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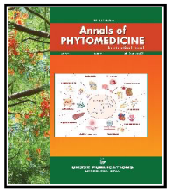

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Methanolic extract of rhizospheric fungus as antimicrobial agents: GC-MS and *in silico* analysis

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Abstract

Antimicrobial drugs are used for the inhibition and management of communicable ailments in flora and fauna. Antimicrobial resistance (AMR) occurs when bacteria no longer respond to antimicrobial medicines leading to increased menace of illness extend, dreadful infection, incapability and expiry. AMR is a usual procedure that develops gradually involving genetic changes in the microorganism. Human interaction, particularly the improper utilization of microbicidal to regulate illnesses in in flora and fauna promotes their establishment and spread. In the present investigation, the methanolic extract of rhizospheric fungus was examined for antioxidant and antibacterial activity. The two rhizospheric fungal species, *Fusarium incarnatum* and *Aspergillus ochraceous*, were distinguished on the basis of distinct and microscopic features. The 51 compounds from above rhizospheric fungi were inspected by the technique gas chromatography-mass spectrometry (GC-MS). There was a significant zone of inhibition of 25 mm contrary to *E. coli* and 26 mm against *Bacillus subtilis* for crude extracts of *Fusarium incarnatum* as compared to *Aspergillus ochraceous*. Further *in silico* docking study showed binding energy in between -6.3 kcal/mol to -3.9 kcal/mol for all the compounds against tetracycline (*i.e.*, 4.95 kcal/mol), which is one of the antimicrobial approved drugs by the food and drug administration (FDA).

1. Introduction

The chemical variability and diverse biological actions of bioactives inventing from natural sources serve as crucial for therapeutic methods in the fight against antibiotic resistance (Irfan *et al*., 2022). Various microbes, including bacteria, fungi, sponges, and many more, were known to produce bioactive chemical substances (Saldívar *et al*., 2022; Stincone and Brandelli, 2020). It has been reported more than ten thousand antibiotics have been discovered in fungus and bacteria (Manganyi and Ateba, 2020). However, a wide range of potential in biotechnology, medicine, food, and agriculture have been documented from the secondary metabolites rhizospheric fungus (Bokhary *et al*., 2021). A study on antibiotic resistance conducted between 2000 and 2010 revealed a significant overuse of antibiotics (76%) in India, China, Brazil, Russia, and South Africa (Chandra *et al*., 2021). Additionally, the overall high consumption of antibiotics worldwide scaled up 65% demarcated daily doses in developing and underdeveloped countries (Gulwako *et al.,* 2023). Consequently, the prevalent issue of antibiotic resistance has been recognized as one of the most significant areas of research for not only the European Union Commission but also other nations (Ferri *et al*., 2017). Therefore, natural bioactive ingredients might serve as an efficient alternate approach for alleviating MDR challenges.

The novel antibacterial compounds from rhizospheric fungal strains require extra attention (Mantravadi *et al*., 2019) to investigate their antibacterial activity. Rhizospheric fungi have been established to serve as plant symbionts, colonizing the roots of more than eighty per cent of all terrestrial plants and promoting a robust soil ecology (Adedayo and Babalola, 2023). Rhizospheric fungi, especially *Fusarium* and *Aspergillus* have been significantly proven to contain antibacterial compounds. One of the notable antibacterial compounds produced by *F. incarnatum* is fusaric acid. The metabolite has been shown to be effective against *S. aureus* and *E. coli*, two types of Gram-positive and Gram-negative bacteria (Shen *et al*., 2020). Another study showed significant antibacterial activity of cyclic hexadepsi peptides enniatins produced by *Fusarium* species. Potential bioactive compounds, such as penicillic acid and xanthomegnin, have been isolated from methanolic extracts of *A. ochraceous* exhibited antibacterial activity against various pathogenic bacteria. Computational molecular interaction and absorption distribution metabolism excretion toxicity (ADMET) assessment has additionally been demonstrated to be effective methods to determine the potential inhibitory impact of crude extracts of rhizospheric fungal insulates against pathogenic bacteria.

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Consequently, the present study focuses on both the *in vitro* and *in silico* antibacterial studies of bioactive compounds derived from selected rhizospheric fungal detached crude extracts as an alternative therapeutic agent which might be used against antibiotic resistance.

2. Materials and Methods

Both *in silico* and *in vitro* approaches (Figure 1) were supposed to be employed for the identification of antibacterial bioactives derived from fungal extract. The antibacterial efficiency and GC-MS profiling of the extracts were performed.

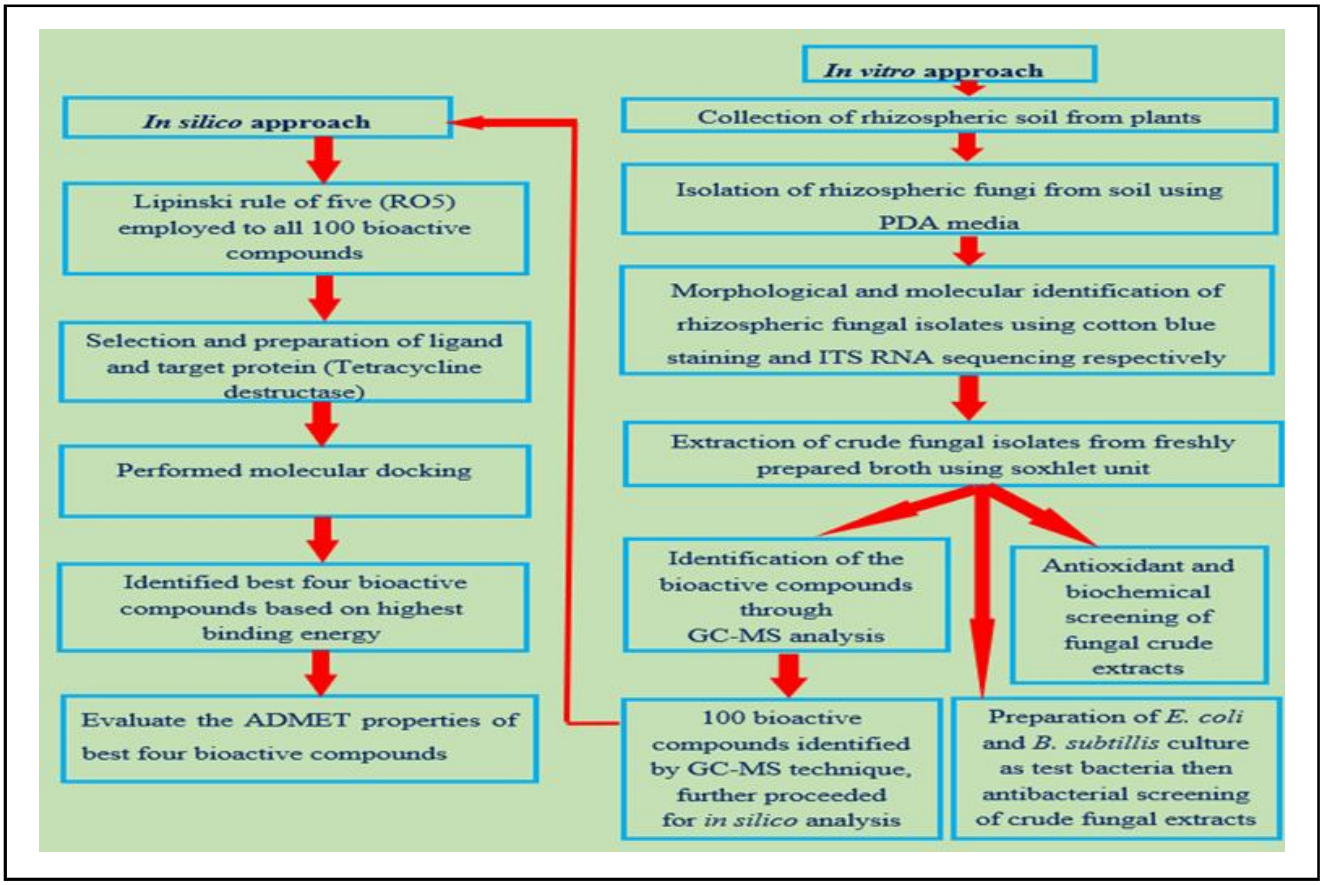


Figure 1: ***In vitro*** **and** ***in silico*** **steps involved in the characterization and isolation of fungi from rhizospheric soil and their bioactive compounds analysis.**

2.1 *In vitro* approach

2.1.1 Sample collection, storage and primary screening for rhizospheric fungal isolates identification

The rhizospheric soil samples (each bag 100 g) were aseptically collected from the rhizospheric areas of two selected plants; namely, *Chrysopogan zizanioides* (*Poaceae*) and *Andographis paniculata (Acanthaceae),* sited at the Central Institute of Medicinal and Aromatic Plants (CIMAP) Lucknow during the rainy season and collected (Kalimuthu *et al*., 2022) in well labelled bags (each bag 100 g). A required amount of freshly cultured fungus was stained with lacto phenol cotton blue reagent to examine the morphological conidial structures with a compound microscope at 40X magnification (Nafaa *et al*., 2023; Khan *et al*., 2024). Presumptive identification was done based on the morphological analysis.

2.1.2 Process for selection of test microorganisms

The preliminary screening for antibacterial activity of rhizospheric fungal crude extracts was evaluated using two bacterial strains. One of these bacterial strains, Gram-negative *E. coli* (MCC-3109), was purchased from National Center for Microbial Resource (NCMR), Pune and another culture *B. subtillis* (MTCC-441), from Institute of Microbial Technology (IMTECH), Chandigarh, respectively.

2.1.3 Minimum inhibitory concentration (MIC) determination assay

The MIC is the lowermost value of an microbicidal that suppresses the apparent proliferation of a microbe resulting from an overnight incubation (Silao *et al.,* 2024). Briefly, 50 µl of freshly cultured test bacteria at a standard concentration of $5x10^8$ colonies/ml were aseptically transferred and spread over solidified nutrient agar (NA) plates. Two methods; namely, macrodilution and microdilution at different concentrations of crude extracts were applied for MIC. Subsequently, each fungal isolate was prepared (3.125, 6.25, 12.5, 25, and 50 mg/ml) in 1 mg/ml DMSO. Tetracycline was practiced as a positive control.

2.1.4 Biochemical characterization of fungal isolates

The crude extracts from fungal isolates were used for different biochemical tests, and the individual results were recorded (Fernandes

et al., 2009). The biochemical tests were performed for alkaloids, flavonoids, steroids, saponins and terpenoids (Falalu *et al*., 2024; Sapunyo *et al*., 2023).

2.1.5 Assessment of bioactive compounds using GC-MS techniques

Rhizospheric fungal crude extracts of *Fusarium incarnatum* and *Aspergillus ochraceous* were analyzed from Central Instrumentation Laboratory (CIL) Bhatinda through GC-MS (Analytical 1, AOC-20i+s). A specific chromatogram was achieved and the base peak of each spectrum was matched with the base peak of the chemical fragments in the NIST Ver. 2005 MS data library for observation of effective secondary metabolites.

2.2 *In silico* approach

2.2.1 Selection and preparation of tetracycline destructase as a receptor protein

Tetracycline antibiotics are covalently altered and inactivated by tetracycline destructases (T Dases), which are monooxygenases (FMO) (Sabra *et al.,* 2024). The 3D structure of the tetracycline destructase protein (PDB ID: 5TUF) was utilized as a reference drug, with an X-ray diffraction resolution of 2.25 Å (Williford *et al*., 2023). Biochemical constructions of complexes were organized using PubChem data. Entire isolated ligands were transformed into Pdb.qt arrangement, and molecular complexes were generated by means of the Autodock tool (Kumari *et al.,* 2023).

2.2.2 Molecular docking and ADMET analysis of ligands

A total of 51 bioactive compounds selected from the GC-MS analysis of two different extracts of rhizospheric fungal isolates were prepared as ligands. The 3D structures and atom coordinates of the compounds were retrieved from the PubChem database (Rojas *et al.*, 2022). Subsequently, the sdf files were converted to pdb.qt format and energy minimization was carried out after the addition of hydrogen atoms. Molecular interactions were performed to identify bioactive compounds derived from rhizospheric fungal isolates in reference to drugs using the docking module Pyr x (Tabrez *et al.,* 2022). For tetracycline destructase, the expected grid dimensions were X: 67.29, Y: 87.31, and Z: 52.51. refinement, placement and an aggregate of 10 conformations of each compound kept in an exclusive database file in a.pdb format (Nayab *et al*., 2020). The binding energy and binding affinity of the protein-ligand complexes were calculated using Discovery Studio (Ramachandran *et al*., 2021). The ADMET properties of all 51 selected bioactive compounds such as hepatotoxicity, cytotoxicity, mutagenicity, immunotoxicity, and lethal dose (LD_{50}) value, were analyzed using the ProTox II server (Karakoti *et al*., 2022).

3. Results

The results section underwent thorough examination, organized into two segments to emphasize distinct analytical approaches. The first part extensively covered *in vitro* analysis, while the second part explored *in silico* analysis.

3.1 *In vitro* results

3.1.1 Isolation and identification of rhizospheric fungal isolates

A total of 10 rhizospheric fungal isolates were observed using serial dilution range 10^{-2} to 10^{-5} from rhizospheric soil of two different plants. Further identification of rhizospheric fungal isolates using ITS rRNA sequencing shown in Table 1 revealed two the most common isolates *F. incarnatum* and *A. ochraceous*. This result evaluates the predominance of rhizospheric fungal communities (*Aspergillus* and *Fusarium* species) associated with the soil of medicinal plants during rainy season which enhances plant growth promotion, antimicrobial and anticancerous activity. The rhizospheric fungal isolates were mainly associated to classes namely: Eurotiomycetes (*A. ochraceous*) and Sordariomycetes (*F. incarnatum*). Both of two isolated rhizospheric fungi were related to ascomycetes.

Table 1: Identification of fungal isolates from rhizospheric soil of plants using solvent and ITS region of rRNA gene sequences

S. No.	Plant sources	Unknown sample fungal isolates	Fungal species identified	Length (bp)	Identity
1.	*Chrysopogan zizanioides*	C3	*Fusarium incarnatum*	550 bp	99.82%
2.	*Andrographis paniculata*	KA3	*Aspergillus ochraceous*	593 bp	99.83%

3.1.2 Evaluation of antimicrobial activity of crude rhizospheric fungal isolates by agar well diffusion method

The crude fungal extracts were prepared at a concentration of 25 mg/ml (Table 2). The methanol extract of the cell culture of *F. incarnatum* exhibited the highest zone of inhibition against *E. coli* (25 mm) and *B. subtillis* (26 mm) indicating that the metabolites are effective against human pathogenic bacteria. *A. ochraceous* showed a minimum zone of inhibition against *B. subtillis* (24 mm) and *E. coli* (21 mm).

Table 2: Fungal isolates showing zone of inhibition against *E. coli* and *B. subtillis*

Test microorganism	Zone of inhibition (mm)		
	Fusarium incarnatum (C3)	*Aspergillus ochraceus* (KA3)	Tetracycline (positive control)
B. subtillis	26	24	10
E. coli	25	21	11

3.1.3 MIC determination test

3.1.3.1 Macrodilution method

For the selected fungal isolates (n = 2), the MIC values ranged from 50 to 150 µg/ml (Table 3) for Gram-positive and Gram-negative bacteria. Fungal isolate *F. incarnatum* (C3) showed MIC at 50 µg/ml against *B. subtillis* and 75 µg/ml against *E. coli.*

3.1.3.2 Microdilution method

The 96 well plate microdilution method was used in all three fungal isolates for evaluation of the MIC value (Table 4). The methanol

fungal extract of *F. incarnatum* had the lowest MIC value, 20 µg/ml against *B. subtillis*. While the MIC of *A. ochraceous* against *E. coli* was established 40 µg/ml.

3.1.4 Biochemical characterization of rhizospheric fungi

Various biochemical tests were performed to identify the nature of the bioactive compounds. Rhizospheric fungi contain numerous biochemical constituents responsible for diverse pharmacological and antimicrobial activities. Rhizospheric fungus may therefore generate volatile chemicals, release secondary metabolites, and compete with bacteria for resources to sustain plant growth (Koza *et al.,* 2022). Our results exemplified the significant presence of saponins, phenols, steroids and flavonoids in methanol extracts of selected fungi in significant amounts.

Table 3: Showing MIC against *E. coli* and *B. subtillis* bacteria using macrodilution method

Test microorganism	MIC (µg/ml)		
	Fusarium incarnatum (C3)	*Aspergillus ochraceous* (KA3)	Tetracycline (positive control)
B. subtillis	50	100	100
E. coli	75	100	150

Table 4: MIC against *E. coli* and *B. subtillis* bacteria using microdilution method

Test microorganism	MIC values of selected fungal isolates (µg/ml)		
	Fusarium incarnatum (C3)	*Aspergillus ochraceous* (KA3)	Tetracycline (positive control)
B. subtillis	20	40	40
E. coli	40	80	80

3.1.5 Free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) dependent antioxidant potential

The antioxidant activity of crude extracts of two rhizospheric fungal isolates was determined through DPPH assay. *F. incarnatum* showed the highest antioxidant activity at 78% at 517 nm (Table 5). While *A. ochraceous showed* 76% inhibition. There has been enormous scope of natural antioxidants produced from rhizospheric fungi to neutralize the reactive oxygen species (ROS). As a study, the antioxidant activity of *A. flavus* petroleum ether extract proved to be moderate, with an IC_{50} of 272 ± 3.7 µg/ml, and ethyl acetate, at 901.3 ± 42.8 µg/ml (Abo El-Nasr *et al.,* 2023). Adekola *et al*. (2017) proposed that exopolysaccharide from *Aspergillus species* unveiled significant anti free radical action with a medium active concentration (EC_{50}) of 573.6 ìg/ml by means of DPPH free radical assay (Adekola *et al*., 2017). Another study estimated DPPH inhibition (%) of Snef1216 (*P. chrysogenum*) as 63.86 ± 0.82 (El-Ghonemy, 2021).

Table 5: IC_{50} value of antioxidant activity of selected fungal isolates

S.No.	Concentration	Name of isolated fungus			
		Fusarium incarnatum		*Aspergillus ochraceous*	
		Absorbance	Antioxidant activity (%)	Absorbance	Antioxidant activity (%)
		Absorbance of control ascorbic acid (AA) 0.118			
1.	20	0.0648	45	0.0605	49
2.	40	0.0531	55	0.0511	57
3.	60	0.0481	59	0.0472	60
4.	80	0.0311	73	0.0322	72
5.	100	0.0253	78	0.0281	76

4. *In silico* investigation of bioactive compounds identified in rhizospheric fungi *via* GC-MS

4.1 Molecular docking scores

A protein tetracycline destructase (PDB ID: 5TUK) might bind a ligand at an orthosteric pocket based on the size, structure, functional groups, and interactions (Szlenk *et al*., 2019). Molecular docking (Table 6) determines the possibility and compatibility of interactions between a protein (receptor) and ligand in a complex (Chen *et al*., 2023). Although the correlation between binding affinity and inhibitory potential may not be straightforward in all cases, research has underscored the significance of comprehending the structural interactions between inhibitors and enzymes to formulate successful therapeutic strategies (Hanson *et al*., 2004; Ma *et al*., 2021). Molecular docking of the studied compounds on tetracycline destructase exhibited the best binding affinity with phenol, 2,4-bis (1,1-dimethylethyl) (-6.3), followed by 9,12-octadecadienoic acid (Z, Z)-, (-5.5), hexadecanoic acid, methyl ester (-4.6) and tetradecane (-3.9) as shown in Table 7. However, two ligands namely phenol, 2,4-bis (1,1-dimethylethyl (-6.3) and 9,12-octadecadienoic acid Z, Z (-5.5) showed a higher binding affinity than control tetracycline -4.95 kcal/mol (Figure 2).

4.2 Molecular interactions of protein ligand assessment

Numerous interactions such as carbon hydrogen bond, conventional H bond, unfavourable donor-donor bond and pi alkyl bonds were observed between the ligands and tetracycline destructase, as shown in Table 7.

Table 6: List of common compounds derived from isolated rhizospheric fungi; namely, *F. incarnatum* and *A. ochraceous*

S. No.	Name of compound (Chemical Id)	Molecular formula (gm/mol)	Molecular weight	Retention time (min)	Peak area (%)	RO5
1.	Tetradecane (CID 12389)	$C_{14}H_3O$	198.39	9.51	4.92	yes
2.	Phenol, 2,4-bis (1,1-dimethylethyl) (CID 7311)	$C_{14}H_{22}O$	206.32	11.98	2.94	yes
3.	Hexadecanoic acid, methyl ester (CID 8181)	$C_{17}H_{34}O_2$	270.5	21.2	1.85	yes
4.	9,12-octadecadienoic acid (Z, Z)-, (CID 5280450)	$C_{18}H_{32}O_2$	280.4	24.6	3.19	yes

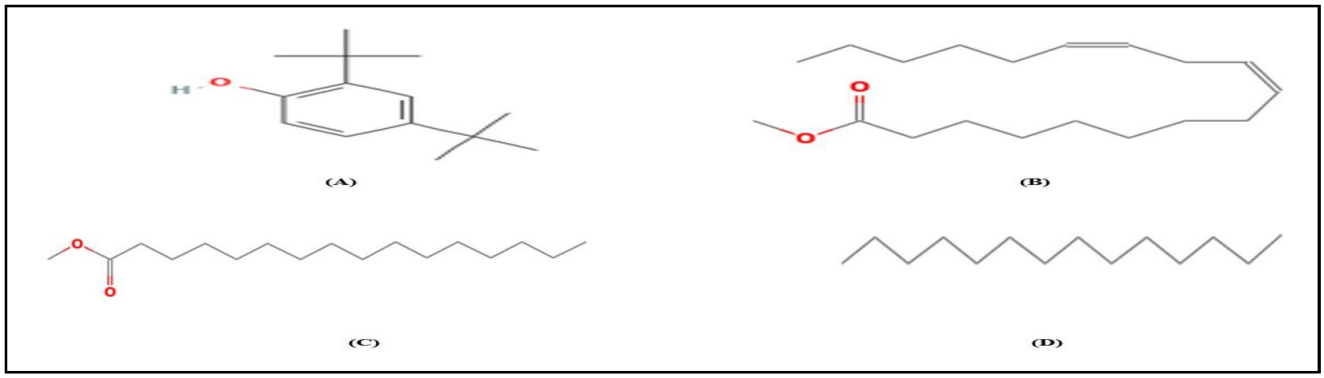
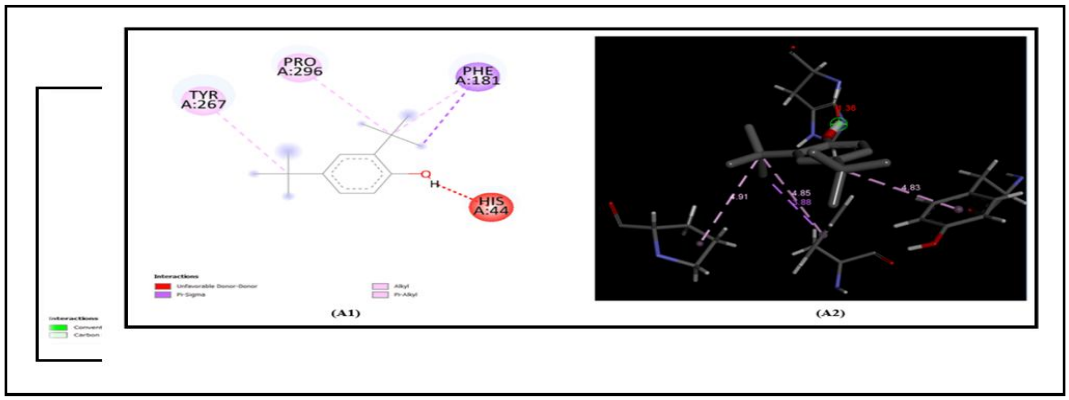


Figure 2: Showing 2D chemical structure of 4 common compounds with best binding affinity (A. Phenol, 2,4-bis (1,1-dimethylethyl) B. 9,12-octadecadienoic acid (Z, Z)-, C. Hexadecanoic acid, methyl ester and D. hexadecanoic acid, methyl ester) isolated from crude rhizospheric fungal extracts applying GS MS techniques.

Table 7: Binding energy of *in silico* screened common bioactive compounds derived from *F. incarnatum* and *A. ochraceous* with their RO5 assessment

S.No.	Bioactives with chemical ID	Chemical formula	Lipinski's rule of five (RO5)					
			MM (g/mol)	HBD	HBA	LogP	MR	Docking score
1.	Phenol, 2,4-bis (1,1-dimethylethyl (7311)	$C_{14}H_{22}O$	206	1	1	-3.98	65.50	-6.3
2.	9,12-Octadecadienoic acid (Z, Z)-, (5280450)	$C_{18}H_{32}O_2$	280	1	2	-6.8	83.37	-5.5
3.	Hexadecanoic acid, methyl ester (8181)	$C_{17}H_{34}O_2$	270	0	2	-7.9	80.33	-4.6
4.	Tetradecane (12389)	$C_{14}H_{30}$	198	0	0	-7.2	66.47	-3.9
5.	Tetracycline	$C_{22}H_{24}N_2O_8$	444	6	9	-0.34	110.79	-4.95

MM: molecular mass, HBD: hydrogen bond donor, HBA: hydrogen bond acceptor, Log P: partition coefficient, MR: molar refractivity



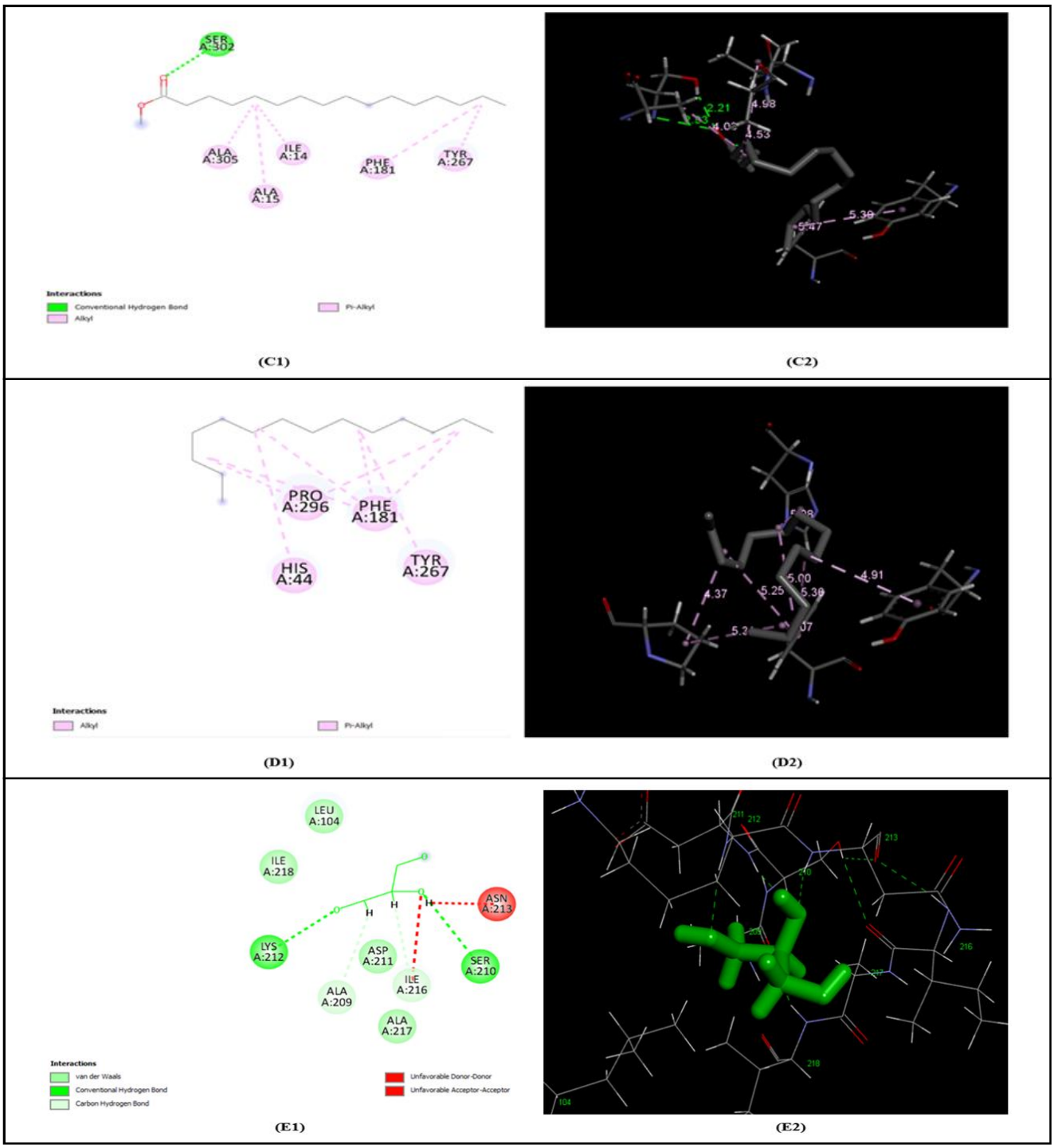


Figure 3: 2D projection of interactions (left) and 3D docked ligand at the binding site (right) of (A) phenol, 2,4-bis (1,1-dimethylethyl (B) 9,12-octadecadienoic acid (Z, Z)-, methyl ester (C) hexadecanoic acid, methyl ester and (D) tetradecane (E) tetracycline (control) with tetracycline destructase.

Some ligands showed hydrophilic interactions, as there is the formation of hydrogen bonds, representing the polar nature of ligands. Apart from that the ADMET property of the above-mentioned top four bioactive compounds were exhibited in the Table 8.

The hydrophobic interaction plays a significant role in the ligand-tetracycline destructase interaction. The major interactions were pi alkyl interactions shown by TYRA: 267, PROA: 296, and PHEA: 181, followed by conventional hydrogen bonds shown by SER A: 302 and carbon hydrogen bonds exhibited by LYSA: 212 and SERA: 302, ALAA: 209 and ILEA: 218 were also frequently observed in other literature and are analogous to our findings (Kupliñska and Rz'd, 2021) (Table 9). Non-polar amino acid residues prefer non-polar ligands.

Table 8: List of ADMET and toxicity properties of the best four compounds and control drug

S. No.	ADMET parameters	Name of bioactive compounds				
		Phenol, 2,4-bis (1,1-dimethylethyl) (CID 7311)	9,12-Octadecadienoic acid (Z, Z)-, methyl (CID 5280450)	Hexadecanoic acid, methyl ester ester (CID 8181)	Tetradecane (CID 12389)	Tetracycline (CID 54675776)
1.	LD50 (mg/kg)	700	10000	5000	750	440
2.	Lipinski rule (RO5)	yes	yes	yes	yes	yes
3.	Carcinogenicity	inactive	inactive	inactive	inactive	inactive
4.	Immunotoxicity	inactive	inactive	inactive	inactive	**active**
5.	Mutagenicity	inactive	inactive	inactive	inactive	inactive
6.	Cytotoxicity	inactive	inactive	inactive	inactive	inactive
7.	BBB penetration	yes	yes	yes	yes	inactive
8.	CYP2C19	inactive	inactive	inactive	inactive	inactive
9.	CYP2C9	inactive	inactive	inactive	active	inactive

Table 9: Molecular interaction of best 4 bioactive compounds with tetracycline destructase protein receptor and their corresponding amino acids active site residues

S. No.	Compound name (ligands)	Binding energy (- kcal/mol)	Hydrogen bond participation	Active site residues (Distance Å)	Common active site residues
Control	Tetracycline	-4.2	2	LEUA: 104ILEA: 216 ASPA: 211ALAA: 217 ASNA: 213SERA: 302	PHEA: 181TYRA: 267 HISA: 44PROA: 296
1.	Phenol, 2,4-bis (1,1-dimethyl ethyl)(CID 7311)	-6.3	0	TYRA: 267, PROA: 296, PHEA: 181, HISA: 44	
2.	9,12-Octadecadie-noic acid (Z, Z)-methyl ester, (CID 5280450)	-5.5	1	TYRA: 267, PROA: 296, PHEA: 181, HISA: 44, SERA: 302	
3.	Hexadecanoic acid, methyl ester (CID 8181)	-4.6	1	SERA: 302, PHEA: 181, TYRA: 267, ALAA: 305, ILEA: 14	
4.	Tetradecane (12389)	-3.9	0	TYRA: 267, PHEA: 181, PROA: 296, HISA: 44	

5. Discussion

More than 3.8 million fungal diversities have been detected and recorded in several documents in both terrestrial and aquatic habitats (Aafreen and Haneef, 2023). *F. incarnatum* and *A. ochraceous* are two common fungal species that can be found in various environments. There are 300 recognized *Fusarium* species. However, almost fifty percent of them have not been researched.

ITS sequencing was used for species identification is novel approach for accurate identification of *Fusarium* and *Aspergillus* species. Mega sequencing is an effective method for phylogenetic analysis. In research, these fungi are studied for their pathogenicity, toxicity, and biotechnological applications. They typically reside as chlamydospores, sclerocytes, or conidia in soil or plant waste, where they may survive for long periods of time. Additionally, in a recent study, *Aspergillus* species were found to constitute about 12 to 20% of the total fungal population in the rhizosphere of medicinal plants like Ashwagandha (*Withania somnifera*) and Tulsi (*Ocimum sanctum*). In a study *Fusarium* species accounted for 10 to 18% of the rhizospheric fungi in plants such as Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale*). Thus, the presence of *Aspergillus* and *Fusarium* species in the rhizosphere of medicinal plants has a significant impact on plant health as well as finding ridiculous source of bioactive compounds.

Recent studies have proved the efficacy of Soxhlet extraction to isolate fungal secondary metabolites, such as alkaloids, terpenoids, and phenolics, which have important therapeutic potential. Further, alkaloids were effectively extracted from *Aspergillus fumigatus* using Soxhlet extraction with ethanol, showing reliability of this technique. Another study revealed potent antioxidants from *Aspergillus niger* using a methanol solvent.

An observation exposed that the extracts of *Fusarium incarnatum* are quite effective in methanol solvent as compared to results in n-hexane in the range of 25 mg/ml (Mohd Israfi *et al.*, 2022). A recent study investigated *Aspergillus ochraceous* having antibacterial activity against *S. aureus*, *B. subtillis and E. coli* with inhibition zones ranging between 9.3 ± 0.5 to 20.8 ± 0.3 nm (Fouda *et al.*,

2022). Moreover, *Aspergillus* and *Penicillium* demonstrated significant antibacterial activity towards *S. aureus* ATCC 25923, *Shigella* species, *Salmonella* species, and *E. coli*, with inhibition diameters ranging from 18 to 29 mm (Rasiya *et al.,* 2021). The difference in the zone of inhibition may be due to the application of different concentrations and test organisms from variable sources during the antimicrobial assay and the use of different extraction methods.

The MIC represents the lowest concentration of bioactive agent that inhibits visible growth of a microorganism after a definite incubation period. A study showed the MIC of chloroformic extract of *Chaetomium globosum* ranging from 0.05 to 5 mg/ml. Moreover, *A. flavus*, *A. aculeatus* and *P. rubens* exposed sensitivity to *M. tuberculosis* at concentrations of 25 to 50 µg/ml. Another study proposed MIC values varying between 12.5 to 50 µg/ml against resistant bacteria, respectively (Mossie, 2024). Future research should concentrate on regulation of MIC determination approaches to facilitate more reliable results.

Different class of bioactives in the crude extracts of rhizospheric fungi was confirmed by different researches (De Mers, 2022). Bioactive compounds from rhizospheric fungal extracts of *Fusarium* sp. exhibited strong antibacterial effectiveness, with an MIC value of 62.50 mg/ml. As a result, rhizospheric fungi promote plant growth and have antimicrobial properties by secreting secondary metabolites, generating volatile compounds, and competing for nutrients.

Rhizospheric fungi, *Fusarium* and *Aspergillus*, are recognized to produce different bioactive compounds with antioxidant potential applied in pharmaceuticals and nutraceuticals. The ethyl acetate extract demonstrated an IC_{50} of 901.3 ± 42.8 µg/ml, while the petroleum ether extract from *Aspergillus flavus* showed slight activity with an IC_{50} of 272 ± 3.7 µg/ml (Abo El-Nasr and Mokhtar, 2023). Additionally, El-Ghonemy (2021) proposed that exopolysaccharides from *Aspergillus* species displayed good antioxidant activity with a median EC_{50} concentration (EC_{50}) of 573.6 µg/ml using the DPPH radical scavenging assay. Another study estimated the DPPH inhibition (%) of Snef1216 (*P. chrysogenum*) as 63.86 ± 0.82.

Analyzing volatile and semi-volatile chemical molecules in complex combinations can be performed with the help of the GC-MS technology. A study investigated the volatile organic compounds such as 3-methylbutanol, hexanol, and octanol from *F. oxysporum* using GC-MS. Further, *Fusarium* extract exposed several phenolic compounds, including gallic acid, ferulic acid, and catechin, with strong antioxidant activities. Chromatographic techniques, and data integration with other omics techniques, such as proteomics and genomics, will enhance our understanding of fungal secondary metabolism and its applications with proper advancements. *Phoma medicaginis* associated with medicinal plants yielded the bioactive compound brefeldine A (Wang *et al*., 2022). In a study carried on crude extract of *A. flavus*, it was observed that the extract is high in flavonoid and phenolic contents (158.33 mg quercetin/ml and 65.77 mg/ml, respectively) (Patil *et al*., 2015). *Penicillium* derived compounds have been searched for potential antibacterial, antifungal and antiviral biological properties. Mycophenolic acid is a potent bioactive compound widely known in medicine derived from *Penicillium* and *Aspergillus* species. Similarly, L-tyrosine exhibited strong antibacterial and antibiofilm activities identified from *R. oryzae* AUMC14899 against multidrug resistant Gram-negative and Gram-positive bacteria (Rabin *et al.,* 2015).

Using Pyrx software, compounds from the GC-MS analysis selected for docking studies to evaluate binding interactions to understand the potent action of the active compounds. All of the specified rhizospheric fungal compounds were docked onto the tetracycline destructase enzyme's active site, which renders them excellent choices for *in silico* antibacterial action. The binding affinities, and energy outlines of compounds, laterally with reference drugs, towards the active site and 3D interactions of the finest principal compounds are summarized in Table 9. Tetracycline destructase binds with tetracycline resulting degradation of antibiotics leading to resistance. However, bioactives from selected rhizospheric fungi have been showed less binding affinity towards selected protein 5TUK. The overall conclusion drawn from the *in silico* studies stated that the common compounds in Table 9 have strong potential to inhibit bacterial growth.

In a study it was observed that identified bioactive compounds from *Aspergillus* species showed strong binding affinities, suggesting their potential as tetracycline destructase inhibitors. Further, enniatin B isolated from *Fusarium* species showed a binding affinity of -7.2 kcal/mol (Yoneyama *et al.,* 2022). These compounds could be further investigated *in vitro* and *in silico* to confirm their inhibitory effects and potential efficiency of tetracycline antibiotics against resistant bacterial strains.

6. Conclusion

The antibacterial activity of crude rhizospheric fungal extracts of *F. incarnatum* and *A. ochraceous* against two bacterial strains reveals higher antibacterial potency compared to the widely used antibiotic, tetracycline. A significant inhibition of bacterial growth, suggesting a promising potential for developing novel approach. These results are valuable in the context of rising antibiotic resistance, as alternative treatments are urgently needed. Further research is required to characterize the specific bioactive compounds and novel techniques responsible for development of effective antibacterial, antiviral and anticancerous drugs.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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