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Suppression of Phytopathogenic Fungi by Plant Extract of Some Weeds and the Possible Mode of Action

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MH, SAA and AAS designed the study, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors MH, SAA and SuAA performed the statistical analysis and managed the analyses of the study. Authors AAS and MFMM managed the systematic classification of the plant species. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The goal of this work was to emulate the antifungal properties of some newly collected weeds against important phytopathogenic fungi: *Pythium ultimum, Penicillium expansum* and *Fusarium solani*. The mode of action through which the extracts could affect the target fungi was studied.
Place and Duration of Study: Department of Biology, Faculty of Science, King Khalid University, Saudi Arabia, between February, 2014 and May, 2015.
Methodology: Ten plant species were obtained from Aseer region. The plant extract obtained by ground dry plant material to be a fine powder and shaking in chloroform, ethanol or hexane. Screening of antifungal activity of the plant extracts was carried out against the phytopathogenic fungi grown on Czapek's agar medium using disc diffusion method and the minimum inhibitory

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concentration was determined. The treated fungal samples were examined and photographed using the scanning electron microscope to define the alteration and malformation in the fungal hyphae. The chemical composition of the most active plant extracts was determined using gas chromatography–mass spectrometry (GC–MS).
Results: Preliminarily test of the crude extract of the weed plants showed antifungal activity against all organisms but in various extent. The crude extract of *Foeniculum vulgare* showed the highest antifungal activity against the three phytopathogens. Scan electron microscopy (SEM) approved a clear morphological malformation in the hyphae and spores supported the assumption that the mechanism involved in killing the fungus includes the altering of cell membrane permeability that leads to the plasmolysis of the cells and changing their shape. Gas chromatography–mass spectrometry (GC-MS) analysis results showed that many aliphatic and aromatic compounds were identified in the different extracts. The main observation was the number and types of the detected compounds have greatly depended on the type of the extract. We assume that many compounds in each extract act in synergetic effect to destruct the fungal cell integrity and involved in their death.
Conclusion: The plant extract of *Foeniculum vulgare* is a promising natural product that could be applied in control of many phytopathogenic fungi as an alternative to hazard chemical fungicides.

Keywords: Antifungal; Fusarium; Penicillium; phytopathogens; Pythium.

1. INTRODUCTION

Weed plants represent an important wealth and economic component of biodiversity. It is essential to make the complete record of the medicinal and weed component of the flora of any country for sustainable use [1]. Some of the medicinal and weed plants are used in traditional medicine in various parts of the world to treat [2,3,4]. Recent research has focused on the role of biological natural compounds to treat many diseases, and overcoming the resistance of new mutants against chemical antibiotics [5]. So, seeking new natural antimicrobial agents has become more important than any time before. Plants with antimicrobial activity are also known to be numerous, yet prior to a decade ago, minimal research had been conducted in the area of antifungal medicinal plants [6,7]. In addition, the suppression of phytopathogenic fungi via application of the plant extract had a very little consideration of research. Use of natural products like plant extracts for the management of fungal diseases in plants is considered as a good alternative method to the synthetic fungicides. Because such natural preparations have less negative impacts on the human and environment [8]. Where, the use of biofungicide based on antagonistic microbes takes a long time from discovery to commercial application, in addition to their expected negative impact on the non-target microorganisms. However, application of plant extract in control of the plant disease offers an effective, safe and cheap alternative.

Weed flora of the Kingdom of Saudi Arabia (KSA) includes about 2100 plant species, from which 35 plant species are endemic. Distribution of these plants species is concentrated in the dry areas of the Kingdom, mainly in low-lying areas where water collects after the rain. Some plant families in Saudi Arabia was proven for their medical effect [6]. Variation in climate, geography, and rainfall in Saudi Arabia led to the diversity of vegetation and make the vegetation differs from region to another [9]. Especially, the south-western part of the KSA that has a unique environmental climate and terrain. These unique conditions could accommodate the growing of specific groups of plants that may have their special phytochemical characteristics.

This work introduces many weeds for biological control of phytopathogenic fungi as well as their mechanism of action as a main aim. It provides considerable information about the antifungal properties, especially phytopathogens, of the weed plant distributed in Aseer region, Saudi Arabia. We assume that the success of this biotechnology could change the strategy of biological control of plant diseases in the near future.

2. MATERIALS AND METHODS

2.1 Tested Plants

Ten plant species (*Forsskaolea tenacissima, Xanthium spinosum, Abutilon pannosum*, *Solanum incanum*, *Foeniculum vulgare*, *Sisymbrium erysimoides, Amaranthus hybridus, Argemone ochroleuca*, *Micromeria biflora* and *Euphorbia triaculeata*) were obtained from different areas of Aseer region (during the period of July-September, 2013). Fresh plant samples

were collected and put in sterilized polyethylene bags and transferred to the laboratory. The plants were identified on the species level by a plant taxonomist with aiding of taxonomical plant guides [10].

2.2 Preparation of the Crude Extract

Enough fresh quantity of each plant species (stem and leaves) was washed thoroughly several times with distilled water and dried in shade and open air at room temperature (25–30℃) for 2 wk. The plant material was ground in a hammer mill to be a fine powder. Fifty grams of each plant was put in a 500-mL flask, and 100 mL of the desired solvent (chloroform, ethanol or hexane) was added. The flask was shaking gently for three days. The produced extract was filtered through filter paper, dried in vacuum at 40ºC, and the obtained dry material was weighed. The crude extract of each plant species was made by dissolving a known weight in a definite volume of dimethyl sulfoxide (DMSO) to give known concentration. The produced extract was sterilized by passing through a 0.45-µm cellulose acetate membrane. All extracts were maintained under cooling (4ºC) until use [11].

2.3 Test Microorganisms

Pythium ultimum, *Penicillium expansum*, *Fusarium solani* were selected as common plant pathogens. These fungi were isolated and identified in our laboratory at the biology department, faculty of science, King Khalid University during previous works. Czapek's agar medium was used for growing the phytopathogenic fungi. This medium is composed of glucose, 10 g; $NaNO_3$, 3 g; $K2HPO_4$, 1 g; Mg SO_4, 0.5 g; KCl, 0.5 g; agar, 15 g and distilled water 1 L and adjusted to pH 6.5 before sterilization at 121ºC for 20 min.

2.4 Screening of Antimicrobial Activity of the Plant Extracts

Antifungal activity of the plant extracts was carried out using disc diffusion method as described by Murray et al. [12] with minor modification. Test plates (diameter: 7 cm) were prepared with Czapek's agar medium and inoculated in surface with a spore suspension in sterile dissolution of 0.9% saline. The concentration was adjusted to 10^4 CFU/mL. Sterile paper disks (diameter: 5 mm), impregnated with 10 µL of a desired plant extract, were applied over the test plates. The negative control was prepared using the solvent, in which the plant extract was dissolved. Standard antifungal Flucoral (10 µg/disc) was used as positive control. Flucoral is an antifungal drug related to the group of triazole. It mainly Inhibits the synthesis of ergosterol disrupting the permeability of the cell wall. It is commonly used against the dermatophytes. The plates were incubated for 3 d at 27℃, and the inhibition zone was recorded. The test was repeated twice in three replicates.

2.5 Minimum Inhibitory Concentration (MIC)

The MIC was determined according to Kariba et al. [13]. Briefly, filter paper discs (diameter, 5 mm) were impregnated with 60 µL of the reconstituted samples at concentrations of (25–150 mg/mL). The discs were transferred aseptically into Petri plates containing Czapek's agar medium that were inoculated previously with the test microbe. The MIC was considered as the lowest concentration that produced a visible zone of inhibition. For each test, triplicates were used. The plates were incubated at 37℃ for 24–48 h.

2.6 Studying the Mode of Action of the Most Active Extract

2.6.1 Scanning electron microscopy

Fungal cultures treated with different concentration of the most active plant extract, based on the screening test, were examined by the scanning electron microscopic (SEM) and photographed. Segments of 5×10 mm were cut from growing cultures and promptly placed in vials containing 3% glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 4℃. Samples were kept in this solution for 48 h for fixation and then washed with distilled water three times for 20 min. The sample was dehydrated in an ethanol series (30, 50, 70, and 95%), for 20 min in each alcohol dilution and finally with absolute ethanol for 45 min. Samples were dried at the critical point in liquid carbon dioxide. Following drying, the prepared samples were mounted on standard SEM stubs using double-stick adhesive tabs and coated with gold-palladium electroplating (60 s, 1.8 mA, 2.4 kV) in a Polaron SEM coating system sputter coater. All samples were viewed in JEOL JSM 5300 scanning electron microscope, and the samples were examined at 15 kV and photographed.

2.6.2 GC–MS analysis of the plant extracts

The chemical composition of the most active plant extracts was determined using gas chromatography–mass spectrometry (GC–MS). One µL of solvent containing the plant extract was injected into the GC–MS (6890 N/5975B). The HP-5MS column was 30 m in length, 0.25 mm i.d., and 0.25 mm in thickness. The carrier gas was helium with average velocity 36 cm sec^{-1} and flow 1 mL min^{-1}. The operating condition of GC oven temperature was maintained as follows, initial temperature 40℃ for 9 min, 150℃ for 8 min, at 15℃ min^{-1} up to final temperature 310℃ with isotherm for 3 min at 25℃ min^{-1}. The injector and detector temperatures were set at 250 and 280℃, respectively, according to the standard method 8270 EPA [14]. Identification of the components of the prepared extract was assigned by comparison of their retention indices, relative to a series of n-alkane indices on the capillary column and GC–MS spectra from the Wiley 6.0 MS data.

2.7 Statistical Analysis

Treatments were arranged in a completely randomized design. Analysis of variance was performed using the SPSS software package. Analysis of variance (ANOVA) was performed on the data to determine the least significant difference (LSD) among treatment at $P < 0.05$ and Duncan's multiple range tests were applied for comparing the means.

3. RESULTS

3.1 Antifungal Activity of the Testes Plants

Results of the screening test showed that out of 33 tested plants, only 10 species had antifungal activity against the phytopathogenic fungi: *Pythium ultimum, Penicillium expansum* and *Fusarium solani.* Data in Table 1 demonstrated that the crude extract of the 10 plants suppressed the three phytopathogenic fungi but in various extent. Chloroform was the best extractant that inhibited the three fungi and produced the widest inhibition zone (IZ) in almost cases. The plant extract of *Forsskaolea tenacissima* was the most effective among the ten plants. It inhibited the three fungi and produced inhibition zone ranged from 4.20-5.46 cm in diameter. *Penicillium expansum* was the most sensitive fungus to the chloroform extract of this plant. Hexane was the second most effective extract and exerted its maximum inhibitory effect against *Pythium ultimum* (IZ= 4.23 cm). Ethanol extract of the same plant produced IZ 3.43–3.53 cm. *Xanthium spinosum, Abutilon pannosum* and *Solanum incanum* had a good inhibitory effect against the three fungi. However, the chloroform extract was the best extractant that produced IZ more than 4.0 cm against all fungi. *Penicillium expansum* was the most sensitive fungus to the chloroform extract of *X. spinosum* (IZ=5.30 cm) while it showed a slight resistance to the same extract of *A. pannosum* (IZ=4.06 cm). The lowest IZ was obtained in the case of *P. ultimum* when it was treated with the ethanolic extract of *S. incanum. F. solani* was the most resistant fungus against the hexanic extract of *A. pannosum* (IZ=2.56 cm). *Foeniculum vulgare* and *Sisymbrium erysimoides* exhibited a moderate inhibitory effect against the tested three fungi. The measurement of IZ produced, as the result of application of the crude extract of these plants, did not exceed 3.33 cm. The ethanolic extract *F. vulgare* was the most effective one against *F. soalni* (IZ=3.33 cm) while, hexanic extract of *S. erysimoides* was the most effective against *P. ultimum* (IZ=3.3 cm). *Amaranthus hybridus*, *Argemone ochroleuca, Micromeria biflora* and *Euphorbia triaculeata* displayed a weak antifungal activity against the target fungi. Inhibition zone produced as the result of application of any extractant of these plants did not exceed 1.56 cm. The lowest effect was accomplished by the hexanic extract of *M. biflora.*

3.2 Minimum Inhibitory Concentration (MIC)

Based on their effectiveness to suppress the three fungal pathogen, six plants were selected to determine their MIC (Table 2). The results indicated the extract of *F. tenacissima* was the best one that suppressed the three fungi in very low concentration, however, chloroform was the most effective extractant among the three applied solvents. *Pythium ultimum* was the most sensitive fungus to this extract that was inhibited by a concentration of 0.021 mg/mL, followed by *P. expansum* (0.091 mg/mL) and *F. solani* (0.093 mg/mL). The crude extract of *Xanthium spinosum* inhibited the three fungi in a higher concentration compared with this achieved in the case of *F. tenacissima*. The MIC of *X. spinosum* ranged between 0.112 mg/mL and 0.134 mg/mL when chloroform was the extracting solution. When hexane was applied

as a solvent, the MIC was 0.111–0.213 mg/mL, however, MIC was 0.104–0.221 mg/mL in the case of ethanol. *P. expansum* was the most sensitive fungus that was inhibited by 0.104 mg/mL of ethanol extract of this plant. The MIC of *Solanum incanum* fluctuated between 0.111 mg/mL and 0.316 mg/mL. The most sensitive organism to this extract was *P. ultimum,* which was inhibited by 0.111 mg/mL of chloroform extract. MIC of the other three plants; *Abutilon pannosum, Foeniculum vulgare* and *Sisymbrium erysimoides* ranged from 0.213 to 0.491 mg/mL, from 0.210 to 0.565 mg/mL, and from 0.247 to 0.401 mg/mL, respectively.

3.3 Scanning Electron Microscopy (SEM)

The treated hyphae of *F. solani, P. expamsum* and *P. ultimum* with the crude extract of *F. tenacissima* were examined by the scanning electron microscope (SEM) to give a clear image about how the plant extract could alter the fungal cell. Results established a clear difference between the treated and untreated mycleia. The untreated mycelia *F. solani* were appeared as well-developed, inflated having a smooth wall and definite septation (Fig. 1A, B). Conversely, the treated mycelia with the crude extract of *F. tenacissima* showed plasmolysis, distortion and squashing. Almost hyphae were appeared empty, collapsed and completely dead (C–F). Fig. 2 showed the scanning electron micrographs of *P. expansum* that was treated with the same crude. The untreated mycelia were well-developed inflated having normal wall and normal conidia (A and B), however, the treated mycelia showing plasmolysis, distortion, squashing and collapsing (C–F). Amplification of the mycelia clearly approved their death as the final step.

Table 1. Antifungal activity of crud extract of the tested plants against different microorganisms (inhibition zone in cm)

Fusarium solani	*Penicillium expansum*	*Pythium ultimum*	Solvent	Plant species
4.20	5.46	5.26	Chloroform	*Forsskaolea tenacissima*
3.56	3.60	4.23	Hexane	
3.50	3.53	3.43	Ethanol	
4.13	5.30	4.26	Chloroform	*Xanthium spinosum*
4.13	4.20	3.46	Hexane	
4.53	4.43	3.43	Ethanol	
4.30	4.06	4.23	Chloroform	*Abutilon pannosum*
2.56	3.30	2.93	Hexane	
3.96	3.73	3.03	Ethanol	
4.26	4.53	4.16	Chloroform	*Solanum incanum*
3.56	3.06	3.13	Hexane	
3.46	3.13	1.40	Ethanol	
2.96	2.10	3.00	Chloroform	*Foeniculum vulgare*
2.16	2.96	2.16	Hexane	
3.33	1.43	2.63	Ethanol	
2.80	3.16	3.20	Chloroform	*Sisymbrium erysimoides*
2.56	2.90	3.30	Hexane	
2.93	3.06	3.10	Ethanol	
0.70	0.56	0.93	Chloroform	*Amaranthus hybridus*
0.93	0.80	0.86	Hexane	
1.40	1.00	0.96	Ethanol	
0.66	0.67	1.16	Chloroform	*Argemone ochroleuca*
0.60	0.54	1.56	Hexane	
0.76	0.55	1.13	Ethanol	
0.63	0.93	0.93	Chloroform	*Micromeria biflora*
0.50	0.56	0.53	Hexane	
0.66	0.66	0.73	Ethanol	
0.54	0.66	0.83	Chloroform	*Euphorbia triaculeata*
0.73	0.83	0.86	Hexane	
0.63	0.80	0.60	Ethanol	
6.00	5.80	6.20	-	Flucoral
1.223	1.474	1.486		LSD ($P < 0.05$)

Table 2. Minimum inhibitory concentration (MIC) of crude extract of the tested plant against different microorganisms (concentration in mg/mL)

Fusarium solani	*Penicillium expansum*	*Pythium ultimum*	Solvent	Plant species
0.093	0.091	0.021	Chloroform	*Forsskaolea tenacissima*
0.109	0.103	0.108	Hexane	
0.106	0.105	0.109	Ethanol	
0.112	0.112	0.134	Chloroform	*Xanthium spinosum*
0.113	0.111	0.213	Hexane	
0.151	0.104	0.221	Ethanol	
0.213	0.213	0.381	Chloroform	*Abutilon pannosum*
0.342	0.491	0.319	Hexane	
0.292	0.216	0.214	Ethanol	
0.125	0.121	0.111	Chloroform	*Solanum incanum*
0.291	0.316	0.209	Hexane	
0.311	0.301	0.210	Ethanol	
0.232	0.253	0.210	Chloroform	*Foeniculum vulgare*
0.412	0.411	0.427	Hexane	
0.565	0.391	0.456	Ethanol	
0.247	0.267	0.247	Chloroform	*Sisymbrium erysimoides*
0.315	0.365	0.341	Hexane	
0.401	0.347	0.367	Ethanol	
0.013	0.012	0.011		LSD ($P < 0.05$)

The mycelia have clearly seemed as devoted of cytoplasm. Electron micrographs of *P. ultimum* that was treated with the crude extract of the same plant approved the fungitoxic effect of this extract on the mycelia of this fungus (Fig. 3). The untreated mycelia had a healthy appearance, inflated having a normal wall and the sporangia were also well developed (A and B). But, the treated mycelia were plasmolyzed, shrunk and crimpy (C–F). Then, the hyphae become completely flat, empty and dead. Sporangia were clearly affected by the treatment and they are cut or burst.

3.4 GC-Mass Analysis of the Plant Extracts

The crude extract of *Forsskaolea tenacissima* in different solvents was analyzed using GC-MS to explore their fine chemical composition that may help in understanding the mode of action of these plant extracts against the microorganisms. Result exposed the presence of 38 chemical compounds in the three solvents. These compounds included aliphatic and aromatic components including; alcohols, alcohol derivatives, carboxylic acids, ketones, aldehydes, esters, sulfur compounds, amino compounds and cholesterol derivatives. Chloroform extract had 15 compounds that represent the highest number among the three solvents (Table 3; Fig. 4). The major components in this extract were dimethyl sulfone (30.731%), dimethyl sulfide (12.642%), butane, 2,2-dimethyl (8.179%), nonane, 1-iodo (7.614%), isopropyl lactate (7.087%), 6-fluoro-2-trifluoromethylbenzoic acid, 3-fluorophenyl ester (6.911%) and 4-heptanone, 2-methyl (6.467%). However, the other 8 compound were detected in lower concentration (1.363% – 6.349%). Hexane extract contained 13 compounds. The main component in this extract was 2-hexaneone, 3,3-dimethyl (20.131%), ethanone, 1-(3-ethyloxiranyl) (14.652%), docosane (12.868%). The 11 compounds remaining have the concentration ratios vary between (1.108% – 11.423%) (Table 4, Fig. 5). Analysis of ethanol extract of *F. tenacissima* approved the presence of 12 compounds. The major component in this extract was dimethyl sulfone (22.63%), 4H-1,3,2-dioxaborin, 6-ethenyl-2-ethyl-4-(2-methylpropyl) (22.09%), diethyl phthalate (11.58%), oxalic acid, isobutyl pentyl ester (9.368%), undecane (6.288%), while the lowest one was cholesta-8,24-3-ol, 4-methyl,(3.beta, 4.alpha.) (2.012%) (Table 5, Fig. 6).

4. DISCUSSION

Forsskaolea tenacissima is a member of the non-stinging nettles genus *Forsskaolea* and is in the same family as the stinging kind, urticaceae. Described as "looking like a tough character that does not want or need a caress", *F. tenacissima* makes its home where not many plant species survive, in stony soils, road edges, in the gravel wadi and "in the rock crevices and water-receiving depressions" above the stone pavements of the Hamadas [15].

Table 3. GC-MS analysis of chloroform extract of *Forsskaolea tenacissima*

Percentage (%)	Retention time	Compound name
12.642	6.084	Dimethyl sulfide
6.349	6.300	Ethylbenzene
30.731	7.060	Dimethyl sulfone
5.408	8.159	3-Hexaneol
7.087	8.564	Isopropyl lactate
8.179	13.646	Butane, 2,2-dimethyl
1.363	13.973	3-Buten-2-ol
3.210	16.298	3-Hexaneone
7.614	27.873	Nonane, 1-iodo
Trace	-	3-Ethyl-3-methylheptane
6.467	41.858	4-Heptanone, 2-methyl
6.911	43.144	6-Fluoro-2-trifluoromethylbenzoic acid, 3-fluorophenyl ester
Trace	-	Propanoic acid, 2-methyl-,anhydride
Trace	-	Phthalic acid, 4-bromophenyl ethyl ester
4.040	54.529	Borane, diethyl (decyloxy)

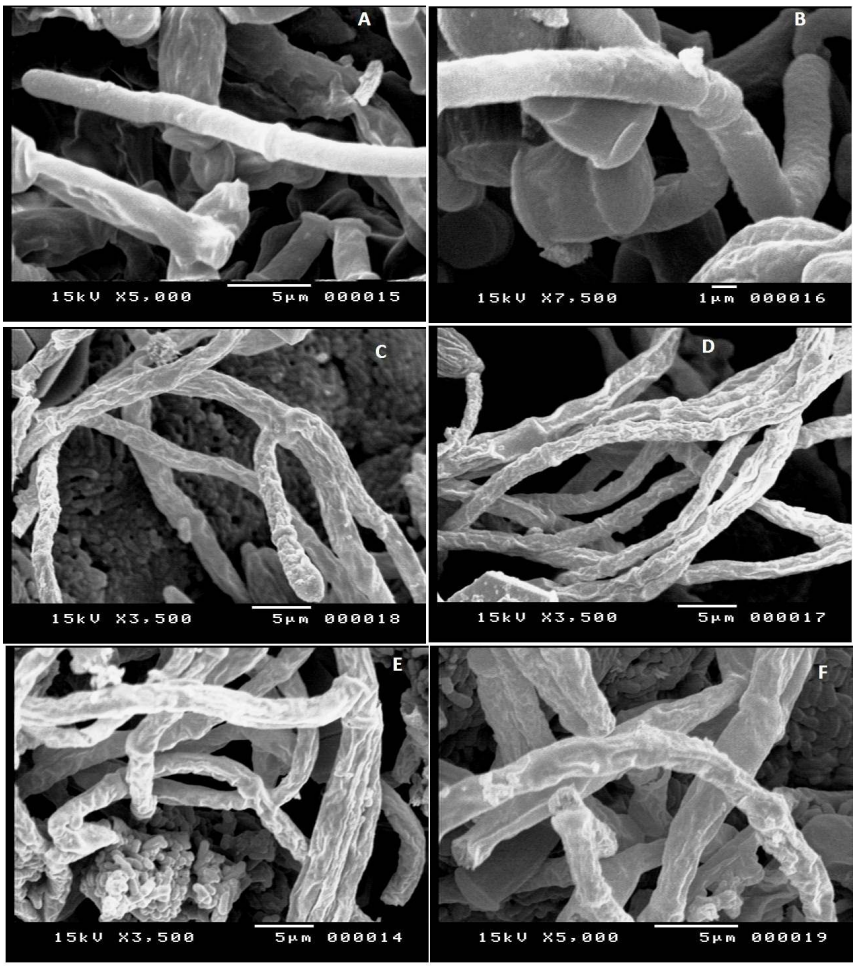

Fig. 1. Scanning electron micrographs of *Fusarium solani* treated with *Forsskaolea tenacissima* extract (0.165 mg/mL). The untreated mycelia are well-developed inflated having smooth wall (A; B). However, the treated mycelia showing plasmolyzed, distorted, squashed and collapsed hyphae (C–F) and completely dead

Table 4. GC-MS analysis of hexane extract of *Forsskaolea tenacissima*

Percentage (%)	Retention time	Compound name
20.131	6.667	2-Hexaneone, 3,3-dimethyl
14.652	7.314	Ethanone, 1-(3-ethyloxiranyl)
1.720	8.150	1-Pentanol, 2,2-dimethyl
1.802	8.556	Hydroperoxide, 1-methylpentyl
3.302	9.157	3-Hexen-2-one
1.108	83.563	Nonadecane
1.328	86.478	13-Docosenamide
3.091	87.796	Heptadecane
8.092	91.885	Tetracosane
11.423	95.820	Octacosane
12.868	99.614	Docosane
11.099	103.276	Tetratetracontane
9.383	106.829	Hentriacontane

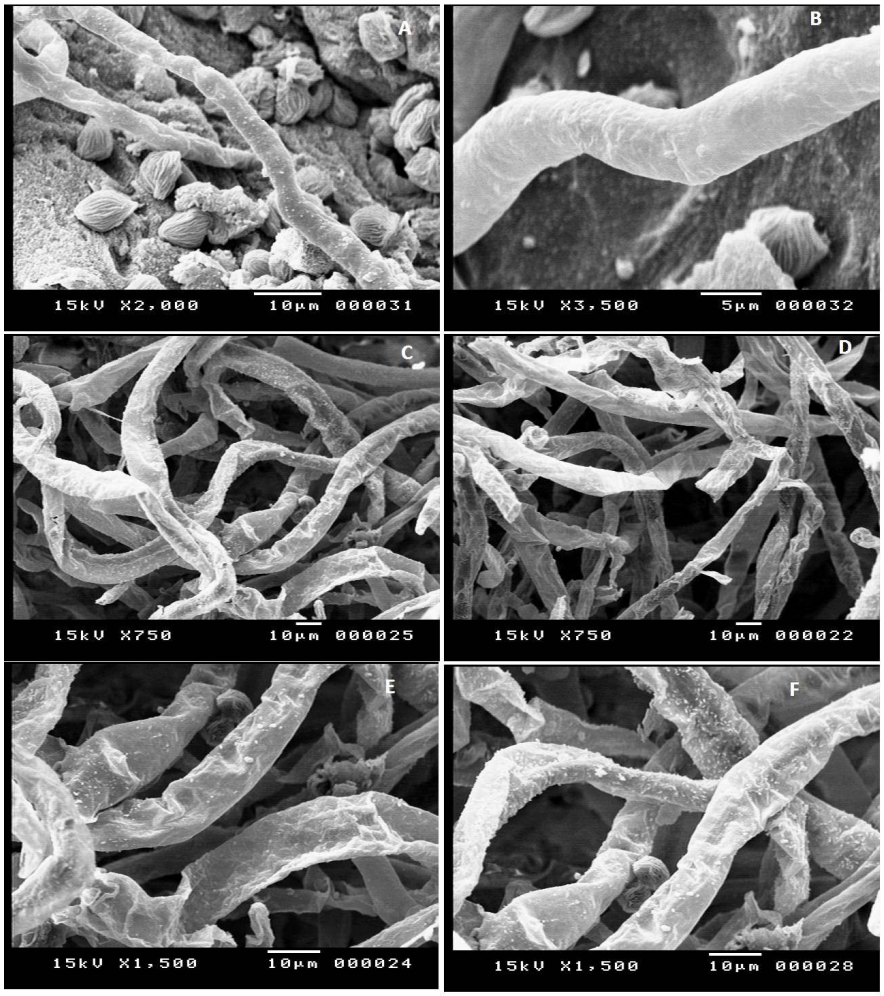

Fig. 2. Scanning electron micrographs of *Pencillium expansum* treated with *Forsskaolea tenacissima* extract (0.165 mg/mL). The untreated mycelia are well-developed inflated having normal wall and normal conidia (A; B). However, the treated mycelia showing plasmolysis, distorted, squashed and collapsed hyphae (C–F) and completely flat and empty dead hyphae

Table 5. GC-MS analysis of ethanol extract of *Forsskaolea tenacissima*

Percentage (%)	Retention time	Compound name
9.368	6.178	Oxalic acid, isobutyl pentyl ester
22.63	7.161	Dimethyl sulfone
6.288	16.144	Undecane
6.227	21.404	Nonane,3,7-dimethyl
11.58	48.156	Diethyl phthalate
3.733	66.181	Methyl tetradecanoate
3.160	68.030	n-Hexadecanoic acid
3.573	74.434	9,15-Octadecadienoic acid, methyl ester
3.842	76.295	Cyclooctene, 3-methyl
5.488	76.526	1,14-Tetradecanediol
22.09	102.838	4H-1,3,2-Dioxaborin, 6-ethenyl-2-ethyl-4-(2-methylpropyl)
2.012	105.289	Cholesta-8,24-3-ol,4-methyl-,(3.beta, 4.alpha.)

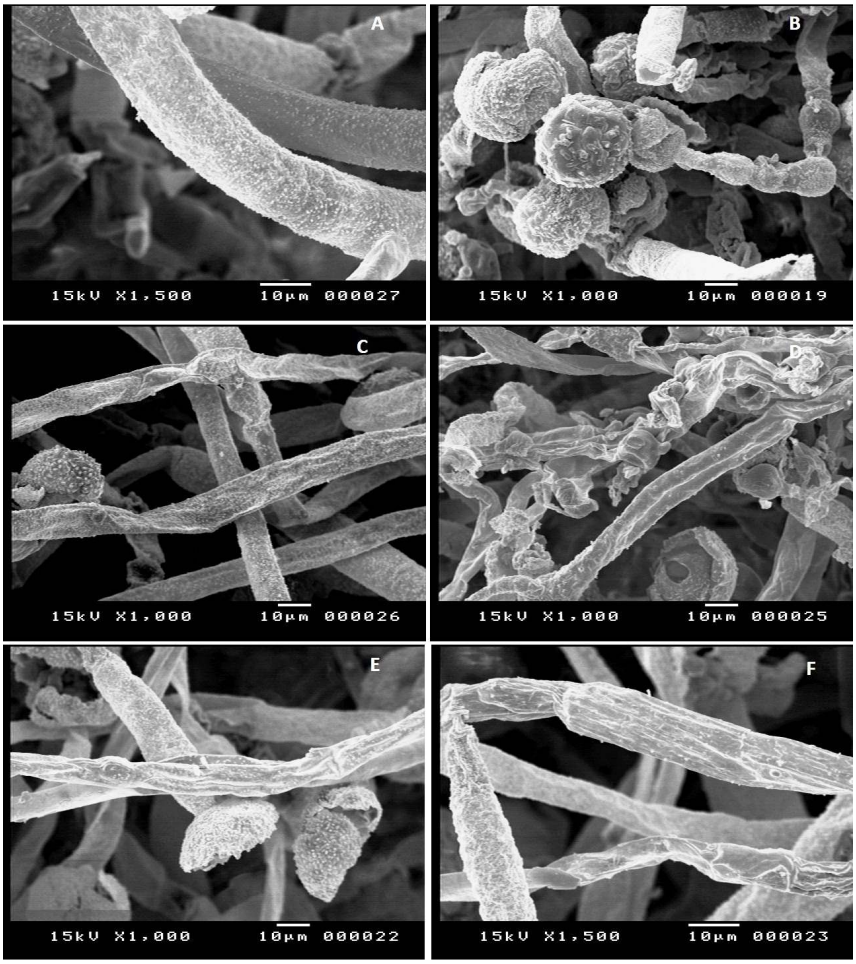

Fig. 3. Scanning electron micrographs of *Pythium ultimum* treated with *Forsskaolea tenacissima* extract (0.287 mg/mL). The untreated mycelia are well-developed inflated having normal wall and well developed sporangia (A; B). However, the treated mycelia showing plasmolysis, distorted, squashed and collapsed hyphae (C–F) and completely flat and empty dead hyphae. Sporangia are affected clearly by the treatment and they are ruptured and cut

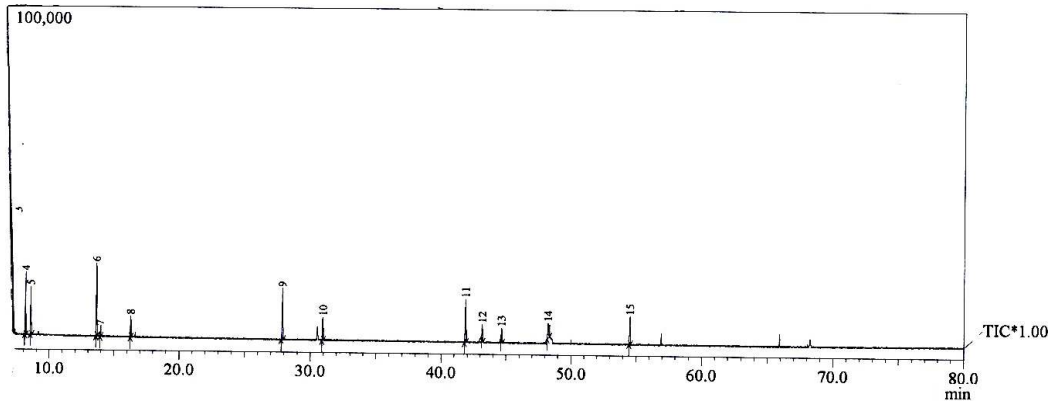

Fig. 4. Chromatogram of GC-MS analysis of chloroform extract of *Forsskaolea tenacissima*

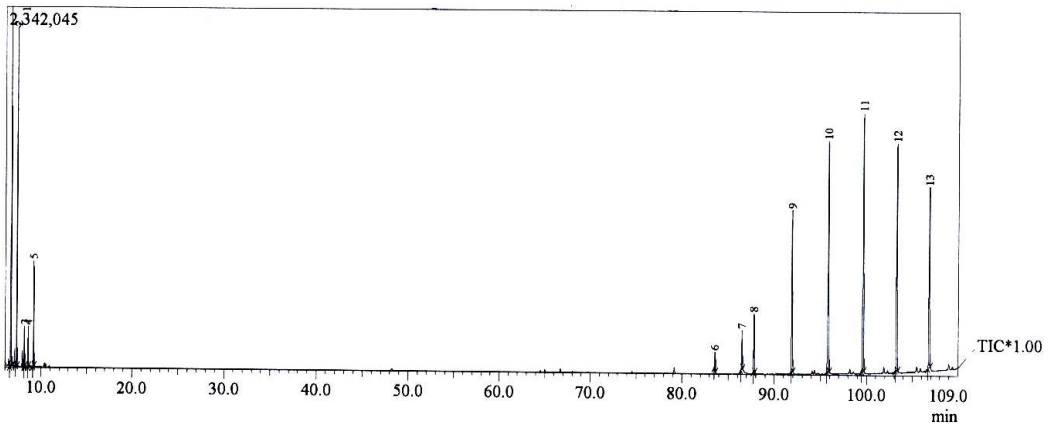

Fig. 5. Chromatogram GC-MS analysis of hexane extract of *Forsskaolea tenacissima*

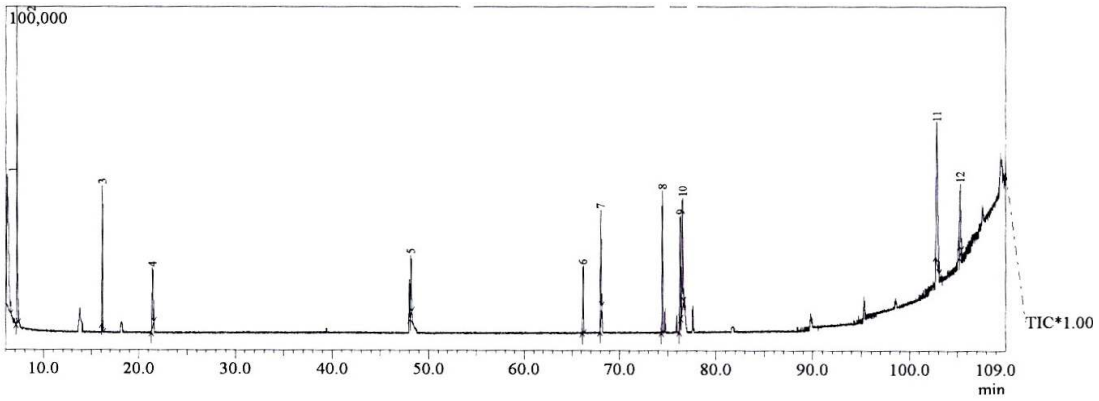

Fig. 6. Chromatogram of GC-MS analysis of ethanol extract of *Forsskaolea tenacissima*

Chloroform was the best extractant that resulted in the highest suppressive effect on the three phytopathogenic fungi. Chloroform extract *F. tenacissima* inhibited the three fungi and produced inhibition zone ranged from 4.20–5.46 cm in diameter. *Xanthium spinosum,* *Abutilon pannosum* and *Solanum incanum* also inhibited the three fungi and chloroform was the extractant and produced IZ more than 4.0 cm against all fungi. The inhibitory effect of the tested plants varied depending on the plant species and the extracting solvent. This

observation was confirmed by many findings of many authors. In this respect, Zaidi [16] observed that the growth of *Aspergillus flavus*, *A. niger*, *Fusarium solani* and *F. moniliforme* was inhibited by the methanolic extract of *Grewia erythraea,* while the growth of *F. moniliforme* and *F. oxysporum* var. *lycopersici* was inhibited by the extract of *H. sessilifolius* and *V. stocksii*. He mentioned that the methanolic extract of *Zygophyllum fabago* had a strong antifungal activity against *Candida albicans,* while the extract of each of *Hymenocrater sessilifolius* and *Vincetoxicum stocksii* exhibited an inhibitory antifungal activity against *C. albicans* and *Microsporum canis*. Kumar et al. [17] approved that the antifungal activity of methanol extract of *Bauhinia racemosa* against *Aspergillus niger*, *A. flavus* and *Alternaria solani* strains. The extract of *Rumex vesicarius* showed good activity against *F. moniliforme* and *F. oxysporum* and it was also significant active against *Bacillus subtilis*, *B. cereus*, *Corynbacterium ulcerans*, *Staphylococcus aureus*, *Serratia marcescens*, *Trichophyton mentagrophytes*, *T. rubrum*, *Micosporum audouinii* and *Chrysosporium tropicum* [18]. Olufolaji et al. [19] evaluated the extracts of leaves of *Ocimum gratissimum*, *Chromolaena odorata*, *Cymbopogon citratus*, seeds of *Eugenia aromatica*, *Piper guineense*, and nuts of *Garcinia kola* against *Pyricularia oryzae*. They reported that significant inhibition on mycelial growth and sporulation of *Pyricularia oryzae*.

We examined the treated hyphae of the phytopathogenic fungi with the crude extract of *F. tenacissima* by SEM to obtained an image about how the plant extract could affect the fungal cell. Result of SEM approved the toxic effect of the plant extract of *F. tenacissima* on the three phytopathogenic fungi. It was clear that hyphae and conidia of *Fusarium solani*, *Pencillium expansum* and *Pythium ultimum* were distorted and squashed. Complete death of fungal hyphae confirmed the toxicity of such plant extracts that could act as fungicidal agents. Based on the microscopic examination, various phenomena associated with cell damage observations could be speculated [20]. Our observation was in agreement with many findings of other researchers, who examined the effect of the crude extract of many plants against fungi. In a previous work, Hashem [11] reported that scanning electron microscopy (SEM) have proved that *Ballota undulate* ethanol extract exhibits fungicidal effect on *Paecilomyces lilacinus* through alterations in hyphal structures including budding of hyphal tip, anomalous structure, such as swelling, decrease in cytoplasmic content, with clear separation of cytoplasm from cell wall in hyphae. These results also were confirmed by De Billerbeck et al. [21], who tested *Cymbopogon nardus* essential oil against *Aspergillus niger*, and Fleming et al. [22], who estimated *Tagetes patula* extract on *Pythium ultimum*. Getie et al. [23] reported that the hyphae and conidia of *Microsporum gyseum* treated with methanol extract of *Acorus calamus* were shrunk and collapsed, which might be due to cell fluid leakage. Girish and Satish [24] approved that SEM showed the entity of the morphological alterations of *P. ultimum* correlates with extract concentration. The fact that deepest alterations are observed in the irradiated samples suggests an involvement in the extract action of thiophenes, that possess phototoxic properties [25].

The mode of antifungal activity of such extracts could be due to cell wall attack and retraction of cytoplasm in the hyphae and ultimately death of the mycelium [26]. Such modifications induced by the interference of plant extract components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth. It has been demonstrated that monoterpene hydrocarbons and oxygenated monoterpenes in the flower essential oils are able to destroy the cellular integrity and thereby inhibit respiration and ion transport processes [27,28]. Because of the low number and expensive of natural antifungal agents the effective plants extract against fungi could be employed for the development of cheap and effective new antifungal agents [4].

The crude extract of *F. tenacissima* was analyzed using GC-MS to detect the main chemical components that could play a key role in suppression of the tested fungi. Results approved the presence of many aliphatic and aromatic compounds in the different extracts. It was observed that the number and types of the chemical compounds depended on the type of the extract. This could interpret the difference in the inhibitory rate of the different extracting solvents of the same plant. This hypothesis could be strengthened by finding by [29], who reported that the differences in the composition in the extracted compounds could be correlated with the antimicrobial properties of each extract. Our result approved the presence of some effective compounds such as 2-heptene, heptadecane, 3-ethyl-3-methylheptane. These results are agreed

with those of Miliauskas [29], who confirmed the presence of 5-hepten-2-one, 6-methyl in their samples that had an antimicrobial effect. This compound was previously detected as a major component in other samples of natural origin, like apricot fruit pulp [30] or *Anthemis tenuisecta* plant essential oils [31]. According to the assumption of some authors, no data are available about the specific activity of either compound alone, without the synergistic effect of other compounds present in the plant preparation. As the literature data confirm, the synergistic effect can enhance the antimicrobial effect of some natural extracts [29]. Edris and Farrag [32] reported about the synergistic effect of linalool and eugenol on some fungal species. These data support our finding that many compounds in each extract in synergetic effect as antimicrobial agents.

5. CONCLUSION

The study concludes that the crude extract of *Forsskaolea tenacissima*, extracted in either chloroform, ethanol or hexane, is an effective antifungal agent that could be applied practically to suppress the phytopathogenic fungi: *Fusarium solani*, *Penicillium expansum* and *Pythium ultiumu*. We recommend the applying of this preparation to use in control of plant disease as a good alternative to the hazard fungicides especially in organic farming and specifically to control the diseases caused by the tested fungi such as root rot and damping-off as well as the postharvest decay in fruits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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