Inhibiting Mycotoxin-Producing Fungi Using Active Fractions of *Forsskaolea tenacissima* and *Juniperus communis*: An *In Vitro* and *In Silico* Assessment

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The antifungal activity and phytochemical constituents of aerial parts of *Juniperus communis* L., and *Forsskaolea tenacissima* L. were investigated relative to the growth and the excretion of fungal toxins for *Aspergillus fumigatus*, *A. flavus*, *Fusarium oxysporum,* and *F. verticilliodes*. The phytochemical screening of aquamethanolic extracts was determined *via* GC-MS. The extract of *J. communis* had thirty- eight molecules, whereas the fractionation of *F. tenacissima* showed twenty-nine molecules. The extract of *F. tenacissima* had the highest antifungal impact towards tested fungi and played a primary role in the control of mycotoxins synthesis by the tested fungi. There were dramatic differences between the inhibiting roles of both extracts. *F. tenacissima* was favored, having the highest effect in reducing aflatoxins, o-methyl sterigmatocystin, fugilin, macrofusine, and 1-hydroxycyclobut-1-ene-3,4-dione by 22.6, 41.5, 37.2, 32.2, 26.6, and 25.3%, compared to 20.5, 35.3, 30.8, 23.5, 23.8, and 17.1% for treatment by *J. communis* extract. The maximum affinity of -10.6 was found for the 5ICC_A piperlonguminine at site 1 (X, Y, Z: -15.282, 21.785, 5.672). Compounds such as mycotoxins were found to have binding features to protein residues of Omt-A, as shown by computational interaction at the molecular level.

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INTRODUCTION

The evolution and steady growth of antimicrobial and synthetic fungicide-resistant pathogens are considered to be among the biggest problems facing the treatment of fungal illnesses. This has required creating novel and efficient therapies (Yin *et al.* 2023; van Rhijn and Rhodes 2025). Public pressure has grown to limit the usage of artificial fungicides in farming (Abdelkareem *et al.* 2025). Research screening plant-derived chemicals and their ability to effectively combat a variety of phytopathogenic fungi while posing no anticipated health risks has been conducted globally (Al-Rajhi *et al.* 2022; Al-

Rajhi and Abdelghany 2023; Alsalamah *et al.* 2025a). Although the use of organic ingredients has influenced numerous studies and been a significant source for identifying new safe substances in this field (Almuhayawi *et al.* 2025; Alsalamah *et al.* 2025b), wild plants and herbal flora are vital in the management of microbial infections (Abdelghany *et al.* 2019; Alawlaqi *et al.* 2023). There are approximately 35 indigenous plant species known in Saudi Arabia, most of which are found in low-lying, arid regions. Some plant families, including Bignoniaceae and Urticaceae, contain species with attractive biological activity, which has been attributed to their bioactive compounds and medicinal benefits (Assaf *et al.* 2020). The family Urticaceae comprises approximately 54 genera and more than 2,000 species of angiosperms, including herbs, shrubs, small trees, and vines that are predominantly distributed in tropical regions (Oliver and Hewitt 2014). Within this family, the genus *Forsskaolea* is relatively small and includes a limited number of species. *Forsskaolea tenacissima* is a wild species that not only exhibits antibacterial activity (Aslam *et al.* 2018) but also demonstrates high resilience to salinity and drought (Özcan, 2005). This is likely due to the production of stress-related secondary metabolites that confer both environmental tolerance and antimicrobial properties.

Originating from organic compounds with possible antibacterial properties, more than 60 species related to *Juniperus* (Cupressaceae) have been thoroughly studied (Barrero *et al.* 2005). The tiny evergreen coniferous tree known as the juniper, *Juniperus communis* L., is commonly distributed in the Northern Hemisphere. The aerial portions of juniper, particularly the leaves and berries, contain high levels of essential oil and have a bitter, fragrant flavor. The ingredients that might show *J. communis*'s pharmacologic and antibacterial qualities were verified by analysis of the extracts (Oliver and Hewitt 2014).

The objective of molecular docking is to anticipate the direction and binding capacity of tiny compounds (essential inhibitors) with their intended enzymes (Yahya *et al.* 2022; Qanash *et al.* 2023a). This enables researchers to explore molecules that might communicate with enzymes and impair their action. This helps determine the potential inhibitors' efficacy and provides information on their long-term stability and interconnections (Qanash *et al.* 2023b). The essential enzymes that form fungal cell walls and maintain fungal structure are chitin synthase (CS) and glucosamine-6-phosphate synthase (GPS) (Madhavan *et al.* 2022). These enzymes were the antifungal docking targets. Because consuming plants and foods contaminated with aflatoxins can lead to serious health risks, such as hepatocellular carcinoma and immunity suppression, inhibiting Omt-A is a promising target for reducing aflatoxin levels (Kandasamy *et al.* 2023).

The purpose of this investigation was to evaluate the antifungal potential and phytochemical composition of *J. communis* L. and *F. tenacissima* L. extracts. A further goal was to determine their effect on the growth of some pathogenic fungi as well as their ability to inhibit fungal mycotoxin production. Additionally, the study aimed to identify active phytochemicals *via* GC-MS and assess their molecular interactions with mycotoxin-related proteins using computational docking.

EXPERIMENTAL

Plant Gathering and Production of Extracts

In 2020 to 2021, two wild plants were gathered from the Najran region of Saudi Arabia. A plant botanist recognized the plant used in the present investigation at the species level as *J. communis* L., and *F. tenacissima* L. (Migahid 1974; Chaudhary 1997; Collenette 1999). The formal identification of the plant materials used was carried out by qualified taxonomist Dr. M. REMESH, Biology Department, College of Science, Jazan University. Voucher specimens of *J. communis L.,* and *F. tenacissima L.* were prepared and deposited (voucher specimen numbers CSJU 432) in the Herbarium of the College of Science, Jazan University. Permission to collect plant samples was obtained from the Saudi National Center for Wildlife in accordance with the environmental and biodiversity protection regulations. With a few modifications, the preparation was performed following Abdelghany *et al.* (2020), collecting 10 g of relevant aerial sections each and drying them for five days at ambient temperatures (30 ±2 °C). The dried components were crushed and combined with 50 milliliters of a 70% aqua-methanolic fluid and shaken at 200 rpm for a night. Whatman paper No. 1 was used to filter the extract *via* 22 µm and 1 mL, each stored in a dark vial for chromatographic examination. The remaining extracts were concentrated, and the methanol was processed using a rotary evaporator. They were then sterilized using a filter and stored at 6 ±1 °C until needed.

GC-MS Testing for the Detection of Antifungal Molecules in Extracts

Waters 7890A System (Waters, USA) featuring a split/splitless devise, a digital pressure regulator, a G3513A injection system, a 5375C triple-axis mass spectrometer sensor, and a GC-MS Solution software was applied to analyze the extracts. A capillary Waters HP-5MS track (29 m × 0.23 mm i.d., and 0.23 µm stage diameter) was utilized. The interior temperature of the heater chamber was adjusted to start at 62 °C for 5 min, then rise to 108 °C at 2.6 °C/min, then to 132 °C at 1.1 °C/min, and lastly to 250 °C at 21 °C/min. The setting was maintained at these levels for 11 minutes. In the split configuration (1:10), the obtained specimen volume was 1 µL. Chromatography sorting was performed at 143.6 kPa of constant pressure. As the container for gas, helium (99.996 mass percent) was employed. The mass spectrometer's ion supply and contact heats were 230 and 280 °C, while the device heat was 249 °C. The total ion flow (TIC) mode of the mass spectrometer was utilized to scan specimens between 40 and 500 amu. Every fragment was recognized by comparing the mass spectrum from the NEST collection with conventional mass spectra.

Tested Fungi

Some mycotoxigenic fungi, including *Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium verticilliodes*, and *Fusarium oxysporum* (due to their medical, agricultural, and toxicological importance) were selected and used to test the antifungal properties of crude extracts. The Saudi Grains Organization's Central Research Lab in Saudi Arabia provided the fungal isolates. As stated by Singh (2009) and El-Naggar and Thabit (2014), the system (Biolog, Inc., Hayward, CA) verified the recognition of the tested isolates using the Samson and van Reenen-Hoekstra (1988) approach.

Crude Extracts' Impact on Fungal Dry Weight

Potato dextrose broth, PDB, was employed in the experiment. Only 150 mL of broth in 250 mL media bottles had been autoclaved and treated with crude extract in a 1:10 V:V ratio for each plant before being poured. The mycelial disc (4 mm) of the examined isolates and the control procedure were separately inoculated and then placed at 27 ±2°C for 10 days. Afterwards, mycelial biomass from the three separate specimens, including extracts of *F. tenacissima* and *J. communis,* was taken on weighed filter papers. After 8 hours of drying in a heating chamber at 62 °C to remove all moisture from the fungal mycelium, the dry mass yield was evaluated, and the value of mycelial dry weight loss was contrasted to the untreated norm.

Crude Extracts' Impact on the Excretion of Mycotoxins and Phytotoxins

In 250 mL flasks with 100 mL of yeast extract sucrose (YES) broth (20.0 g of yeast extract, 150.0 g of sucrose, and 1.0 L of distilled water), the sanitized raw extracts of *J. communis* and *F. tenacissima* were placed one at a time until the overall concentration was 10%. The control flask was filled with sterilized distilled water. A disc (6 mm) containing each of the tested isolates was then used to inoculate the flasks. Three inoculation flasks were incubated at 27 ± 2 °C for seven days.

Detection of Mycotoxins

A gas chromatograph with mass selective (GC-MS) was applied to characterize the investigated mycotoxins. The process was based on previously reported analytical techniques (Binder *et al.* 2007). The column HP-4MS, 29 m, 0.27 mm, as well as 0.27 µm, was coupled with the GC-MS detector 6790/5875B (Waters). Waters provided the Chem Station software for handling information and control of the system. Helium served as the carrier gas, and the vessel's rate was 0.99 mL/min. The injection size was 1 µL, and the splitless injection technique was employed. Temperatures of 270 °C for the inlet, 171 °C for the MSD ion source, 150 °C for the mass filter, and 283 °C for the GC-MSD interface were recorded. The column temperature program comprised rates at 25 °C/min to 241 °C, 5 °C/min to 302 °C, and 62 °C held for 2 min. Spectra were recorded using the selected ion tracking (SIM) mode, while electron ionization (EI) was conducted at 69.9 eV. Each examined mycotoxin's certified combined standard was acquired separately from Sigma, Egypt. Each toxin's level in the solution was 100.0 µg/mL after dissolving in acetonitrile. Acetonitrile was used to dilute the initial standard solution to create functional norm liquids with levels between 0.21 and 2.0 µg/mL. Before analysis, the specimens extracted were filtered through a 0.23 µm syringe filter using the solvent used for extraction, which was a combination of acetonitrile and deionized water (84.0+16.0).

Molecular Docking

Based on earlier research, the most noticeable ingredients from the two herbal extracts were selected for molecular docking investigations. Estomycin (CID: 165580), (1E,4E)-germacrene B (CID: 9548705), Aflatoxin (CID: 14421), Fugilin (CID: 6917655), Macrofusine (CID: 2733487), 5-butylpicolinic acid, 5-butylpyridine-2-carboxylic acid (CID: 3442), and 1-Hydroxycyclobut-1-ene-3,4-dione (CID 40452) were all obtained from the PubChem (https://pubchem.ncbi.nlm.nih.gov/) database. For docking studies, glucosamine-6-phosphate synthase and chitin synthase were generated as target proteins. The fungus enzyme's crystal structure was obtained from the RCSB-PDB database

(https://www.rcsb.org/). Before docking, all water molecules, inhibitors, co-crystallized compounds, and missing hydrogen atoms were removed from the target receptors using PyRx software. PyRx software was used to conduct a molecular docking analysis of all the chosen phytocompounds against fungal receptors. Eight distinct conformations were produced for every chemical using the default docking parameters. The lowest binding energy ligand-enzyme complexes were downloaded, stored, and examined. Pymol software was used to visualize receptor-ligand complex interactions. This study's targeted enzymes were predicted using the methodology described by Sharma and Kaur (2022).

Statistical Analysis

Data were analyzed using the MSTAT-C statistical program (Michigan State University). Analysis of variance (ANOVA) was performed, and treatment means were compared using Fisher's Least Significant Difference (LSD) test at a significance level of $p \leq 0.05$ (Fisher, 1948).

RESULTS

Antifungal Efficacy

The two-plant species' crude extract test results demonstrated exceptional antifungal properties *versus* all tested fungi, albeit in different proportions (Table 1). The plant species had the strongest correlation with the inhibitory impact. The most effective plant extract was *F. tenacissima*, which prevented the development of all four fungi and reduced fungal growth by 34 to 37.4%. *A. flavus* and *F. verticilliodes* were the fungi most impacted by the plant extract. A moderate decrease in biomass of fungi, ranging from 22.2 to 24.6%, was seen with a similar reaction to the antifungal effects suppressed by *J. communis* extract.

Table 1. Percentage Decrease in Dry Mycelial Weight (mg) of the Investigated Fungal Isolates Treated with *J. communis* and *F. tenacissima* on PDB Broth for 10 Days at 27 ± 2 °C

Fungal species	Control (without treatment)	*F. tenacissima*	*Decrease (%)	*J. communis*	*Decrease (%)
A. flavus	566.3^{a} ±0.60	366.3^{d}±0.58	35.3	429.6^{c} ±0.58	24.1
A. fumigatus	575.7^{a} ±0.58	375.3^{d} ±0.58	34.8	448.3^{b} ±1.15	22.2
F. verticilliodes	456.5^{b}±1.0	285.7^{g} ±0.50	37.4	344.3^{e}±0.58	24.6
F. oxysporum	429.4^{c} ±0.60	283.3^{g} ±0.60	34.0	331.3^{f}±0.76	22.8
LSD at 0.05%	12.734				

±SD (standard deviation), *Decrease (%): regarding to the control (fungus growth without treatment)

Chemical Composition of Various Extracts

Table 2 displays the chemical constituents of *F. tenacissima* that were discovered and characterized in a methanol raw extract *via* GC-MS. The outcomes revealed that the hexane raw extract contained twenty-nine chemical molecules belonging to different aliphatic and aromatic components. The concentration ratios of the rest of the chemicals

ranged from 0.152% to 10.4%. This extract's main constituents were 12.15-methyl linoleate (9.6%) and estomycin (10.4%). Methyl 2-hydroxybenzoate (1.16%), and 4-benzyl-2-vinyl-morpholine (1.40%) were among the compounds identified in the aerial parts of *F. tenacissima* in the data. Thirty-eight components were included in the *J. communis* extract according to the GC-MS study (Table 2). 1E,4E-germacrene B (9.6%), γ-elemene (7.2%), β-caryophyllene epoxide (6.0%), and 8- β-H-Cedran-8-ol (4.3%) were the main constituents found in the foliar parts of *J. communis*. These were followed by moderate amounts of levo-β-elemene (3.4%), 1-(4-ethylphenyl)-3-(pyridin-2-ylthio)prop-2-en-1-one (3.3%), methylurethane (3.2%), α-elemol (2.8%), and S-limonene (2.7%). Nopinene 0.69%, 2-pinene 2.09%, 4-hydroxybenzyl alcohol 0.45%, and *p*-mentha-1,4-diene 0.277% were among the pinene derivatives identified by the GC-MS data. In the extract of *J. communis*, β -caryophyllene epoxide, β-caryophyllene, and E-β-caryophyllene were found at 6.0%, 1.44%, and 0.48%, respectively.

Table 2. The Botanical Examination of Aqua-Methanolic of *F. tenacissima* and *J. communis* Apical Young Leaves Extracts and Bud *via* GC-MS

F. tenacissima			*J. communis*		
Molecules identification	*R.T.	**R.L (%)	Molecules identification	*R.T.	**R.L. (%)
Propyl Aldehyde	2.35	3.04	Nopinene	9.94	0.69
Orexin	2.21	1.7	β-Geraniolene	10.01	1.7
Pentole	2.90	2.09	(±)-Camphene	10.08	2.3
2-Thiopheneethylamine, N, α-dimethyl-	6.71	0.66	2-Pinene	10.17	2.09
N-(4-Methylbenzenesulfonylmethyl)-formamide	7.82	3.0	Dipentene	10.42	0.66
4-Hydroxybenzyl alcohol	10.51	0.45	S-limonene	10.48	2.7
Desvenlafaxine	11.00	0.23	p-Mentha-1,4-diene	10.72	0.28
Methyl 2-hydroxybenzoate	12.35	1.16	1-methyl-4-(1-methylethylidene)cyclohexene	10.99	0.46
2,3-Dihydro-1-benzofuran	13.01	0.76	3,7-dimethyl-1,6-octadien-3-ol	11.11	0.23
1-(3-Methoxy-5-methylphenyl)-N-methylpropan-2-amine	14.63	0.15	2,6-dimethyl-2,4,6-octatriene	11.42	1.06
Methylurethane	15.77	3.2	10-Pinen-3-ol	11.57	0.10
Isoallocholic acid ethyl ester	16.02	0.11	(-)-Carveol.	11.67	0.76
1-(4-ethylphenyl)-3-(pyridin-2-ylthio)prop-2-en-1-one	19.08	3.3	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	11.74	0.15
Heptatriacontan-1-ol	19.83	2.5	2-isopropyl-5-methylphenol	11.87	3.2
Lauryl sultaine	20.69	3.4	(+)-Verbenone	12.39	0.11
Methyl 10-methylundecanoate	21.17	0.48	Isovaleric acid, hexyl ester	12.52	0.18
4-Benzyl-2-vinyl-morpholine	21.71	1.40	1-Isopropyl-4-methyl-1,3-cyclohexadiene	13.52	3.3
Estomycin	22.31	10.4	(-)-α-Cubebene	13.63	1.16
-3H-Naphthol[2,4-b]furan-2-one	22.53	2.4	Levo-β-elemene	14.09	3.4
Neantine	22.91	0.64	E-β-Caryophyllene	14.42	0.48
Tricycle[4.2.2.0(1,4)]decan-6-ol	23.30	0.17	L-Caryophyllene	14.47	1.4
2H-Benzo[f]oxirenol[2,4-E]benzofuran-7(9h)-one	25.31	6.04	γ-Elemene	14.53	7.2
Butyl ethylhexyl phthalate	28.80	3.2	A-Humulene	14.83	0.65
3-[cyclopropyl(hydroxy)methyl]butanoic acid	29.92	0.44	Aglaiene	15.01	1.2
12,15- methyl linoleate	33.12	9.6	α- Cedrene	15.04	0.16
9,12,15,- methyl linoleate, (Z,Z,Z)-	33.25	3.6	δ-amorphene	15.43	1.9
Isoallocholic Acid Ethyl Ester	38.64	0.18	β -caryophyllene epoxide	15.47	6.0
1-H-Cyclopropa[3,4]benz[1,4-e]azulene-6,7b,8,9a-tetrol	40.76	0.97	Selina-4(14),11-diene	15.56	1.03
-	-	-	D-γ-cadinene	15.60	3.2
-	-	-	B1-Cadinene	15.65	0.29

-	-	-	(4aR,8aα)-Decahydro-4aβ-methyl-1-methylene-7-(1-methylethylidene)naphthalene	15.68	0.44
-	-	-	(1E,4E)-germacrene B	15.71	9.6
-	-	-	α- Elemol	15.78	2.8
-	-	-	γ -Cadinol	16.04	0.80
-	-	-	(+)- Isospathulenol	16.26	0.18
-	-	-	γ-Amorphene	17.18	0.81
-	-	-	(+)- Ledol	17.40	0.16
-	-	-	8- β-H-Cedran-8-ol	18.10	4.3

*R.T., retention time; **R.L., Relative level trans-, β-, and epoxy

Table 3. The Inhibitory Impact of Raw Extract of *F. tenacissim* and *J. communis* Individually on the Excretion of Mycotoxins of Tested Fungi on YES Broth for 10 Days of Incubation at 25 ±2 °C

Producer	Mycotoxins ngml^{-1}	Treatment			LSD at 0.05%
		Control	*F. tenacissim*	*J. communis*	
A. flavus	Aflatoxin	725.81^{a}±1.1	561.63^{c} ±0.6	576.09^{b} ±0.2	1.557
	Reduction %	-	22.6	41.5	
	o-Methylsterigmatocystin	14.67^{a} ±0.5	8.57^{c}±0.2	9.48^{b} ±0.3	0.380
	Reduction %	-	20.49	34.3	
A. fumigatus	Fugilin	620.73^{a} ±1.2	390.53^{c} ±1.1	430.30^{b} ±0.8	2.057
	Reduction %	-	37.2	30.8	
F. verticilliodes	Macrofusine	1232.77a ±2.8	835.83f ±0.9	943.50c ±0.7	2.658
	Reduction %	-	32.2	23.5	
	5-Butylpicolinic acid, 5-butylpyridine-2-carboxylic acid	99.87^{a} ±2.2	78.57^{d} ±1.0	82.87^{c} ±0.3	2.032
	Reduction %	-	21.3	17.0	
	1-Hydroxycyclobut-1-ene-3,4-dione	869.27^{a} ±2.8	660.11^{d} ±1.1	726.93^{c} ±0.9	2.604
	Reduction %	-	24.1	16.4	
F. oxysporum	Macrofusine	1179.17^{b} ±1.5	852.53^{e} ±1.0	932.76^{d} ±1.1	2.658
	Reduction %	-	27.7	20.8	
	5-butylpicolinic acid, 5-butylpyridine-2-carboxylic acid	87.83^{b} ±0.7	64.42^{f} ±0.5	65.95^{e} ±1.2	2.032
	Reduction %	-	26.7	23.8	
	1-Hydroxycyclobut-1-ene-3,4-dione	795.57^{b} ±1.1	594.31^{e} ±0.7	689.33^{d} ±1.1	2.604
	Reduction %	-	25.3	17.1	

Impact of Crude Extracts on Excretion of Mycotoxins

Table 3 displays the results pertaining to the generation of toxins. The GC-MS results demonstrated that plant extracts influenced the mycotoxin synthesis of *A. fumigatus, A. flavus*, *F. verticilliodes*, and *F. oxysporum*. They also showed a similar pattern of plant species' inhibitory effect on fungal biomass. The outcomes showed that the methanolic extract of both plants marginally decreased the production of all mycotoxins. However, when compared to *J. communis* extract, the extract of *F. tenacissima* showed a strong inhibitory impact on mycotoxin synthesis. Mycotoxin and phytotoxin production in *F. tenacissima* extract treatments was reduced by 21.3% for phytotoxin 5-butylpicolinic acid and 5-butylpyridine-2-carboxylic acid synthesized by *F. verticilliodes* and 41.5% for STC mycotoxin synthesized by *A. flavus* when compared to the control samples. However, a decrease was noted with *J. communis* extract treatment, which reported 16.4% for *F. oxysporum* synthesized moniliformin and 35.3% for STC for *A. flavus* (Table 3). In both plant extracts, the decrease in o-methyl sterigmatocystin synthesis was more noticeable.

Molecular Docking

The fungal targets used in the molecular docking studies were chitin synthase, glucosamine-6-phosphate synthase, and O-methyltransferase A, which are important in fungal cell wall and mycotoxin synthesis. The 3D models of the fungal receptor chitin synthase with highly affinized ligands are displayed in Fig. 1. The selected compounds had the highest antifungal activities against *A. flavus* and *F. verticillioides*. The three-dimensional structure of CS (AF_AFP30584F1) had a resolution of 1.75A and R-value free of 0.187. The total structure weight of the selected protein was 113.81 kDa, with a total atom count of 8,003. The theoretical isoelectric point (pI) of the protein was 6.00, which arranged the protein in a stable form. There were 69 negatively charged residues and 94 positively charged residues in total. The estimated half-life is over ten hours. With a calculated instability index (II) of 37.98, the protein is categorized as stable. The interacting chain with Estomycin is chain B, with hydrogen bonds, salt bridges, and Pi-Pi stacked (Fig. 1A). The salt bridge interactions were between residues LYS 337; ARG 331, and ARG 211 with a distance of 3.38 atoms and a total number of 3,427. The conventional hydrogen bonds in between were GLC 803, TYR 514, THR 515, and GLY 42, with one bond of Pi-Pi stacked and carbon hydrogen bond in between TYR 493, ALA 329, and PHE 481. The interacting chain B and D with (1E,4E)-germacrene B were attached with hydrogen bonds, salt bridges, and attractive charges (Fig. 1B). The salt bridge interactions were between residues LYS 301D; ARG 582D, ARG 579D, and ARG 586D. The conventional hydrogen bonds were between HIS 297D, GLY 292B, HIS 291D, and GLY 294D. The docking analysis outcome of the two compounds with GPS is summarized in Figs. 2C and D, indicating that all the ligands were efficiently docked with fungal receptors and different bonds.

For Paromomycin, the chain A interacted with hydrogen, hydrophobic, and water bridges. The hydrophobic bonds were between LEU 69 and ILE 131 A. The hydrogen bonds were configured at ASP 130, ILE 131, VAL 133, GLN 138, GLY 139, LYS 141, LEU142, and GLY143. The water bridges were between TYR 514, TYR 515, and TYR 493. The interacting bonds between GPS and (1E,4E)-germacrene B were hydrogen, hydrophobic, and π stacking bonds. The hydrophobic and π stacking bonds were between HIS 127A, ARG211, GLN 510, and TYR 572. The hydrogen bonds were distributed between HIS 127 A with a distance of 3.57 A.

Table 4. The Detailed Structure of the Docked Grid Boxes of CS and GPS with Estomycin and Germacrene

Structure features	Grid boxes			
	Grid 1	Grid 2	Grid 3	Grid 4
Dimension	X:25.00 Y: 25.00 Z: 25.00	X: 11.6990 Y: 2.9001 Z: 2.5014	X: 18.7001 Y: 14.7736 Z: 27.8239	81.7001 45.7336 27.8000
Spacing (Aº)	0.3750	0.2000	0.2000	0.6034
Sequence	DFVRMNAEGKHLS QCTIYPW	RENQGLHVKM AWFCITSPDY	PGVLITHAQWNS DREFY	SLYFIGRDAET HVKMPQWNC
Cavity volume	1453	1718	1471	1859
Cavity point	-23.355 -4.981 66.403	-18.981 -16.341 55.711	4.375 -5.286 68.499	-4.879 11.606 60.540

Table 5. Molecular Docking with PyRx Software

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Estomycin -CS	-5.6	7.79	4.523
Germacrene-CS	-4.5	20.234	15.9
Estomycin –GPS	-7.4	3.245	2.009
Germacrene-GPS	-6.9	3.289	2.114

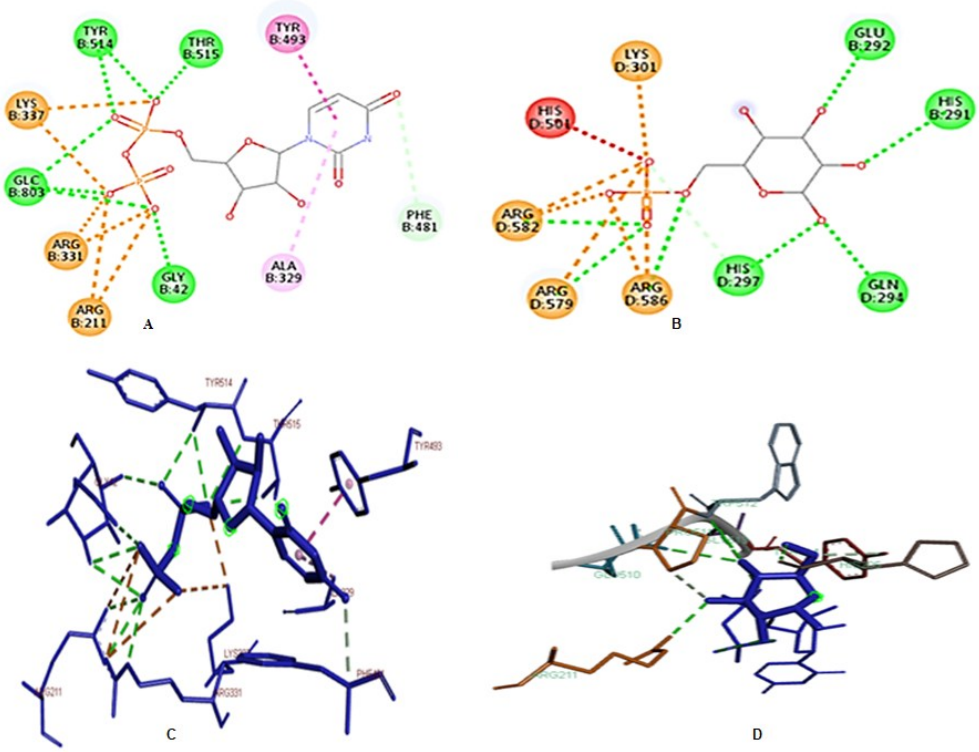

Fig. 1. Docked conformation of the selected compounds at inhibition bounding site of CS (A) Estomycin, B) (1E,4E)-germacrene B GPS, C) Estomycin, and D) Germacrene

For docking data, the generated grid boxes of the docked Estomycin with CS were Grid 1 and Grid 2. The dimension of Grid 1 was X: 25.0000, Y: 25.0000, and Z: 25.0000A (Fig. 2A). The dimension of Grid 2 for (1E,4E)-germacrene B was X: 11.6990, Y: 2.9001, and Z: 2.5014A (Fig. 2B). The docked structures of GPS with Estomycin and (1E,4E)-germacrene B were X: 18.7001, 81.7001; Y: 14.7736, 45.7336; and Z: 27.8239, 27.8000 (Fig. 2C, D). All the detailed structures of the Grid boxes with the volume of cavity and sequences were shown in Table 4. Table 5 summarizes the results of the docking analysis, showing that (1E,4E)-germacrene B and estomycin docked with CS effectively. Estomycin had the lowest binding energy with CS, while the two prevalent phytochemicals had binding potentials with PyRx ranging from -5.6 to -4.5. The binding energies of the two ligands were determined to be -7.7 and -7.4 using GPS.

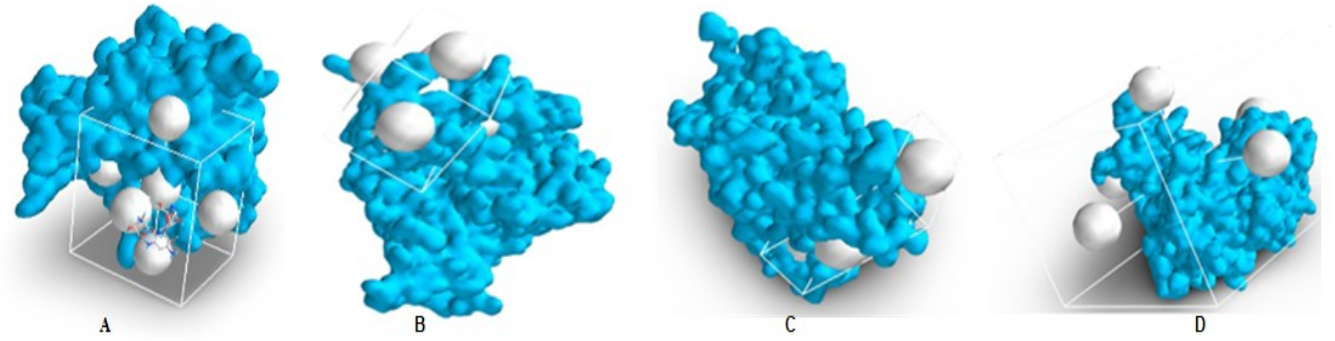

Fig. 2. The three dimensions of grid box showing the axes X, Y, and Z with white circles. A) Estomycin with CS B) (1E,4E)-germacrene B with CS, C) Estomycin with GPS D) Germacrene with GPS

Omt-A Protein Molecular Docking Analysis

A distant homologue and Ramachandran plot, which showed 98% residues in the most preferred areas, was used to validate the model after OmtA (6J1O) was chosen for modeling. The percent of residues found in areas that were prohibited was 0.0%. The Omt-A structural model was refined, and its ligand binding sites were targeted with effective mycotoxins. The protein has a molecular weight of 786.90 kDa and a calculated pI of 4.37. There were two negatively charged residues and one positively charged residue. The instability index (II) was computed to be 5.26, classifying the protein as stable. Chain A was used in the docking modeling. The chain has five crucial sites, of which sites 1 and 2 were the most suitable for molecular docking.

The area, volume, description, and position of the five active sites were illustrated (Table 6). The interacting chain with aflatoxin is chain A with hydrogen bonds, salt bridges, and ionic interaction (Fig. 3A). The salt bridge interactions were between residues ASP 238, MET239, LEU219, HIS133, and SER 38A. The conventional hydrogen bonds were between TRP 149, SAH401, GLY 227, ASN 257, and PHE 276. The ionic bonds were between Phe 162, TRP252, ASP42A, TYR71A, and LEU120A. Fugilin was docked within active site 2 with hydrophobic and ionic bonds and no hydrogen interactions were found. The interacting hydrophobic bonds were ALA345, LEU 346, LEU 138, PHE 189, and ARG 291 (Fig. 3B). The ionic bonds were PHE 276, HIS274, LEU 253, ASP 252, and ARG 275. HIS 40 was a primary amino acid residue, involved in a hydrogen bond with the Macrofusine VAL 42, ASP 140, SAH 224, ASP 142, and GLN 90. These were crucial

amino acid residues behaving as a hydrophobic interaction with the ligand (Fig. 3C). The 5-butylpicolinic acid and 5-butylpyridine-2-carboxylic acid docked within the active site 1 with hydrogen bonds and ARG 291 as a primary amino acid, alongside others, were crucial as hydrophobic interactions as PHE 276, GLY 227, GLY 228, and HIS 277. LEU 253 and ASP 257 behaved as ionic interactions (Fig. 3D).

1-Hydroxycyclobut-1-ene-3,4-dione docking results, illustrated in Fig. 3E, showed hydrogen, ionic, and hydrophobic bonds. The docking analysis outcome is summarized in Table 7, indicating that all ligands (Mycotoxins) efficiently docked with OmtA. The ligands were found to have binding potentials with PyRx. With 5-butylpicolinic acid and 5-butylpyridine-2-carboxylic acid having the lowest binding energy (-6.2).

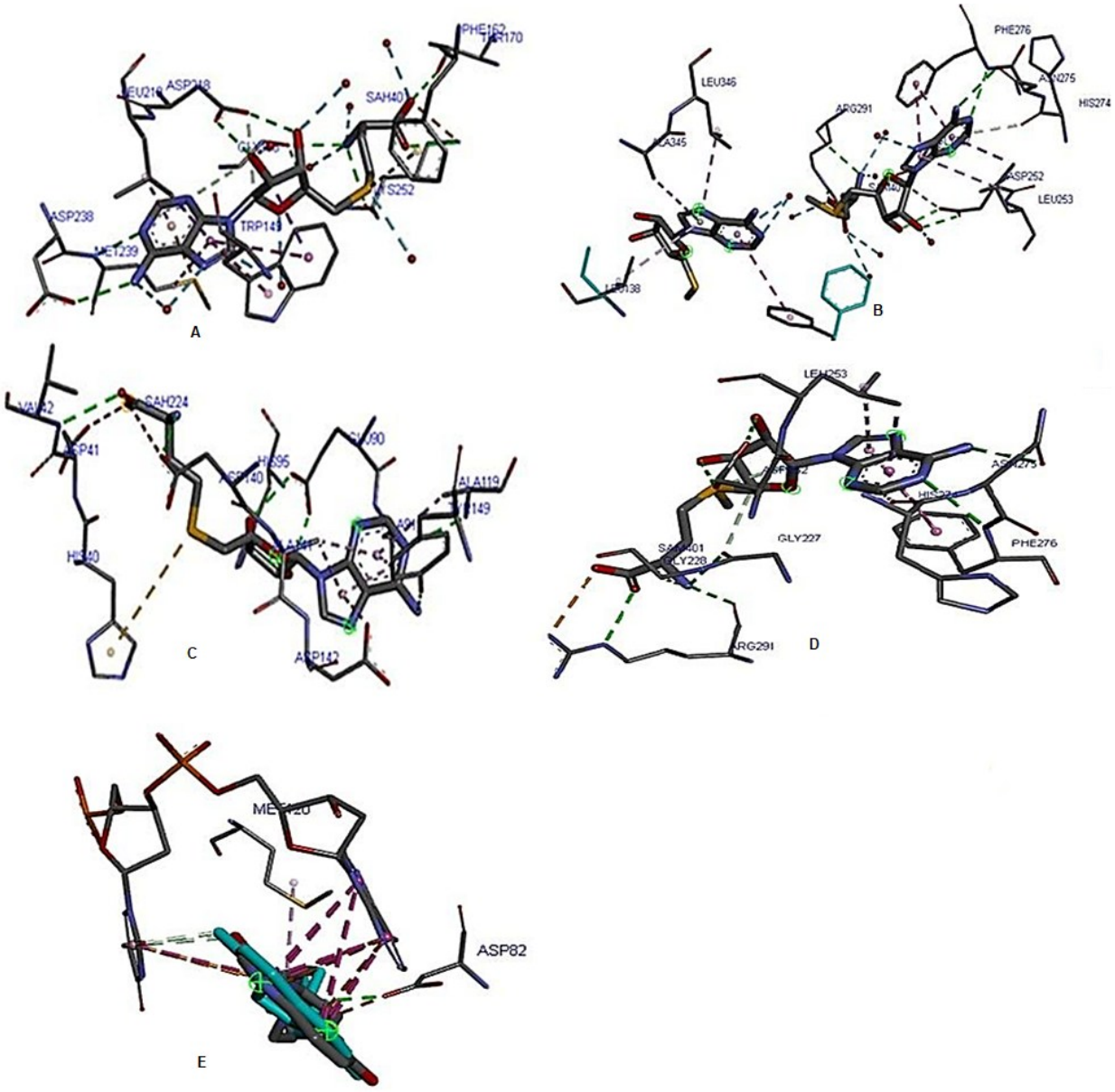

Fig. 3. Docked conformation of the mycotoxins compounds at inhibition bounding site of OmtACS, A) aflatoxins, B) Fugilin, C) Macrofusine, D) 5-butylpicolinic acid, 5-butylpyridine-2-carboxylic acid, and E) 1-Hydroxycyclobut-1-ene-3,4-dione

Table 6. Details of the Five Active Sites of OmtA Showing the Active Sites in the Docking Study

Pocket ID	Area (A^2)	Volume (A^3)	Region of interest	Position (s)	Description
1	1623.130	1210.126	2, 70, 76, 94, 98, 118	175-192	Substrate binding
2	1651.026	1199.507	2, 5, 6, 12, 39, 70, 73, 92, 95, 99, 102, 103, 105, 106	135-148	Substrate binding
3	497.119	505.095	2	227-228	S-adenosyl-L-methionine
4	371.388	243.548	2	252	S-adenosyl-L-methionine
5	137.218	181.659	2,34	275-276	S-adenosyl-L-methionine

Table 7. The Binding Energies of the Selected Ligands Showed Their Affinity (kcal/mol) With the Amino Acids Residue Took Part in Interaction with Respective Sites in Chain A

Ligands	Energy	rmsd/ub	rmsd/lb	Hydrophobic energy	Hydrogen bonds	Ionic interactions
Aflatoxin	-5.5	11.226	7.726	LEU68A	VAL42A LEU68A TYR72 ALA91A ASP142	ASP42A TYR71A LEU120A
Fugilin	-4.5	6.744	5.562	GLY69A LEU120A ASP 41A	ASP286A HIS294A ARG300 A	LYS29A LYS158A THR115
Macrofusine	-5.4	3.113	2.816	VAL 42 ASP 140 SAH 224 ASP 142 GLN 90	HIS40 VAL149.	TRP142A LYS252A ASP252A GLY162A THR200A HIS221A
5-Butylpicolinic acid, 5-butylpyridine-2-carboxylic acid	-6.2	2.231	1.955	VAL 42 ASP 140 SAH 224 ASP 142 GLN 90	PHE276 GLY227 GLY228 HIS 277	LEU 253 ASP 257
Monilifomin	-4.7	12.644	11.54	VAL 83 ASP 56 SAH 78	ASP82 MET 20	ASP 34 VAL 45 GLN 90

DISCUSSION

Plants contain a wide variety of biochemical substances and metabolite derivatives that have been widely employed as biofungicides. The experimental outcomes confirmed that the crude extract of the plants *J. communis* and *F. tenacissima* was effective towards four fungi: *A. flavus*, *A. fumigatus*, *F. verticilliodes*, and *F. oxysporum*. Antifungal properties against all four pathogenic species were demonstrated by the aqua-methanolic extract of *J. communis* and *F. tenacissima*. Compared to the extract of *J. communis*, the extract of *F. tenacissima* was generally more effective at inhibiting the growth of the fungi, with reductions ranging from 34.0% to 37.3%. Numerous data (Aslam *et al.* 2018; Abdelghany *et al.* 2019) supported the studied plants' inhibitory action. Hesham *et al.* (2016) reported that the hyphae and conidia of *Penicillium expansum*, *Fusarium solani*, and *Pythium ultimum* were completely killed by the toxic effects of *F. tenacissima* extracts, which may have fungicidal properties. The cytoplasmic retraction in the attack hyphae's cell wall and subsequent mycelium death may be the mechanism of such extracts' antifungal action (Bazaid *et al*. 2025). GC-MS was applied in the current work to establish the qualitative and quantitative phytochemical research for the accurate characterization of bioactive chemicals in plant extracts. The GC-MS chromatograms of the *F. tenacissima* methanol fraction show that 29 different chemicals were identified. Estomycin, methyl esters of fatty acids, 12-15-methyl linoleate, methylurethane, methyl 2-hydroxybenzoate, and 4-benzyl-2-vinyl-morpholine are among the six prominent compounds shown to have antibacterial and antifungal properties in many studies (Francis *et al.* 2021). It is known that fatty acid methyl esters, such as 12-15-methyl linoleate, have antifungal qualities (Agoramoorthy *et al.* 2007). Estomycin may operate on fungal spores by impairing membrane permeability, making it simple for the antibiotic to reach delicate intracellular locations, such as the cytoplasmic and mitochondrial protein synthesis systems (Rawn and Etten 1978). Numerous detected compounds (39) were found in the *J. communis* extract according to GC-MS analysis. Germacrene, γ-elemene, β-caryophyllene epoxide, and 8-β-H-Cedran-8-ol were found to be the main elements in the foliar sections of *J. communis*. These were followed by moderate levels of β-elemene, α-terpinene, methylurethane, α-elemol, and S-limonene.

The findings concur with those of other studies that reported noteworthy qualitative and quantitative aspects of *J. communis.* According to earlier findings, thymol exhibited the strongest antifungal against *Botrytis cinerea*, the pathogen responsible for grey molds in agricultural goods (Zhang *et al.* 2019). Thymol interfered with the amino acid associated with germination and impacted the enzymatic cell system (Nasser *et al.* 2017). The pinene derivatives were identified by the present GC-MS data. According to some publications, the essential oil extracted from *J. communis* branches has a similar makeup (Raal *et al.* 2010). The aerial portions of four *Juniperus* species—*J. procera, J.* excels, *J. virginiana*, and *J. communis*—were shown to contain trans-, β-, and epoxy caryophylene (Hadaruga *et al.* 2011).

Caryophyllene was detected in the present extract. Previously Barrero *et al.* (2005) demonstrated its antibacterial and antifungal properties. According to the results of GC-MS mycotoxin assays, the generation of all mycotoxins was marginally decreased when *F. tenacissima* and *J. communis* methanolic extracts were used. To combat fungal phytopathogenicity, the antifungal and anti-mycotoxigenic qualities of herbal plants were examined (Bakri *et al.* 2020; Abdelghany *et al.* 2020). The harmful and genotoxic

consequences of mycotoxins may be compromised by some botanical constituents found in plants (Powers *et al.* 2019). Furthermore, Abdelghany (2014) illustrated that *J. procera* extract inhibits *A. flavus* toxins, reducing the generation of aflatoxins B1 and completely inhibiting aflatoxins B2 after treatment. The fungitoxic properties of *J. procera* extract may be the cause, as it blocks the metabolic route of aflatoxins manufacture (Sánchez *et al.* 2005). The results obtained are comparable to those of Abdelghany (2014) and Bakri *et al.* (2020), where the impact of *J. procera* extract is evaluated on the decline in the production percentage of some mycotoxins, such as 5-methyl-picolinate, 5-n-butylpyridine-2-carboxylic acid, o-methyl sterigmatocystin, cyclopiazonic acid, and aflatoxins B1 and B2. According to a recent study, *J. procera* fruit extract suppressed the synthesis of gliotoxins, nivalenol, and neosolaniol toxins (Bakari *et al.* 2020). Through various fractions of *Juniperus* extracts of leaves and bark, numerous studies have confirmed that essential oils effectively regulate mycotoxigenic fungi growth and their related mycotoxins (Powers *et al.* 2019).

Molecular docking is important for predicting the binding interactions between phytochemicals and target proteins (Alsalamah *et al*. 2025b; Binsaleh *et al*. 2025), thereby providing mechanistic support for the observed antifungal and anti-mycotoxigenic activities. To evaluate and examine how effectively a molecule can act as a therapeutic agent, it is crucial to recognize the binding mechanism and probable binding site of a drug within the protein frameworks (Selim *et al.* 2025). Structure-based drug design (SBDD) is the most prevalent computational method employed for drug development based on three-dimensional structures (Geoghegan *et al.* 2017). Bioactive molecules that exhibit the lowest docking scores with their targets suggest the greatest potential for inhibition. Molecular docking is a powerful computational technique used to forecast and determine the possible binding mode between a chemical compound and a specific target protein or receptor (Al-Rajhi *et al.* 2025). With a docking score of 3,670, glucosamine-6-phosphate synthase stood out among the other enzymes for its significant docking contact with 1,8 cineole. Hydrophobic and hydrogen bond interactions were shown to mediate the interaction of 1,8 cineole in the active sites of chitin synthase and glucosamine-6-phosphate synthase. The strength of binding is determined by the number of hydrogen bonds that exist between the ligand and the enzyme (Khan *et al.* 2017).

CONCLUSIONS

1. This study identified the bioactive compounds in *F. tenacissima* and *J. communis* extracts responsible for their diverse biological and antifungal activities.
2. The phytoconstituents and bioactive compounds demonstrated strong anti-mycotoxigenic properties, suggesting their potential use as biofungicides and alternatives to synthetic antifungals.
3. Docking studies confirmed the fungicidal potential of estomycin and (1E,4E)-germacrene B. *F. tenacissima* showed the greatest potential for reducing aflatoxins through interactions with the Omt-A protein.

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