growth of 5-6 of bacterial species tested

Table 3. Antibacterial effects of some species of endophytic fungi isolated from *Withania somnifera*

Endophytic species	Antibacterial activity							
	Enterobacter aerogenes	***Enterococcus faecolli***	***Escherichia coli***	***Klebsiella pneumonia***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Salmonella typhimurium***	***Shigella flexneri***	***Staphylococcus aureus***
Acremonium butyri [(b)]	8	N.I.	N.I.	10	9	N.I.	9.5	8	10
A. cerealis [(c)]	N.I.	20	7.5	N.I.	N.I.	N.I.	N.I.	17	13
A. kiliense [(b)]	8	11.5	N.I.	11.5	10	N.I.	10	N.I.	8
A. rutilum [(b)]	10	11.5	N.I.	11.5	N.I.	N.I.	10	9.5	N.I.
Alternaria alternata [(d)]	N.I.	N.I.	N.I.	8.5	N.I.	N.I.	N.I.	N.I.	10
A. citri [(e)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
A. raphani [(c)]	9	8	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	8
Aspergillus flavo-furcatis [(a)]	8	8	10	N.I.	8	8	9	9	10
A. niger [(d)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	12
A. ohraceous [(c)]	N.I.	N.I.	8	10	10	N.I.	9	N.I.	N.I.
A. terreus var. *auraus* [(a)]	9	9.5	10	N.I.	8.5	9	9.5	9	9
Cadophora meleini [(b)]	N.I.	N.I.	10	N.I.	8	8	8	N.I.	8
Chaetomium globosum [(d)]	11	N.I.	9	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Cladosporium spongiosum [(b)]	9	N.I.	N.I.	12	9	N.I.	N.I.	8	12
C. uredinicola [(a)]	8	8	9.5	N.I.	9	10	8	10	8
Cochliobolus bicolor [(d)]	9	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
C. spicifer [(d)]	10	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Curvularia ovoidea [(d)]	N.I.	N.I.	8	N.I.	N.I.	N.I.	N.I.	11.5	N.I.
Emericella nidulans var. *lata* [(e)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Memnoniella levispora [(d)]	N.I.	N.I.	8	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Mucor hiemalis [(d)]	N.I.	8	8	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Myrothecium state of *Nectria bactridioides* [(a)]	8	9	10	N.I.	8	8	8	8	8
Nigrospora sphaerica [(c)]	N.I.	N.I.	8	N.I.	8	N.I.	N.I.	N.I.	8
Penicillium erythromellis [(b)]	10	N.I.	N.I.	11.5	8	N.I.	9	N.I.	8
P. funicolusum [(d)]	N.I.	N.I.	8.5	N.I.	N.I.	N.I.	11.5	N.I.	N.I.
Quambalaria cyanbstens [(e)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Scopulariopsis brevicaulis [(b)]	N.I.	8.5	N.I.	N.I.	8	9	8	9	8
Setosphaeria rostrata [(e)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Stemphyllium sarciniforme [(a)]	8.5	8	10	N.I.	8	9	8	N.I.	8
Sterile Mycelium black [(e)]	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.	N.I.
Ulocladium tuberculatum [(a)]	11	N.I.	8	11.5	9	N.I.	10	11	8
Chloramphenicol	37	35	12.5	32	20	32	32	34	32

N.I. = no inhibition (a) Highly antibacterial activity: Endophytic fungal extract inhibited growth of 7-9 of bacterial species tested. (b) Moderate antibacterial activity: Endophytic fungal extract inhibited growth of 5-6 of bacterial species tested. (c) Narrow antibacterial activity: Endophytic fungal extract inhibited growth of 3-4 of bacterial species tested. (d) Weak antibacterial activity: Endophytic fungal extract inhibited growth of 1-2 of bacterial species tested. (e) None-active antibacterial activity: Endophytic fungal extract did not inhibit growth of any bacterial species tested

3.4 Antibacterial Effect of Fungal Extract from *W. somnifera*

The data obtained in Table 3 showed that, Six endophytic fungal extracts showed highly antibacterial activity against tested bacterial species and these were: *Aspergillus flavo-furcatis, A. terreus* var. *auraus*, *Cladosporium uredinicola*, *Myrothecium* state of *Nectria bactridioides*, *Stemphyllium sarciniforme* and *Ulocladium tuberculatum* with inhibition zones ranged from 8 to 10 mm. Seven endophytic fungal extracts including *Acremonium butyri*, *A. kiliense*, *A. rutilum*, *Cadophora meleini*, *Cladosporium spongiosum*, *Scopulariopsis brevicaulis* and *Penicillium erythromellis* showed moderate antibacterial activity on tested bacterial species with inhibition diameter ranged from 8 to 12 mm. The remaining fungal species showed narrow, weak and no inhibitory effects on the growth of different species of bacteria (Table 3).

The above results were agreement with obtained by several workers [17,30,34,37,43,44]. Idris et al. [45] which assessed the extracts of endophytic fungi isolated from medicinal plant (*Kigelia Africana*) for antibacterial activity against three standard pathogenic bacterial strains: *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli.* Most of the extracts showed *in vitro* inhibition of bacterial growth. Ramesha and Srinivas [39] screened 24 endophytic fungi from *P. obtusifolia* for antimicrobial activity, 16 endophytic isolates demonstrated activity. The antimicrobial potential of endophytic fungi from *P. acuminata* was assessed and it was found that 10 isolates from 17 endophytic fungi demonstrated activity against the pathogens.

3.5 Gas Chromatography-mass Spectrometry (GC-MS) Analysis

Eight extracts of endophytic fungi isolated from *P. dioscoridis* plant were completely inhibited all tested bacterial species, from which *Alternaria alternata* and *Microascus trigonosporus* were chosen randomly to gas chromatography coupled with mass spectrometry (GC-MS) analysis to determine the active antibacterial ingredients. Seventeen and twenty-nine compounds were identified from extracts of *A. alternata* and *M. trigonosporus*, respectively (Tables 4 and 5). From these compounds, 2,4-Bis (1,1-dimethylethyl) phenol, Phenol, 3,5-dimethoxy; Phloroglucinol dimethyl ether, Hexadecanoic acid; Palmitic acid and Isoquinoline, 2-(2,2-dimethyl-1-oxopropyl)-1,2,3,4-tetrahydro-6,7-dimethoxy were previously reported as antibacterial compounds [46,47,48,49]. Phenol, 3, 5-dimethyl-2-nitro, Phenol, 2-methyl-5-(1-methylethyl); Carvacrol, 4-Chromanone, 7-methoxy-2,2-dimethyl and 7-methoxy-6-ethoxy-2,2-dimethyl-2H- chromene possessed antimicrobial potential [50,51,52]. Also, P-Hydroxyphenol; Arctuvin inhibit mitotic division of cell and considering bacteriostatic agents [53].

Table 4. GC/MS Analysis of *Alternaria alternata* extract

Retention time (min)	Name of compound	% of total	Molecular weight
16.481	2,4-Bis(1,1-dimethylethyl) phenol	0.81	206.167
21.304	Pyrrolidino[1,2-a]piperazine-3,6-dione	1.02	154.074
22.311	Phenol, 3,5-dimethoxy; Phloroglucinol dimethyl ether	0.30	154.063
23.21	Pyrrolidine, 1,5-dimethyl-3,3-diphenyl-2-ethylidene	0.15	277.183
23.21	Isoquinoline, 2-(2,2-dimethyl-1-oxopropyl)-1,2,3,4-tetrahydro-6,7-dimethoxy	0.15	277.168
23.313	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	0.80	210.137
23.456	Benzyl alcohol, 3-hydroxy-4-methoxy	0.72	154.063
23.599	Hexadecanoic acid; Palmitic acid	1.35	256.24
25.47	Octadecanoic acid, methyl ester	0.12	298.287
29.372	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)	0.34	244.121
30.145	Phenol, 2-amino-5,6-dicyano-4-methoxy	0.86	189.054
30.374	3-benzyl-1,4-diaza-2,5-dioxobicyclo[4.3.0]nonane	0.77	244.121
30.534	trans-3-methylthiochroman-4-carbonitrile	2.92	189.061
30.534	3-Methylphenol	2.92	108.058
32.01	1,2-Benzenedicarboxylic acid, diisooctyl ester	0.11	390.277
32.376	Phenol, 2-(1-methylethoxy)-, methylcarbamate	0.15	209.105
32.897	4-Chromanone, 7-methoxy-2,2-dimethyl	0.37	206.094

Table 5. GC/MS analysis of *Microascus trigonosporus* extract

Retention time (min)	Name of compounds	% of total	Molecular weight
16.469	2,4-Bis(1,1-dimethylethyl)phenol	0.85	206.167
20.52	Mononoylphenol	0.06	220.183
20.566	3-(2-Pyrrolidinyl)propanoic acid	0.33	143.095
20.566	Phenol, 3,5-dimethyl-2-nitro	0.33	168.079
20.967	Phenol, 2-methyl-5-(1-methylethyl); Carvacrol	0.13	150.104
21.052	7-methoxy-6-ethoxy-2,2-dimethyl-2H-chromene	0.11	234.126
21.052	Phenol, 2,6-bis(1,1-dimethylethyl)-4-ethyl	0.11	234.198
21.281	Cycloglycylproline; Glycyl-L-proline lactam	0.80	154.074
21.916	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	1.11	210.137
22.3	Phenol, 3,5-dimethoxy; Phloroglucinol dimethyl ether	0.32	154.063
22.946	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl	0.31	220.183
23.204	2-Pyrroline-3-carboxylic acid, 4-(4-chlorobenzylidene)-2-methyl-5-oxo-, methyl ester	0.20	277.051
23.41	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a;1',2'-d]pyrazine	0.37	250.168
23.41	2-Pyrrolidinylmethanamine	0.37	100.1
23.41	2-Pyrrolidinylmethanol	0.37	101.084
23.444	3,9-diazatricyclo[7.3.0.0(3,7)]dodecan-2,8-dione	0.40	194.106
23.444	5,10-Diethoxy-2,3,7,8-tetrahydro-1H,6H-dipyrrolo[1,2-a:1,2-d]pyrazine	0.40	250.168
24.085	Phenol, 3-methoxy-2,4,6-trimethyl	0.20	166.099
26.368	p-Phenylphenol; Paraxenol	0.49	170.073
30.356	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)	1.01	244.121
30.62	1-(1-Methylvinyl)pyrrolidin-2-one	0.01	125.084
32.376	P-Hydroxyphenol; Arctuvin	0.17	110.037
32.903	Phenol, 4,4'-methylenebis[2,6-bis(1,1-dimethylethyl)	0.18	424.334
32.903	Coumarin-6-ol, 3,4-dihydro-4,4,5,7-tetramethyl-, methylsulfate(ester)	0.18	298.087
33.91	Pyrimidine-2(1H)-thione, 4,4,6-trimethyl-1-(1-phenylethyl)	0.46	260.135
33.91	4(1H)-Pyrimidinone, 2-amino-6-hydroxy-5-methyl	0.46	141.054
33.91	4-Amino-5-methyl-2(1H)-pyrimidinethione	0.46	141.036
34.299	5H-Pyrrolo(3,2-d)pyrimidine-2,4-diamine	0.40	149.07
35.157	5-Methyl-7-amino-s-triazolo(1,5-a)pyrimidine	0.77	149.07

A number of possible mechanisms are suggested for the antibacterial activity of fungal extract involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability, alteration of protein structure and finally ion leakage from the cells [54,55,56]. Joseph and Priya [57] reported that many antimicrobial compounds isolated from endophytes, belonged to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinones, and flavonoids. [48] reported that various heterocyclic compounds have shown antimicrobial potential and quinoline is one of the most promising heterocyclic nuclei having prominent antibacterial and antifungal activity. It is known that certain natural and synthetic chromene derivatives possess important biological activities such as antimicrobial [52].

4. CONCLUSION

Endophytic fingi commonly present in almost all plants are frequently considered a rich source of bioactive metabolites which used as antibacterial, anticancer, antifungal or antitumor. In this study endophytic fungi have potential antibacterial activities against 9 pathogenic bacteria species.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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