

ANTIBACTERIAL PROPERTY AND MOLECULAR DOCKING STUDIES OF METHANOLIC FUNGAL PHYTOCHEMICALS OF *CLAVICEPS PURPUREA* INFECTED TO BAJRA (*PENNISETUM GLAUCUM*) CROP

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ABSTRACT

Objective: The objective of this study is to study the antibacterial activity, minimum inhibitory concentration, preliminary phytochemical studies, molecular docking studies, and high-resolution liquid chromatography mass spectrometer (HR-LCMS) analysis of the methanol fungal extract of *Claviceps purpurea*.

Methods: Mass production of the fungal mat of *C. purpurea* was established on potato dextrose broth medium. The dried methanol extract of fungus was subjected to HR-LCMS analysis; antibacterial screening of the extract was carried out against pathogenic bacteria. Molecular docking study of HR-LCMS identified compounds was performed by docking with bacterial enzyme DNA gyrase.

Results: HR-LCMS analysis of methanol extract of *C. purpurea* fungus shows that the compounds arecoline, benperidol, felbamate, solanidine, and triparanol as the major constituents. The antibacterial screening of methanol extract of fungus against bacterial pathogens showed a significant bactericidal activity against the strains *Pseudomonas aeruginosa* (14.40±0.04 mm), *Xanthomonas campestris* (13.60±0.06 mm), *Escherichia coli* (13.40±0.06 mm), *Salmonella Typhi* (11.50±0.05 mm), *Staphylococcus aureus* (11.37±0.04 mm.), and as matched to the standard drug ciprofloxacin. The molecular docking of triparanol against the bacterial enzyme DNA gyrase showed good binding affinity of -6.2 kcal/mol, good drug likeness (7.3767), 2 hydrogen bonds, and hydrophobic interaction with 8 amino acid residues so that triparanol shows good inhibitor as compared to other 4 compounds.

Conclusion: Methanol fungal extract of *C. purpurea* showed significant antibacterial activity against pathogenic bacteria. Triparanol is the major compound present in *C. purpurea* fungus which showed good inhibitory activity against bacterial enzyme DNA gyrase. The methanol extract of *C. purpurea* fungus displayed a good antibacterial drug.

Keywords: *Claviceps purpurea*, Methanol extract, Absorption, distribution, metabolism, excretion and toxicity, DNA gyrase, Molecular docking.

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INTRODUCTION

Bajra is a principal source of energy, protein, vitamins, and minerals for millions of the poorest people in the regions where it is cultivated [1]. Pearl millets are known to increase insulin sensitivity and lower the level of triglycerides. Pearl millet is very effective for controlling diabetes. Because of its high-fiber content, it digests slowly and releases glucose into the blood at a slower rate as compared to other foods. This effectively helps in maintaining the blood sugar level constant in diabetes patients for a long period of time [2].

Claviceps purpurea, an ergot fungus, is able to infect rye and other grains and has caused several epidemics, particularly during the middle ages, due to the consumption of rye and other products contaminated with *C. purpurea* sclerotia (ergots). The resulting disease is called ergotism or St. Anthony's fire. Patients show various symptoms depending on the amount and kind of alkaloids they consume. Painful spasms, diarrhea, paresthesia, nausea and vomiting, and headache or psychosis are typical convulsive symptoms, and gangrenous symptoms are observed, especially for fingers and toes [3].

Ergot alkaloids show strong interactions with serotonin, dopamine, and adrenergic receptors of the central nervous system and also with adrenergic receptors in blood vessels. Therefore, they can act as potent drugs. Examples with pharmaceutical applications are methylethylergometrine used in gynecology to stop bleeding after childbirth,

ergotamine used to treat migraines, and the semisynthetic derivative bromocriptine used to treat Parkinson's disease. The pharmacological activities can be explained by the structural similarity of ergot alkaloids with the three neurotransmitters [4].

The phytochemical analysis of methanol crude extract of *C. purpurea* fungus showed the presence of alkaloids, tannins, steroids, glycosides, triterpenoids, and phenols.

C. purpurea fungus contains ergot alkaloids and other some compounds. The biogenetically closely related elymoclavine was less active, whereas the lysergic acid amides ergometrine and ergotamine showed almost no influence on the growth of the bacteria. It was demonstrated that no essential metabolization of these clavines took place during incubation so that there was no doubt about the direct antibiotic activity of the two clavines certain 1-alkyl and 6-alkyl-6-nor derivatives of festuclavine showed a further enhancement of this effect. This turned out to be also true for human pathogenic bacteria species [5].

Over the past period, much attention has been placed on the study of phytochemicals for their antibacterial activity, especially against multidrug-resistant Gram-negative and Gram-positive bacteria [6]. The appearance of multidrug-resistance among bacteria has challenged the use of antibiotics in the advent of modern medicine and as such, antibiotic resistance has become one of the most serious health-care

problems in the world [7]. Considering the above, there is a need to develop new effective antibacterial agents that circumvent the emergence of resistance. Nevertheless, the discovery of new antibiotics is very expensive and time-consuming, requiring about 10 years bringing a new antibiotic to market [6].

In addition, *in silico* prediction of the absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties plays an important role in antibiotic drug discovery process. Now-a-days, ADMET is applied at an early phase of the drug development process to remove molecules with poor ADMET properties from the drug development pipeline and leads to significant savings in research and development costs. Lipinski "rule of five" is widely used as a filter for drug-like properties [8]. Molecular docking is a frequently used method for evaluating the complex formation of small ligands with large biomolecules [9]. Insight of the above, the present study was conducted to isolate and characterize antibacterial compounds from the *in vitro* derived methanol extract of *C. purpurea* fungus and to verify the antibacterial property against human pathogenic clinical isolates [10].

METHODS

Preparation of fungal extract

Sixty-five gram of dried fungal mat was subjected for cold extraction with methanol for about 48 h. The extract was sieved (Whatman filter paper 1) and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and dried at desiccator.

Preliminary phytochemical screening

The preliminary phytochemical analysis of *C. purpurea* fungal extract for the presence of desired secondary metabolites was carried out using standard methods described by Harborne 2005 [11].

High-resolution liquid chromatograph mass spectrometer (HR-LCMS) analysis of fungal extract

The bioactive components of methanol extract of *C. purpurea* fungus were analyzed by HR-LCMS G6550A system (Agilent technologies). The method used for chromatography was 30 min±electrospray ionization 10032014_MSMS.m. The gas temperature used for analysis was 250°C. The theoretical mass of protonated compound was used for identification. HR-LCMS analysis of fungal extraction from methanol was performed at sophisticated analytical instrument facility, Indian Institute of Technology, Mumbai, India. The compounds were identified by comparison with their retention time and mass with stored metlin library available with IIT, Mumbai.

Antibacterial activity

Microbial strains

The antibacterial activity of the fungal extract was individually tested against a set of five bacterial pathogenic isolates obtained from microbial type culture collection center, Chandigarh, namely *Escherichia coli* (MTCC-1599), *Staphylococcus aureus* (MTCC-4734), *Pseudomonas aeruginosa* (MTCC-1934), *Salmonella Typhi* (MTCC-734), and *Xanthomonas campestris* (MTCC-2286) bacterial isolates were cultured overnight at 37°C in nutrient agar (NA) media.

Agar well diffusion assay

The antibacterial activity of aqueous and solvent extracts was determined by the agar well diffusion method. Inoculum containing 106 colony-forming units (CFUs)/ml of each bacterial culture to be tested was spread on NA plates with a sterile swab moistened with the bacterial suspension. The extract was dissolved in dimethyl sulfoxide (DMSO) at different concentrations (25, 50, 75, and 100 mg/ml of DMSO mg). Wells were made on agar plates using sterile cork borer, and 20 µl of fungal extract of each concentration were introduced into appropriately marked wells, ciprofloxacin (20 µg/ml) was taken as a positive control. Then, culture plates were incubated for 24 h at 37°C. Antibacterial activities were evaluated by measuring the diameter of the

growth inhibition zone in millimeters for the test organisms compared to the controls. The activity index (AI) was calculated for comparison of the zone of inhibition (ZI) of test material with standard antibiotic using the formula $AI = ZI \text{ of test} / ZI \text{ of standard}$ [12].

Minimum inhibitory concentration (MIC)

Antibacterial activity was confirmed by determining the MIC using microdilution method with resazurin. Bacterial suspensions were prepared by direct colony method. The turbidity of initial suspension was adjusted by comparing with 0.5 McFarland's standard and initial bacterial suspensions contain about 106 CFUs/ml and then 1:100 diluted in sterile 0.85% saline. A two-fold serial dilution of methanol fungal extract was made in a concentration range from 20 mg/ml to 0.0012 mg/ml in sterile 96-well plates containing Mueller–Hinton broth. A 10 µl of diluted bacterial suspension was added to each well to give a final concentration of 5×10^6 CFU/ml. Finally, 10 µl of resazurin solution, as a display of microbial growth, was added to each well. The inoculated plates were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of tested compound that prevented resazurin color change from blue to pink. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software and Microsoft excels to determine the mean and standard error [13].

Molecular docking studies

Lipinski "rule of five" is commonly used as a filter for drug-like properties [14]. *In silico* pharmacokinetic properties and ADMET analysis were predicted using DataWarrior (<http://www.openmolecules.org/datawarrior.html>). DataWarrior tries to evaluate the toxicity risk by finding substructures within the chemical structure being indicative of a toxicity risk within one of said four major toxicity classes. The chemical structure of HR-LCMS identified compounds, namely arecoline, benperidol, felbamate, solanidine, and triparanol. The standard drug ciprofloxacin was drawn using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) assigned with proper two-dimension (2D) orientation, and structure of each was checked for structural drawing error. The energy of each molecule was minimized using ChemBio3D. The energy minimized ligand molecules were then used as input for AutoDock Vina to carry out the docking simulation. The protein data bank match file with the name "2XCT.pdb" was used as receptor molecule [15], and all the water molecules were removed from the receptor. The graphical user interface program MGL tool was used to set the grid box for docking simulations. The grid was set so that it surrounds the region of interest in the macromolecule. The grid box volume was set to 8, 14, and 14 Å for x, y, and z dimensions, respectively, and the grid center was set to 3.194, 43.143, and 69.977 for x, y, and z center, respectively, which covered all the ten amino acid residues in the considered active pocket. The docking algorithm provided



Fig. 1: (a): Mass production of *Claviceps purpurea* (b): Mycelial mat of *C. purpurea* (c): Grinding of mat with methanol solvent (d): Crude extract of *C. purpurea*

Table 1: Phytoconstituents of methanol extract of *Claviceps purpurea* from high-resolution liquid chromatograph mass spectrometer

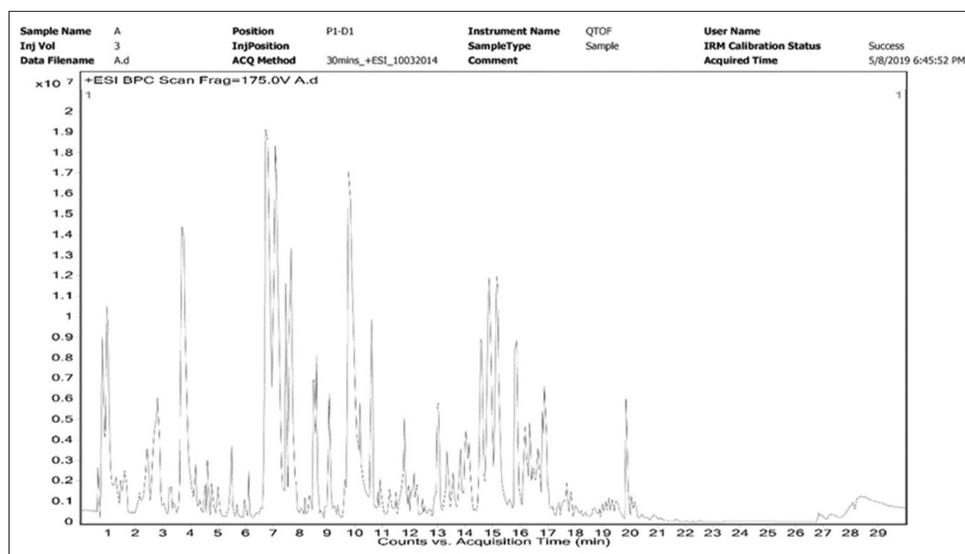
Compound label	RT	Mass	Formula	DB difference (ppm)	Hits
Cpd 1: Triparanol	0.912	143.0972	C7 H13 N O2	-18.25	2
Cpd 2: Azoxy-2-procarbazine	0.935	235.1314	C12 H17 N3 O2	3.04	7
Cpd 3: Isopentenyladenine	0.952	203.1182	C10 H13 N5	-5.31	2
Cpd 4: 1.054	1.054				
Cpd 5: Arecoline	1.072	155.0968	C8 H13 N O2	-14.07	3
Cpd 6: 6,8,10,12-Pentadecatetraenal	1.585	218.1682	C15 H22 O	-5	2
Cpd 7: 5,8,11-heptadecatriynoic acid	2.525	258.1636	C17 H22 O2	-6.13	4
Cpd 8: Acetyltryptophanamide	2.578	245.1177	C13 H15 N3 O2	-4.99	8
Cpd 9: 3-Hydroxy-7-aminonitrazepam	2.92	267.1001	C15 H13 N3 O2	2.64	8
Cpd 16: 2H-Indol-2-one,	5.56	264.1506	C14 H20 N2 O3	-12.32	3
1,3-dihydro-4-[2-hydroxy-3-[(1-methylethyl) amino] propoxy]-					
Cpd 17: 2-(diethylamino)-4'-hydroxy-propiophenone	5.607	221.1445	C13 H19 N O2	-13.04	3
Cpd 18: Benperidol	5.992	381.187	C22 H24 F N3 O2	-4.51	10
Cpd 19: Benperidol	6.682	381.1871	C22 H24 F N3 O2	-4.81	10
Cpd 20: Pinosylvin methyl ether	6.706	226.0987	C15 H14 O2	2.96	4
Cpd 21: 6.728	6.728				
Cpd 22: 6.793	6.793				
Cpd 23: Solanidine	6.793	397.339	C27 H43 N O	-11.47	2
Cpd 26: GPEtn (6:0/6:0)	7.108	411.2008	C17 H34 N O8 P	3.42	8
Cpd 27: L-4-Hydroxy-3-methoxy-a-methylphenylalanine	7.148	225.1045	C11 H15 N O4	-19.57	3
Cpd 29: 7.150	7.15				
Cpd 30: Felbamate	7.151	238.0918	C11 H14 N2 O4	15.14	6
Cpd 31: 7.243	7.243				
Cpd 33: Digitoxigenin monodigitoxoside	7.537	504.3037	C29 H44 O7	9.87	1
Cpd 35: 7.681	7.681				
Cpd 38: 8.290	8.29				
Cpd 39: Tiapride	8.394	328.1448	C15 H24 N2 O4 S	2.63	4
Cpd 40: Androsta-1,4-dien 3,17-dione	8.396	284.18	C19 H24 O2	-8.44	2
Cpd 41: 8.422	8.422				
Cpd 43: GPGro (18:1 (9E)/0:0)[U]	8.676	510.291	C24 H47 O9 P	9.41	3
Cpd 44: Apramycin	8.854	539.2837	C21 H41 N5 O11	-6.4	1
Cpd 45: 9.065	9.065				
Cpd 47: 2-Hydroxyimipramine glucuronide	9.57	472.2197	C25 H32 N2 O7	2.65	4
Cpd 50: MDL 74156 glucuronide	9.667	488.2151	C25 H32 N2 O8	1.57	10
Cpd 52: 2-Hydroxyimipramine glucuronide	9.828	472.2237	C25 H32 N2 O7	-5.81	6
Cpd 53: Oxybutynin	9.864	357.2254	C22 H31 N O3	14	1
Cpd 54: 9.873	9.873				
Cpd 55: Glimepiride	9.91	490.2321	C24 H34 N4 O5 S	-14.56	2
Cpd 56: MDL 74156 glucuronide	9.947	488.2155	C25 H32 N2 O8	0.67	10
Cpd 57: 2-Hydroxyimipramine glucuronide	10.09	472.2204	C25 H32 N2 O7	1.1	7
Cpd 58: Glimepiride	10.27	490.231	C24 H34 N4 O5 S	-12.21	2
Cpd 59: 2-Hydroxyimipramine glucuronide	10.425	472.2199	C25 H32 N2 O7	2.24	4
Cpd 60: 10.474	10.474				
Cpd 61: 10.525	10.525				
Cpd 62: 10.526	10.526				
2-Hydroxyimipramine glucuronide	10.777	472.2201	C25 H32 N2 O7	1.85	4
Cpd 64: 10.993	10.993				
Cpd 66: 11.299	11.299				
Cpd 67: 11.690	11.69				
Cpd 68: 11.853	11.853				
Cpd 69: 12.080	12.08				
Cpd 70: 12.236	12.236				
Cpd 71: 12.926	12.926				
Cpd 72: 13.233	13.233				
Cpd 74: 13.606	13.606				
Cpd 76: Neu5Aalpha2-3Galbeta1-4Glc	13.869	633.215	C23 H39 N O19	-5.28	2
Cpd 77: 13.919	13.919				
Cpd 78: L-Urobilin	13.972	594.3522	C33 H46 N4 O6	-17.68	1
Cpd 80: 14.254	14.254				
Cpd 82: 14.604	14.604				
Cpd 83: 14.916	14.916				
Cpd 84: 14.921	14.921				
Cpd 85: 15.100	15.1				
Cpd 86: 15.279	15.279				
Cpd 87: 15.611	15.611				
Cpd 89: 15.930	15.93				
Cpd 90: 15.958	15.958				
Cpd 91: 16.117	16.117				
Cpd 93: Troglitazone glucuronide	16.259	616.1898	C30 H35 N O11 S	1628.28	2
Cpd 94: 16.429	16.429				
Cpd 95: 16.578	16.578				

(Contd...)

Table 1: (Continued)

Compound label	RT	Mass	Formula	DB difference (ppm)	Hits
Cpd 96: 16.682	16.682				
Cpd 98: 16.911	16.911				
Cpd 99: GPGroP (16:0/18:1 (9Z))	19.873	828.4767	C40 H78 O13 P2	18.19	2
Cpd 100: 19.911	19.911				

RT: Retention time

Fig. 2: High-resolution liquid chromatography mass spectrometry chromatography of methanol extract of *Claviceps purpurea* fungus

with AutoDock Vina was used to search for the best docked conformation between ligand and protein. During the docking process, a maximum of ten conformers was considered for each ligand. Molecular docking was performed in Corei5 Intel processor central processing unit with 6 GB DDR3 RAM. AutoDock Vina [16] was compiled and run in a Windows 8.0 professional operating system. LigPlot+ [17] and PyMol educational version were used to deduce the 2D and three-dimensional pictorial representation of the interaction between the ligands and the receptor. The ligands are represented in green color, H-bonds with their respective distances are represented with cyan color, and the interacting residues are represented in ball and stick model representation.

RESULTS

Fungal culture

Potato dextrose broth was prepared and supplemented with 0.1 g of yeast extract to this media and sterilized in autoclave at 121°C 15 lbs pressure. After sterilized a loopful of fungal inoculum was inoculated to this broth and incubated at 28°C. After incubation, the filtrate was separated using Whatman filter paper 1. The dried mycelial mat was taken and extracted with methanol (Fig. 1) used for phytochemical analysis and antibacterial screening.

Preliminary phytochemical analysis

The preliminary phytochemical analysis of *C. purpurea* fungus extract showed a positive result for alkaloids, tannins, steroids, glycosides, triterpenoids, and phenols.

HR-LCMS analysis

The results of HR-LCMS analysis of *C. purpurea* resulted the presence of some of the compounds (Table 1) and the chromatogram of the phytoconstituents is shown in Fig. 2. Among them, the compounds arecoline, benperidol, felbamate, solanidine, and triparanol were known for antibacterial properties.

Antibacterial activity

The antibacterial activity of *C. purpurea* fungus was evaluated at the concentrations of 25, 50, 75, and 100 µg/ml of DMSO. 100 µg/ml concentration shows significant antibacterial property noticed against bacterial pathogenic strains are *P. aeruginosa* (14.40±0.04 mm), *X. campestris* (13.60±0.06 mm), *E. coli* (13.40±0.06 mm), *Salmonella* Typhi (11.50±0.05 mm), and *S. aureus* (11.37±0.04 mm.), as compared to the standard drug ciprofloxacin. The MIC assay was performed by modified resazurin assay; the extract shows highest inhibitory activity against *E. coli* with a significant MIC value of 2.01±0.14×10⁻². Inhibitions of bacterial strains are summarized in Table 2.

Toxicity prediction

Arecoline, benperidol, felbamate, solanidine, and triparanol are the five compounds which show pharmacokinetic properties, and toxicity analysis properties identified by HR-LCMS are shown in Table 3. All the 5 compounds conform the Lipinski's "rule of 5 limit better Log S values and were free from mutagenic tumorigenic, reproductive and irritant effect. In general, a poor solubility is associated with bad absorption and the aqueous solubility (Log S) of the compound which significantly affects its absorption and distribution characteristics. Based on the results from the DataWarrior, Log P, better Log S, and good drug score and less toxicity risk parameters are predicted, as shown in Table 4.

Molecular docking

In association with *in vitro* antimicrobial activity, it is useful to carry out *in silico* studies to predict the orientation and binding affinity at the active site of the receptor. The molecular docking of HR-LCMS identified ligand molecules, namely arecoline, benperidol, felbamate, solanidine, and triparanol compound with bacterial enzyme DNA gyrase is shown in Fig. 3. Among them, the compound triparanol showed better docking efficiency with DNA gyrase. It forms two hydrogen bonds with amino acids Asp 437, Gly 459 in the active site of the target protein with bond length 3.09 and 3.27 Å, respectively, with the least binding affinity -6.2 and hence is considered as the best dock conformation (Table 3).

Table 2: Zone of inhibition and MIC values fungal extract against pathogenic bacterial strains

Serial number	Inhibition zone diameter (mm) and MIC (mg/ml ⁻¹)					
	Microorganisms	ZI of fungal extract (100 mg/well)	AI	MIC	ZI of Ciprofloxacin (20 µg/well)	MIC
1.	<i>Escherichia coli</i>	13.40±0.06	0.386	2.01±0.14×10 ⁻²	34.63±0.33	3.14±0.11×10 ⁻³
2.	<i>Pseudomonas aeruginosa</i>	14.40±0.04	0.389	2.94±0.10×10 ⁻²	36.93±0.18	4.2±0.25×10 ⁻³
3.	<i>Salmonella Typhi</i>	11.50±0.05	0.304	2.23±0.14×10 ⁻²	37.73±0.23	5.15±0.12×10 ⁻³
4.	<i>Staphylococcus aureus</i>	11.37±0.04	0.276	2.14±0.01×10 ⁻²	41.10±0.06	3.18±0.51×10 ⁻³
5.	<i>Xanthomonas campestris</i>	13.60±0.06	0.375	4.30±0.15×10 ⁻²	36.23±0.15	3.08±0.32×10 ⁻³

MIC: Minimum inhibitory concentration, ZI: Zone of inhibition, AI: Activity index

Table 3: Molecular docking values of methanol extract of *Claviceps purpurea* fungal compounds obtained from liquid chromatograph mass spectrometer analysis

Ligand	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
Arecoline	-3.9	1	3.01	2XCT: Ser438::1:01	Glu435, Gly436, Asp437, Phe1123
Benperidol	-6.1	2	2.95 3.11	2XCT: Ser438::2:02 2XCT: His1018::2:01	Glu435, Gly436, Asp437, Asp512, Gly1082, Arg1122, Phe1123
Felbamate	-4.5	2	3.08 3.11	2XCT: Ser438::3:04 2XCT: Asp437::3:04	Glu435, Gly436, Gly459, Lys460, Asp512, Ile516, Arg1122, Phe1123
Solanidine	-5.9	1	3.09	2XCT: Asp510::4:0	Glu435, Asp437, Arg458, Asp512, Phe1123
Triparanol	-6.2	2	3.09 3.27	2XCT: Asp437::5:02 2XCT: Gly459::5:02	Glu435, Gly436, Lys460, Asp512, His1081, Gly1082, Arg1122, Phe1123
Ciprofloxacin	-6.4	1	3.04	2XCT: Arg1122::CIP: OAQ	Asp512, ser1084, ser1084

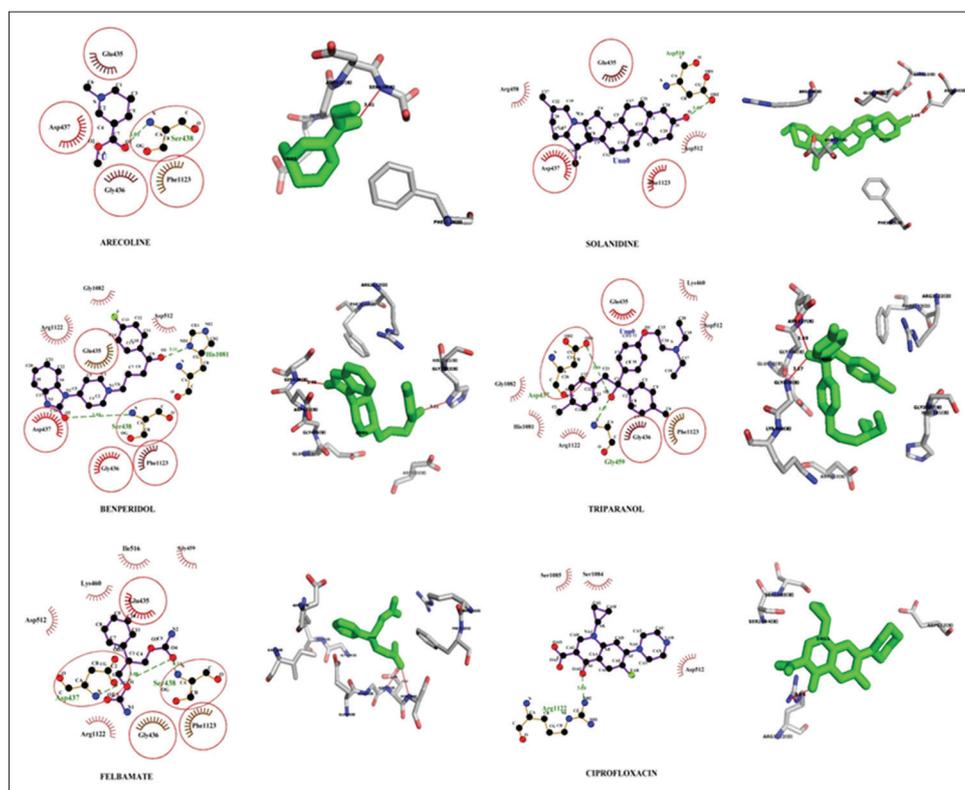


Fig. 3: Two-dimensional and three-dimensional protein-ligand interaction DNA gyrase with the ligands arecoline, benperidol, felbamate, solanidine, and triparanol

The compound benperidol showed the second better docking efficiency with DNA gyrase. It forms two hydrogen bonds with Ser438 and His1018 amino acids with bond lengths 2.95Å and 3.11Å and binding affinity is -6.1. The compound arecoline forms one hydrogen bond

with the amino acid Ser438 with bond length 3.01 Å. The compound felbamate forms two hydrogen bonding with Ser438 and Asp437 and bond length 3.08 and 3.11 Å, respectively. The last compound solanidine forms one hydrogen bond with amino acid Asp510 with bond length

Table 4: *In silico* absorption, distribution, metabolism, elimination and toxicity, and drug likeness prediction using Data Warrior

S. No.	Compound	CLogP	CLogS	H-acceptor	H-donors	TPSA	Ligand efficiency	Drug likeness
1.	Arecoline	0.3138	-0.262	3	0	128.56	0.7048	3.0974
2.	Benperidol	3.3802	-5.191	5	1	288.75	0.2345	6.7132
3.	Felbamate	0.7174	-2.819	6	2	185.82	0.4420	0.8964
4.	Solanidine	5.2031	-5.27	2	1	293.82	0.1978	3.3076
5.	Triparanol	5.5355	-5.116	3	1	350.84	0.2294	7.3767

TPSA: Topological polar surface area

3.09 Å, respectively, in this active pocket. However, all these docked molecules showed more hydrophobic interaction than the standard drug ciprofloxacin. The root mean square deviation (RMSD) has often been used to measure the quality of reproduction of a known binding pose by molecules with ligands. All docked molecules have zero RMSD values, as shown in Table 3.

DISCUSSION

Modern medicines, which are based on synthetic drugs and antibiotics, have only become available during the past 150 years. Previously, humans had to rely on drugs from nature, mostly not only from plants but also from fungi and animals [18].

Microorganisms are significant sources of bioactive natural products with huge potential for the detection of new molecules for drug discovery, industrial use, and agricultural applications. In contrast to other natural sources such as plants, microorganisms are highly diverse but narrowly explored. Studies based on the estimation of microbial populations have discovered that only about 1% of bacteria and 5% of fungi have been characterized, and the rest remain unfamiliar for their contribution to the human welfare [19].

Fungi provide a plentiful and diverse source of unique and often bioactive metabolites, and they have produced a number of medicinally important compounds. The search for new and active compounds from microbial sources is a pursuit for many natural products laboratories. Typically, these efforts will employ a standard culture procedure that most or all microbial strains pass through as a preliminary step to the natural products discovery process [20].

C. purpurea fungal secondary metabolites are very useful to drugs and can be directly extracted from methanol solvent. It contains more phytochemicals as compared to other solvent extracts such as hexane and ethyl acetate [21]. In the present study, HR-LCMS analysis of *C. purpurea* fungus showed the presence of various compounds. Among them, the compounds arecoline, benperidol, felbamate, solanidine, and triparanol are reported as good antibacterial agents [22]. Felbamate is an anticonvulsant drug used in the treatment of epilepsy. It is used to treat partial seizures (with and without generalization) in adults and partial and generalized seizures associated with Lennox-Gastaut syndrome in children. Felbamate is an inhibitor of CYP2C19, and enzyme of the cytochrome P450 system involved in the metabolism of several commonly used medications [23], and triparanol compound is to inhibit the growth of the bacteria [24].

S. aureus bacteremia is a significant cause of morbidity and mortality in neutropenic patients with cancer [25]. In the present study, the metabolites of *C. purpurea* exhibited a significant inhibitory effect on both Gram-positive *S. aureus*, Gram-negative *Salmonella* Typhi, *P. aeruginosa*, *E. coli* and *X. campestris* strains which causes pneumonia (lung infection), osteomyelitis (bone infection), endocarditis (heart infection), phlebitis (infection of veins and blood vessels), mastitis (infection of the breast and formation of abscesses), and meningitis (brain infections).in humans. Antimicrobial activity of secondary metabolites of fungi isolated from the leaves of bush mango against some selected microorganisms [18] and agar well diffusion method was used to study the antibacterial activity of the secondary metabolites and each of the secondary metabolites showed antagonist activity

against the test organisms; Gram-positive bacteria *S. aureus*, and four Gram-negative bacteria *P. aeruginosa*, *Salmonella* Typhi, *X. campestris*, and *E. coli*. These findings from the research indicate that the studies could also pave a way for new therapeutic agents which can be used as potential drugs against the selected microorganisms.

The methanol extract of the fungus *C. purpurea* showed the highest activity against *P. aeruginosa* (14.40±0.04 mm) at a concentration of 100 mg/ml, compared to *Euphorbia hirta* plant ethanol extract showed the highest activity against *P. aeruginosa* (20±0.06 mm) at a concentration of 250 µg/ml [26] and followed by these bacterial strains are *X. campestris* (13.60±0.06 mm), *E. coli* (13.40±0.06 mm), *Salmonella* Typhi (11.50±0.05 mm), *S. aureus* (11.37±0.04 mm.), and ZI.

Arecoline, benperidol, felbamate, solanidine, and triparanol compounds were present in *C. purpurea*; it acts as antibacterial property, and it was supported by molecular docking studies. The *in silico* docking of triparanol and benperidol with the DNA gyrase showed the highest binding affinity and hydrophobic interaction with the amino acids of the active pocket. DNA gyrase is an essential bacterial enzyme that catalyzes the introduction of negative (-) supercoils into chromosomal and plasmid DNA. Gyrase was discovered soon after it was clear that *in vitro* recombination of bacteriophage required a negatively supercoiled DNA substrate. DNA gyrase cleave and transfer DNA to regulate DNA topology and are a major class of antibacterial and anticancer drug targets [14]. The 5 ligand molecules exhibited the antibacterial activity by hindering the function of DNA gyrase.

CONCLUSION

In the present study, methanol extract of fungus *C. purpurea* showed good antibacterial compounds are arecoline, benperidol, felbamate, solanidine, and triparanol. The antibacterial activity was more significant against *P. aeruginosa* (14.40±0.04 mm), and *in silico* docking studies also supported the inhibition of DNA gyrase with highest bonding efficiency and hydrophobic interaction. Due to unscientific overexploitation, many of the medicinal are becoming endangered. The gathering of antibacterial compounds from the *in vitro* grown-up fungus *C. purpurea* methanol extract is a better method to fight infectious microbial diseases.

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AUTHOR CONTRIBUTION

Lokesh ST and Ravikumar S provided substantial contributions in analysis and interpretation of the molecular docking studies. Thippeswamy B contributed in designing and supervision of the work along with drafting the article. Lokesh ST supported in critically evaluating the work and drafting the article. All authors are the guarantors.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

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