International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)

Journal Home page: www.iscientific.org/Journal.html

Antifungal fractionations of some wild flora extracts and their detoxified influence against some mycotoxins

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Abstract

This study investigated the antifungal constituents of *Forsskaolea tenacissima* L. and *Juniperus communis* L. aerial parts on the growth of *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium verticillioides* and *Fusarium oxysporum*, as well as their excretion of mycotoxins. The phytochemical screening of aqua-methanolic extracts was completed using chromatographic analysis (GC/MS). The purified extract of *J. communis* included 39 identified compounds; meanwhile, the fractionation of *F. tenacissima* revealed only 29 constituents and five of them have been reported as antimicrobial substances, namely Paromomycin (10.412%), Octadecadienoic acid, methyl ester (9.654%), methylcarbamate (3.164%), methyl salicylate (1.164%) and 4-Carbomethoxy-4-[2-(2-carbomethoxyvinyl) benzyl] (1.404%). The composition of foliar parts of *J. communis* revealed that Germacrene B (9.654%), γ-Elemene (7.161%), Epoxy caryophyllene (6.036%) and Cedrol (4.301%) are predominant constituents. The aqua-methanolic extract of *F. tenacissima* was the most effective as a growth inhibitor against tested fungi and both extracts had an eliminatory effect against mycotoxins produced by tested fungi. Although, there were significant differences between the inhibitory capacities of the two extracts in favor of *F. tenacissima*, which reduced aflatoxins, sterigmatocystin, fumagillin, fumonisin and monilifomin most effectively by 22.62, 41.54, 37.19, 32.20, 26.65, 25.30 %, respectively. *J. communis* extract treatment led to reductions of 20.49, 35.34, 30.79, 23.46, 23,78 and 17.12% instead. It was found that both extracts significantly reduced the secretion of fusaric acid as one of the phytotoxins and mycotoxins secreted by *F. verticillioides* and *F. oxysporum*.

Keywords: *Forsskaolea*, *Juniperus*, GC/MS, sterigmatocystin, fumagillin, fumonisin.

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1. Introduction

The natural active substances being underway evaluated under the regulatory framework developed for conventional plant protection products in the European Union. Based on these findings, the policy recommendations are suggested develop separate guidelines for natural materials. Much of the work conducted on the use of compounds obtained from plants reveals their effectiveness against many pathogenic fungi and shows that they pose no expected health risks to humans [1-5]. Natural materials have acted as a vital source in the discovery of new and safe compounds in this area, although grasses and wild plants still play a critical role in the control of microbial diseases [6, 7]. In Saudi Arabia, there are about 35 endemic plant species concentrated in the dry regions that have been tested, and their medical efficacy has been proven [8]. Many families include plants of biological importance, such as Urticaceae and Bignoniaceae [9, 10], due to their various phytoconstituents and valuable medicinal uses. It has been reported that Urticaceae plants contain different classes of chemical constituents with several antimicrobial effects. *Forsskaolea* is a small group of 54 genera and more than 2000 species of herbs, shrubs, small trees, and a few vines of the family Urticaceae are distributed primarily in tropical regions [11]. *Forsskaolea tenacissima* is a wild species that is highly resistance to drought and salinity [12, 13] and demonstrates antimicrobial activity [14, 15]. More than 65 species associated to *Juniperus* (Cupressaceae) have been extensively investigated as a source of natural products with potential antimicrobial, acaricidal and insecticidal activities

[16, 17]. The common juniper, *Juniperus communis* L., is an evergreen small coniferous tree that is widely found in the cool temperate northern hemisphere. The aerial parts, especially leaves and berries of juniper, are rich in essential oil characterize by aromatic flavour and bitter taste. Analysis of *J. communis* extracts confirmed the existence of various ingredients that may reflect its pharmacological and antimicrobial properties [8, 18]. This study aimed to confirm the antifungal activity of two herbal wild species extracts against some plant pathogenic fungi. Fractionation of the extracted samples was performed to investigate their potential mode of action as antimicrobial substances against fungal growth and mycotoxins production.

2. Materials and Methods

2.1 Plant collection and extract preparation

Two wild plants (Figure 1) were collected from Najran area, Saudi Arabia, in 2022. The examined plant was collected and identified based on the species level according to taxonomical plant guides [19]. The extraction was conducted according to the method of Abdelghany *et al.* [20]. In total, 10 g of representative leaves of each plant was collected and dried for 5 days at a temperature of (30 ± 2°C) using Perten Laboratory Mill 3600. The dried parts were then mixed with 50 mL of a 70% aqua-methanolic solution and shaking was performed overnight at 200 rpm. The extract was filtrated through Whatman paper No. 1, and then filtered using a pore size of 22 µm; for chromatographic analysis, 1 mL of each sample was kept in the dark. The remaining extracts were concentrated using rotary evaporator equipment, sterilized through a Seitz filter and kept at 5 ± 1 ℃ until future use.

2.2 GC/MS Screening of plant extracts

Extract analyses were performed in the Agilent 7890A system (Agilent Technologies, USA) which includes a split/splitless injector, G4513A autoinjector, mass spectrometer detector 5975C and GC/MS Software solutions. The column used was an Agilent HP-5MS capillary column (30 m × 0.25 mm i.d. and 0.25 µm phase thickness). The programming of the oven temperature was 60 °C for 4 min, which then increased to 106 °C at 2.5°C/min and from 106 °C to 130 °C at 1◦C/min and finally from 130 °C to 250 °C at 20 °C /min; this temperature was kept constant for 10 min. The sample volume was 1 µL in the split mode (1:10). The separation by chromatography was carried out at a constant pressure of 143.6 kPa. Helium (99.996% by mass) was used as the carrier gas. The injector temperature was 250 °C and the ion source and mass spectrometer interface temperatures were 230°C and 280 °C, respectively. The mass spectrometer was operated in a total ion current (TIC) mode and samples were scanned from 40 to 500 amu. All fragments were identified according to the standard mass spectra and compared with the Tandem Mass Spectral library (NIST)

2.3 Tested Fungi

The antifungal effect of *F. tenacissima* and *J. communis* crude extracts was evaluated on tested mycotoxigenic fungi, i.e., *Aspergillus flavus, A. fumigatus, Fusarium verticillioides* and *F. oxysporum.* The fungal isolates were isolated from Genetic modified grains (GMO) corn. The corn samples collected from Riyadh, Al Medina Al Munwrah, Al-Qassim, and Wadi Al-Dawasir in Saudi Arabia. The fungal isolated identified in Central Research Lab., Saudi Grains Organization, Saudi Arabia. The identification of tested isolates was performed according to the method of Samson and van Reenen-Hoekstra [21] and reconfirmed with the Microloge system (Biolog, Inc., Hayward, CA) according to the method of Singh [22] and El-Naggar and Thabit [23].

2.4 Determination of the minimum inhibitory concentration (MIC) of crude extracts and effect on fungal dry weight

Initially, the crude extracts of wild plants were processed as previously mentioned; furthermore, they were used to determine the minimum inhibitory concentration (MIC) or their effect on the linear growth (cm) of *A. flavus, A. fumigatus, F. verticillioides* and *F. oxysporum* after 7 days of incubation at 25 ± 2˚C on Potato Dextrose Agar (PDA) media. Meanwhile, their effect on fungal dry weight was investigated using potato dextrose broth (PDB). The bottles (250 mL) contained 150 mL of broth, were autoclaved and before pouring were treated with crude extracts of each plant at a ratio of (1:10) (V:V). The disc (5 mm) included the tested isolates of mycelium inoculated at 25 ± 2°C for 10 days as well as the control (distilled sterilized water). Mycelial biomass from triplicate samples containing crude extracts of *F. tenacissima* and *J. communis* was collected on pre-weighed filter papers (Whatman No. 1). The dry weight yield was determined after 8 h of oven drying (Memmert Gmbh) at 60 °C, and the percent loss in mycelial dry weight was calculated over the untreated control.

2.5 The effect of crude extracts on mycotoxins and phytotoxin excretion

The sterilized crude extracts of *F. tenacissima* and *J. communis* were added individually to obtain a final concentration of 10 % in 250 mL Erlenmeyer flasks containing 100 mL of Yeast extract sucrose broth (YES) (Yeast extract 20 g; Sucrose, 150 g; Distilled water, 1 L), while the control flask was treated with distilled sterilized water. Then, the flasks were inoculated with a disc (6 mm) of tested isolates individually. The inoculated flasks were triplicated and incubated for 7 days at 25 ± 2°C.

2.5.1 Mycotoxins and phytotoxic determination

The mycotoxins were determined using mass-selective gas chromatography (GC/MS) and the procedure was based on analysis methods described elsewhere [24]. The 6890/5975B GC/MS detector (Agilent Technologies) was combined with the HP-5MS column, 30 m, 0.25 mm and 0.25 µm. The Chem Station program was from Agilent Technologies for system control and data processing. The carrier gas was helium with a column flow of 1 mL/min. The splitless injection mode was used and the injection volume was 1 µl. The inlet temperature was 270 °C, the mass detector (MSD) ion source temperature was 170°C, the mass filter temperature was 150 °C, and the GC–MSD interface temperature was 280 °C. The column temperature schedule was as follows: 60 °C held for 2 minutes, 25 °C/min at 240 °C and 5°C/min at 300°C. Electron ionization (EI) was performed at 70 eV and the spectra were monitored in the Selected Ion Monitoring (SIM) mode. A certified combined standard of tested mycotoxins was obtained

Individually from Sigma-Aldrich Co., Germany. After reconstitution in acetonitrile, the concentration of each toxin

in the solution was 100 µg/ml. Working standard solutions with concentrations of 0.2 and 2.0 µg/mL, respectively, were prepared by diluting the stock standard solution with acetonitrile. The mixture of acetonitrile and deionized water (84 + 16) was used as the extraction solvent and the extracted samples were purified prior to analysis by passage through a 0.22 µm syringe filter.

2.6 Statistical analysis

Analysis of variance was performed with MSTAT-C (statistical package, Michigan State University). The least significant difference procedure was used at a 0.05 level of probability [25].

3. Results

3.1 Antifungal activity

The screening results of the tested crude extracts of the wild species showed remarkable antifungal activity against mycotoxigenic fungi, namely *A flavus, A. fumigates, F. verticillioides* and *F. oxysporum* (Table 1. Furthermore, the minimum inhibitory concentration (MIC) for each species varied, as shown in Figures 2 and 3. In the same trend, these extracts had a role in reducing the biomass and growth ratio of tested fungi. In addition, the inhibitory effect of these extracts was also noticed against dry weight, as shown in Table 1. The extract of *F. tenacissima* was the most effective, and its inhibitory impact ranged between 34 and 37.4 %. In terms of the tested fungi, *F. verticillioides* and *A. flavus* were the most affected by the extract of both plants. The *J. communis* extract exhibited an almost similar response of antifungal activity with a moderate reduction in fungal biomass that ranged from 22.2 to 24.6%.

3.2 GC-Mass analysis of the plant extract

To understand the mode of action of crude extracts of the two plants against the tested Phytopathogenic fungi, their chemical composition was analysed using GC-MS equipment.

3.2.1 Chemical composition of different extracts

The chemical composition of the two plant extracts was analysed using GC-MS to understand their mode of action against some of the tested Phytopathogenic fungi.

3.2.2 Forsskaolea tenacissima extract

The identification and characterization results of chemical compounds of *F. tenacissima* in the methanol crude extract are shown in (Table 2 and Figure 4). The result revealed the presence of 29 chemical compounds belonging to various aliphatic and aromatic components in the crude extract. The remaining compounds have varied concentration ratios of (0.152%–10.412%). The major components in this extract were Paromomycin (10.412%), 12,15-Octadecadienoic acid and methyl ester (9.654%). The data revealed that the arial parts of *F. tenacissima* contain some important compounds, with a broad spectrum antibiotic and antimicrobial activity, i.e., Paromomycin (10.412%); 12,15-Octadecadienoic acid, methyl ester (9.654%); methylcarbamate (3.164%); methyl salicylate (1.164%); 4-Carbomethoxy-4-[2-(2-carbomethoxyvinyl) benzyl] (1.404%).

3.2.3 Juniperus communis extracts

GC/MS analysis of *J .communis* extract reflects the presence of 39 constituents (Table 3) and (Figure 5). The composition of foliar parts of *J. communis* revealed that Germacrene (9.654%), γ-Elemene (7.161%), Epoxy caryophyllene (6.036 %) and Cedrol (4.301%) are the predominant constituents followed by moderate amounts of β-Elemene (3.378%), α-Terpinene (3.265%), Thymol (3.164%), Elemol (2.784%) and L-Limonene (2.720%). The obtained results of GC/MS showed the existence of pinene derivatives including β-Pinene 0.695 % , α-Pinene 2.087% , α-Terpinene 3.265%, α-Terpinolene 0.456% and γ-Terpinene 0.278%. Epoxy caryophyl- lene, β-Caryophyllene and Trans-Caryophyllene, which were also detected in the extract of *J. communis* with 6.036 %, 1.4404 % and 0.481 %, respectively.

3.3 The effect of crude extracts on mycotoxins excretion

Results regarding toxins production can be seen on (Table 4). Results of GC/MS show that mycotoxin production of *A. flavus, A. fumigatus, F. verticillioides* and *F. oxysporum* was influenced by plant extracts and the same trend of the inhibitory effect of plant species on the fungal biomass. The results showed that the production of all mycotoxins was slightly reduced when treated with methanolic extract of both plants, but extract of *F. tenacissima* demonstrated good inhibitory effect on mycotoxins production compared with *J. communis* extract. Compared to the control samples, the reduction in mycotoxins and phytotoxin **(Fusaric acid)** production in *F. tenacissima* extract treatments ranged from 21.33% in case of phytotoxin fusaric acid produced by *F. verticillioides* to 41.54 % for STC mycotoxin produced by *A.flavus*. On the other hand, reduction was observed with *J . communis* extract treatment recorded 16.37 % for Moniliformin produced by *F. oxysporum* to 35.34 % for STC for *A. flavus*. The reduction in sterigmatocystin production was more pronounced in both plant extract.

4. Discussion

As is widely known, plants contain many different biochemical compounds and several metabolite derivatives, which have been extensively used as biofungicides. The obtained results verified the efficacy of the crude extract of *F. tenacissima* and *J. communis* plants against four fungi, i.e., *A. flavus, A. fumigatus, F. verticillioides and F. oxysporum*. The aqua-mthanolic extract of *F. tenacissima* and *J. communis* displayed great potential in terms of antifungal activity against all the four pathogens. In general, the extract of *F. tenacissima* was more effective and inhibited the growth of all tested fungi; the reduction ranged from 34 to 37.4 % compared to *J. communis* extract. The inhibitory effect of the tested plants was confirmed by many findings [14, 20, 26]. Meanwhile, Hesham *et al.* [27] described the toxic effect of *F. tenacissima* extracts against hyphae and conidia of *Fusarium solani, Pencillium expansum* and *Pythium ultimum* as a complete death of fungal hyphae due to the toxicity of these plant extracts, meaning they could act as fungicidal agents.

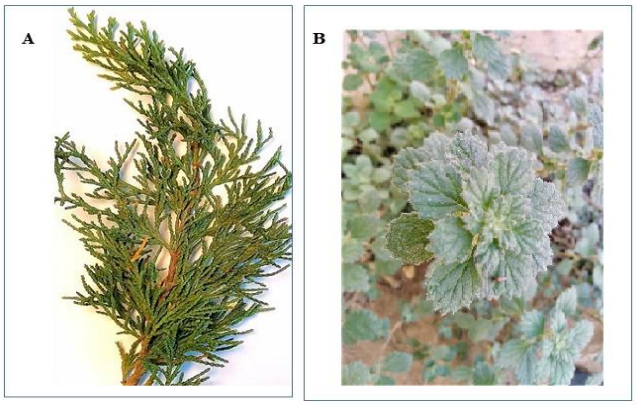

Figure 1: The morphology aerial parts of the tested plants; (A) *Juniperus communis* L. and (B) *Forsskaolea tenacissima* L.

Table 1: The reduction % in dry mycelial weight (mean and standard error) of tested isolates of fungi compared to control after treatment with *Forsskaolea tenacissima* and *Juniperus communis* (Crude Extracts) on PDB broth for 10 days on 25 ±2 °C

Fungal isolates	Control ($\bar{x} \pm SD$)mg	*F. tenacissima* ($\bar{x} \pm SD$)mg	Reduction %	*J. communis* ($\bar{x} \pm SD$)mg	Reduction %
Aspergillus flavus	$566.3^{a} \pm 0.60$	$366.3^{d} \pm 0.58$	35.3	$429.6^{c} \pm 0.58$	24.1
A. fumigatus	$575.7^{a} \pm 0.58$	$375.3^{d} \pm 0.58$	34.8	$448.3^{b} \pm 1.15$	22.2
Fusarium verticillioides	$456.5^{b} \pm 1.0$	$285.7^{g} \pm 0.50$	37.4	$344.3^{e} \pm 0.58$	24.6
F. oxysporum	$429.4^{c} \pm 0.60$	$283.3^{g} \pm 0.60$	34.0	$331.3^{f} \pm 0.76$	22.8
LSD at 0.05%			12.734		

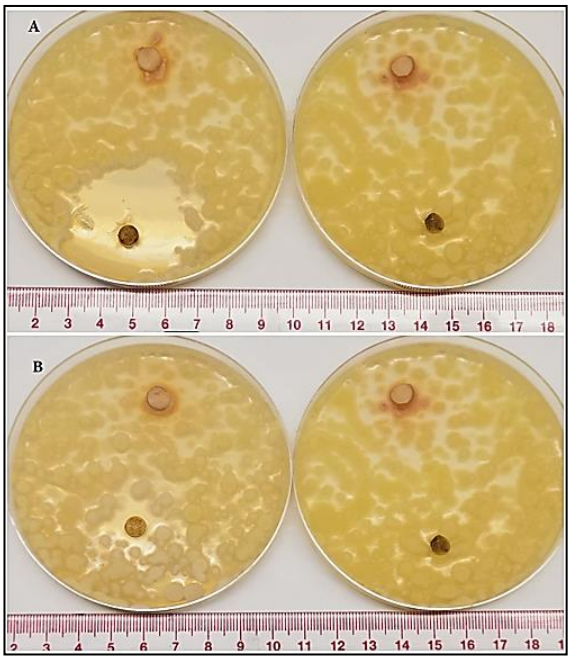

Figure 2: Minimum Inhibitory Concentration (MIC) of crude extracts compared to control. (Sterilized distilled water) against (*A. flavus*). (A) *F. tenacissima* extract; (B) *J. communis extract.*

Table 2: The phytochemical screening of aqua-methanolic (*Forsskaolea tenacissima*) apical young leaves extracts and bud by GC/MS.

No.	Compounds identification	Retention time (Rt)	Relative concentration (%)
1	Propanal	2.36	3.036
2	Dopamine	2.20	1.708
3	1,3-Cyclopentadiene	2.91	2.087
4	Methiopropamine	6.71	0.658
5	N-(4-Tolylsulfonylmethyl) formamide	7.82	2.998
6	Benzenemethanol, 4-hydroxy-alpha	10.51	0.456
7	Norvenlafaxine	11.00	0.228
8	Methyl salicylate	12.35	1.164
9	Benzofuran,2,3dihydro	13.01	0.759
10	1-(3-Methoxy-5-methylphenyl)-N-methylpropan-2-amine	14.62	0.152
11	Methylcarbamate	15.76	3.164
12	Ethyl iso-allocholate	16.03	0.113
13	Propenone,1-(4-ethylphenyl)-3-(2-pyridylthio)	19.08	3.265
14	1-Hepatatriacotanol	19.83	2.480
15	3-(N,N-Dimethyllaurylammonio)propanessulfonate	20.69	3.378
16	Undecanoic acid, 10-methyl- methyl ester	21.17	0.481
17	4-Carbomethoxy-4-[2-(2-carbomethoxyvinyl) benzyl]	21.70	1.404
18	Paromomycin	22.32	10.412
19	-3H-Naphthol[2,3-b] furan-1-one	22.54	2.416
20	Diethyl Phthalate	22.90	0.645
21	Tricycle [4.2.2.0(1,5)] decan-7-ol	23.31	0.164
22	2H-Benzo[f]oxirenol[2,3-E] benzofuran-8(9h)-one	25.32	6.036
23	Phthalic acid, butyl oct-3-yl ester	28.81	3.240
24	Cyclopropanebutanoic acid	29.93	0.442
25	12,15-Octadecadienoic acid, methyl ester	33.13	9.654
26	9,12,15,- Octadecadienoic acid, methyl ester,(Z,Z,Z)-	33.26	3.581
27	Ethyl iso-allocholate	38.64	0.177
28	1-H-Cyclopropa [3,4] benz [1,2-e] azulene-5,7b,9,9a-tetrol	40.75	0.810
29	1-H-2,8a-Methanocyclopenta[a] cyclopropa[a]cyclodecen-11-one	45.58	0.164

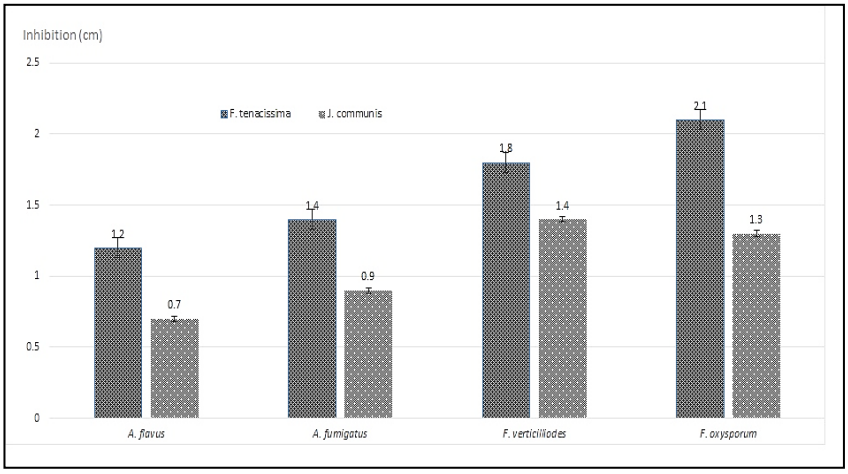

Figure 3: The inhibitory effect of *F. tenacissima* and *J. communis* crude extracts on linear growth (cm) for tested fungi *A. flavus*, *A. fumigatus*, *F. verticillioides* and *F. oxysporum* after 7 days of incubation at 25 ± 2°C on PDA media.

Table 3. The phytochemical screening of aqua-methanolic (*Juniperus communis*) apical young leaves extracts and bud by GC/MS.

No.	Compounds identification	Retention time (Rt)	Relative concentration (%)
1	β-Pinene	9.95	0.695
2	β-Myrcene	10.00	1.708
3	Camphene	10.09	2.341
4	α-Pinene	10.18	2.087
5	D,L-Limonene	10.43	0.658
6	L-Limonene	10.49	2.720
7	γ-Terpinene	10.72	0.278
8	α-Terpinolene	10.99	0.456
9	Linalool	11.11	0.228
10	Allo-Ocimene	11.42	1.063
11	Pinocarveol	11.57	0.101
12	Carveol	11.67	0.759
13	Camphor	11.74	0.152
14	Thymol	11.87	3.164
15	Verbenone	12.39	0.113
16	Hexyl isovalerate	12.52	0.177
17	α-Terpinene	13.52	3.265
18	α-Cubebene	13.64	1.164
19	β-Elemene	14.08	3.378
20	Trans-Caryophyllene	14.43	0.481
21	β-Caryophyllene	14.46	1.404
22	γ-Elemene	14.54	7.161
23	Germacrene	14.71	2.416
24	α-Humulene	14.84	0.645
25	Copaene	15.00	1.151
26	Cedrene	15.04	0.164
27	δ-Cadinene	15.45	1.923
28	Epoxy caryophyllene	15.47	6.036
29	β-Selinene	15.56	1.025
30	γ-Cadinene	15.60	3.240
31	α-Muurolene	15.65	0.291
32	γ-Selinene	15.68	0.442
33	Germacrene B	15.70	9.654
34	Elemol	15.79	2.784
35	α-Cadinol	16.05	0.797
36	Isospathulenol	16.27	0.177
37	α-Amorphene	17.19	0.810
38	Ledol	17.41	0.164
39	Cedrol	18.10	4.301

Table 4: The inhibitory effect of *F. tenacissim* and *J. communis* extracts against mycotoxins excretion of tested fungi on YES broth media after 10 days of incubation at 25 ±2 °C

Fungal isolates		Treatment	Aflatoxin ngml^{-1}	STC** ngml^{-1}	Fumagillin ngml^{-1}	Fumonisin ngml^{-1}	Fusaric acid ngml^{-1}	Monilifomin ngml^{-1}
A. flavus	Control	X`± SD	725.80^{a}±1.2	14.67^{a} ±0.6	-	-	-	-
	F. tenacissim extract	X`± SD	561.63^{c} ±0.6	8.57^{c}±0.2	-	-	-	-
		Reduction %	22.62	41.54	-	-	-	-
	J. Communis extract	X`± SD	577.09^{b} ±0.3	9.48^{b} ±0.3	-	-	-	-
		Reduction %	20.49	35.34	-	-	-	-
A. fumigatus	Control	X`± SD	-	-	621.73^{a} ±1.1	-	-	-
	F. tenacissim extract	X`± SD	-	-	390.53^{c} ±1.1	-	-	-
		Reduction %	-	-	37.19	-	-	-
	J. Communis extract	X`± SD	-	-	430.30^{b} ±0.8	-	-	-
		Reduction %	-	-	30.79	-	-	-
F. verticillioides	Control	X`± SD	-	-	-	1232.77^{a} ±2.8	99.87^{a} ±2.2	869.27^{a} ±2.8
	F. tenacissim extract	X`± SD	-	-	-	835.83^{f} ±0.9	78.57^{d} ±1.0	660.11^{d} ±1.1
		Reduction %	-	-	-	32.20	21.33	24.06
	J. Communis extract	X`± SD	-	-	-	943.50^{c} ±0.7	82.87^{c} ±0.3	726.93^{c} ±0.9
		Reduction %	-	-	-	23.46	17.02	16.37
F. oxysporum	Control	X`± SD	-	-	-	1179.17^{b} ±1.5	87.83^{b} ±0.7	795.57^{b} ±1.1
	F. tenacissim extract	X`± SD	-	-	-	852.53^{e} ±1.0	64.42^{f} ±0.5	594.31^{e} ±0.7
		Reduction %	-	-	-	27.70	26.65	25.30
	J. Communis extract	X`± SD	-	-	-	933.76^{d} ±1.1	66.95^{e} ±1.1	659.33^{d} ±1.2
		Reduction %	-	-	-	20.81	23.78	17.12
LSD at 0.05%			1.557	0.381	2.058	2.659	2.032	2.605

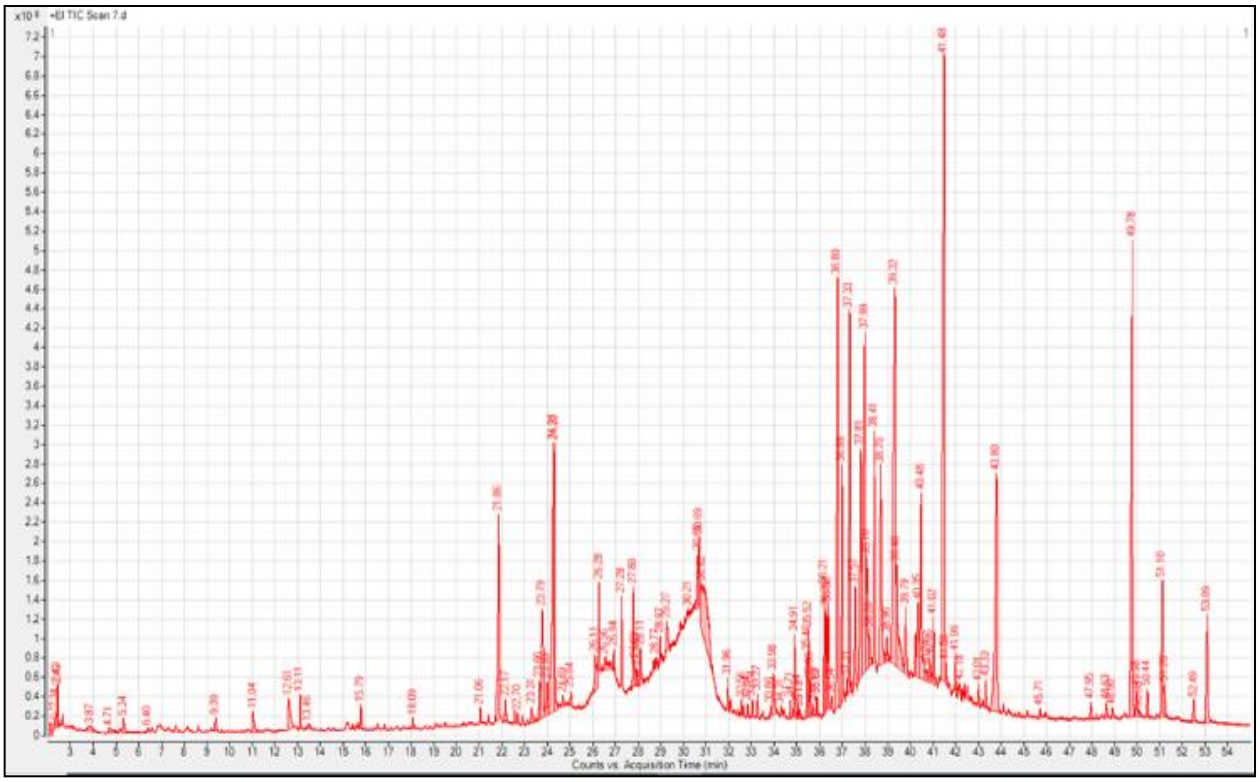

Figure 4: Fractionation of *Forsskaolea tenacissima* extracts on GC/MS (Agilent 7890A), split/splitless injector and capillary column (Agilent HP-5MS 30 m × 0.25 mm).

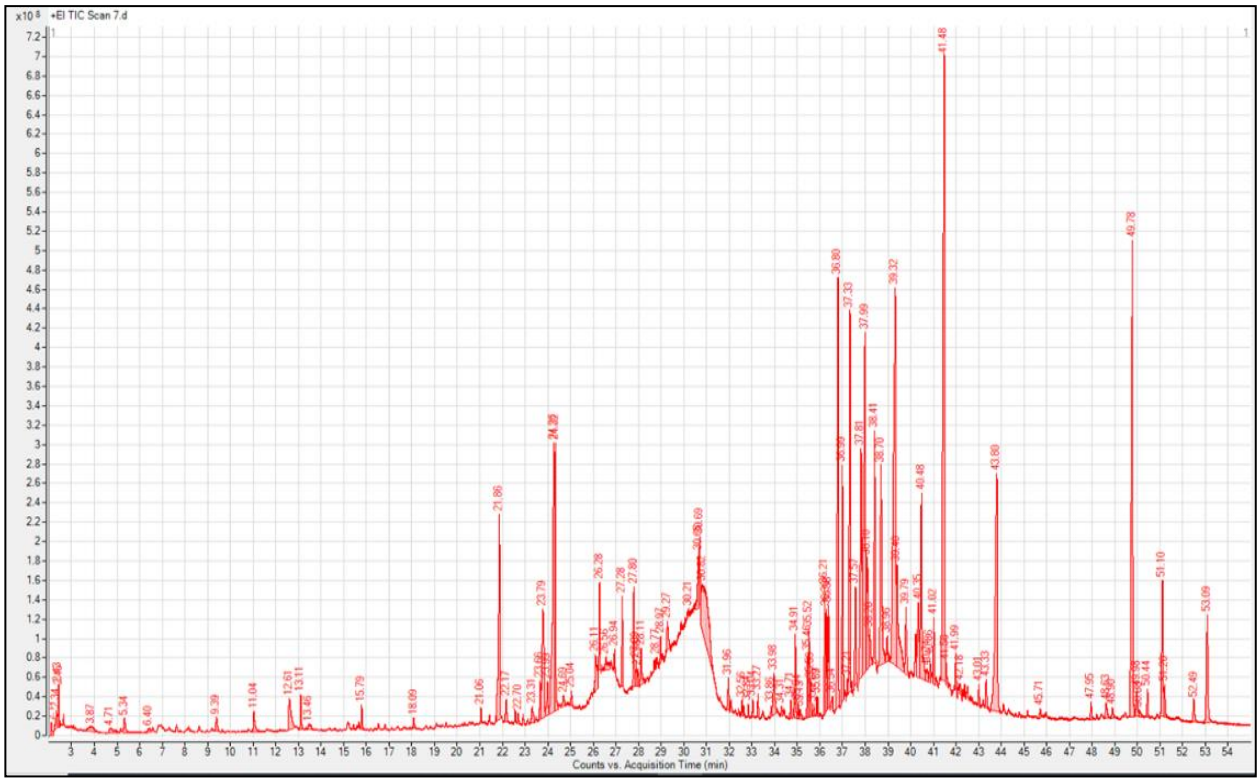

Figure 5: *Fractionation of Juniperus* communis extracts on GC/MS (Agilent 7890A), split/splitless injector and capillary column (Agilent HP-5MS 30 m × 0.25 mm).

The mode of antifungal activity of such extracts could be related to the retraction of cytoplasm in cells of attacked hyphae, and ultimately, the death of the mycelium [15, 28, 29]. This result may be supported by the qualitative phytochemical investigation revealed by chemical analysis of the plant extract, which reflects this finding. In the present investigation, a qualitative and quantitative phytochemical investigation was established using gas chromatography coupled with mass spectrometry (GC-MS) for reliable identification of bioactive compounds existing in plant extracts. The chromatograms of the GC-MS analysis of the methanol fraction of *F. tenacissima* indicate identification of 29 compounds. Six of the prominent compounds were Paromomycin. The fatty acid methyl esters, 12,15-Octadecadienoic acid, methyl ester, methyl carbamate, methyl salicylate and 4-Carbomethoxy-4-[2-(2-carbomethoxyvinyl) benzyl] exhibited antibacterial and antifungal activities in numerous studies [30-32]. Fatty acid methyl esters, such as 12,15-Octadecadienoic acid, are known to possess antifungal properties [33]. The mode of action of paromomycin on fungal spores could be compromised membrane permeability, which provides the antibiotic with easy access to sensitive intracellular sites, including the mitochondrial and cytoplasmic protein synthetic systems [34]. A GC/MS analysis of *J. communis* reflects the presence of 39 constituents. The composition of foliar parts of *J. communis* revealed that Germacrene, γ-Elemene, Epoxy caryophyllene and Cedrol are predominant constituents followed by moderate amounts of β-Elemene, α-Terpinene, Thymol, Elemol and L-Limonene. Our data are in agreement with the previous papers that identified remarkable qualitative and quantitative components from *J. communis*. The previous results revealed that thymol had the highest antibacterial activity [35, 36] and antifungal activity against plant pathogenic fungi [37] and *Botrytis cinerea*, the causal pathogen of grey molds in horticultural products [38]. Thymol affected the enzymatic cell system or interfered with the amino acid involved in germination [39, 40]. Limonene affects membrane integrity, the dissipation of the proton-motive forces, and the inhibition of the respiratory enzymes during its interaction with the cytoplasmic membranes of bacteria [41]. D, L-Limonene, L-Limonene, β-Myrcene, α-Pinene, β- 215 Elemene and some other terpenes compounds enhanced the activity of tuberculostatic antibiotics [41, 42]. Our obtained results of GC/MS demonstrated the existence of pinene derivatives including β-Pinene, α-Pinene, α-Terpinene, α-Terpinolene and γ-Terpinene. Some authors reported a similar composition for the essential oil obtained from branches of *J. communis*. [43,44]. Terpenes may increase the concentration of lipidic peroxides resulting in cell death [45] or inducing leakage of components from the fungal hyphal cytoplasm, causing mycelium death [46]. Trans-Caryophyllene, β-Caryophyllene and Epoxy caryophyl- lene were detected in the aerial parts of four *Juniperus* species, i.e., *J. procera, J. excels, J. virginiana and J. communis* [47]. The antibacterial and antifungal activities of caryophyllene and caryophyllene oxide were exhibited in numerous studies [17]. Results of GC/MS mycotoxin determination showed that the production of all mycotoxins was slightly reduced when treated with methanolic extracts of *F. tenacissima* and *J. communis*. The antifungal and anti-mycotoxigenic properties of herbal plants were investigated against pathogenic [20, 48]. Plants may possess some phytoconstituents capable of compromising the toxic and genotoxic effects of mycotoxins [49, 50]. Abdelghany et al. [20] demonstrated the inhibitory effect of *J. procera* extract on *A. flavus* mycotoxins, where the production of aflatoxins B1 was reduced, while aflatoxin B2 was completely inhibited following treatment with *J. procera* extract. This may be due to blocking of the metabolic pathway of aflatoxins biosynthesis as a result of the fungitoxic effects of *J. procera* extract [51]. Our obtained results are similar to the findings of [20, 48], where the effect of *J. procera* extract was tested on the reduction in productivity percentage of different mycotoxins, including aflatoxins B1, aflatoxin B2, sterigmatocystin, cyclopiazonic acid and fusaric acid. Recently, a study exhibited that the production of Gliotoxin, Nivalenol and Neosolaniol toxins was inhibited with the use of *J. procera* fruit extract [48]. Many studies verified that essential oils can effectively modulate the growth of mycotoxigenic fungi and their associated mycotoxins [52-54] through different fractions of *Juniperus* extracts of leaves and bark.

5. Conclusions

The aim of this study was to confirm the role of plant extracts as antifungal components rather than explore the disadvantages of fungicides. The obtained data verified that the tested plant extracts of *F. tenacissima* and *J. communis* exhibit antifungal effects and contributed to the reduction in mycotoxins excretion for the tested fungi. The study confirmed the bioactive component in the tested plant extract obtained with GC/MS, which is primarily responsible for the reduction in fungal activity.

Funding

This research was funded by the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, grant number (INST080).

Data Availability Statement

Not applicable.

Acknowledgments

The authors extend their appreciation the Deputyship for Research & Innovation.

Conflicts of Interest

There are no conflicts of interest among the authors.

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