Metabolite profiling, In-vitro and insilico assessment of antibacterial and anticancer activities of Alternaria alternata endophytic in Jatropha heynei

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Research Article

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Abstract

Endophytic fungi are the plant symbiont with highly diverse nature and poorly defined ecological importance in host fitness. Although there are the reports on the isolation and characterization of fungal endophytes from a variety of hosts, there is still no report of *Alternaria alternata* from *Jatropha heynei*. Among numerous natural alternative sources, fungal endophytes produce a wide range of structurally diverse bioactive metabolites including antibacterial and anticancer compounds. In this study, an endophytic *A. alternata* was isolated from *J. heynei*. The ethylacetate extract of *A. alternata* was characterized by QTOF-HRLCMS analysis resulted in detection of bioactive compounds include kigelinone, levofuraltadone with antibacterial property and 2-hydroxychrysophanol, isoathyriol, glycophymoline, columbianetin and kaempferol 3-O-β-D-galactoside with cytotoxic properties. The metabolites of *A. alternata* showed significant antibacterial activity against tested clinical bacterial strains by well diffusion method. The high zone of inhibition recorded against Gram positive *Enterococcus faecalis* (14 ± 00 mm), and Gram-negative *Pseudomonas syringae* (19.66 ± 0.57 mm) and *Klebsiella pneumoniae* (14.66 ± 0.57 mm). The *in-vitro* anticancer activity of fungal extract by MTT assay displayed significant cytotoxic effect towards Human lung carcinoma cancer cells (A549) with IC_{50} value of 393.52 $\mu g ml^{-1}$ and no cytotoxic effect to Human breast cancer cells (MCF-7) was observed. Further, antibacterial and anticancer spectral compounds of A. alternata were subjected molecular docking analysis with antibacterial target proteins such as tellurite resistance protein (2JXU), Indole-3-acetaldehyde Dehydrogenase (5IUU), Alkyl hydroperoxide reductase (5Y63) and with anticancer target human apoptotic regulator protein (1G5M). Results of the docking study demonstrated that spectral compounds Kigelinone, Levofuraltadone, 2-Hydroxychrysophanol and Isoathyriol have significant binding modes, with the best binding energy score with their respective antibacterial and anticancer target proteins. The endophytic fungi *A. alternata* in *J. heynei* can be a promising fungus that has broad spectrum antibacterial activity and anticancer property may provide future insight towards the production of bioactive ompounds.

Introduction

Endophytic fungi are a component of the plant microbiome and dwell within plant tissues without causing illness (Suryanarayanan T S 2018). It is believed that more than one million plant-fungal endophytes are dispersed throughout roughly 300,000 terrestrial plant species on earth (Jia et al. 2016). These endophytic fungi are a viable source of antibacterial, anticancer, and other medical therapeutic agents owing to their ability to create several secondary metabolites with diverse structures and activity (Deshmukh et al. 2014; Santos et al. 2015). Endophytes have been the focus of bioprospecting efforts over the past two decades because to their potential to generate metabolites with many desirable bioactivities (Suryanarayanan T S 2018). It is recognised that medicinal plants carry endophytic fungi that are linked with the manufacture of pharmaceuticals (HW S Y and Tan R X 2006). Plants are natural sources of useful molecules that can contribute to the enhancement of innovative medications. Therefore, the plant's antioxidant, antibacterial, wound-healing, antiulcer, anti-inflammatory, and analgesic effects have been thoroughly investigated. However, cultivating the plants and extracting their metabolites are time-intensive processes. Consequently, there is a growing interest in the investigation of new resources, particularly endophytic fungi collected from medicinal herbs that have the capacity to create plant-specific chemicals. Long-term interactions between the

host plant and its endophytes may result in the formation of a symbiotic relationship. This link facilitates the transmission of metabolites between the plant and endophytes. (Aladesanmi et al. 2007).

A number of research have demonstrated that endophytic fungi isolated from medicinal plants can create secondary metabolites with antibacterial and anticancer properties (Lin et al. 2007). Due to the continual evolution of antibiotic resistance in bacterial and fungal pathogens, the demand for antibiotics for human use is increasing, as is the necessity for the creation of novel and effective antibiotic compounds (Santos 2015; Chi W C 2019). Cancer is responsible for 0.3 million annual deaths in India, and the World Health Organization (WHO) expects that cancer will be the leading or second leading cause of death in 2019 (Mann K 2020). Since the discovery of the important medicine Taxol (also known as paclitaxel, a diterpenoid) isolated for the first time from an endophytic fungus *Taxomyces andreanae* recovered from the Pacific Yew bark *(Taxus brevifolia*), endophytic fungi have been recognised as a source of anticancer drugs (Sterile A 1993). Since then, additional anticancer drugs have been isolated from endophytic fungi, including 9-methoxycamptothecin and 10-hydroxycamptothecin from *Fusarium solani* (Shwetha S 2010), camptothecin from *Entrophospora infrequens* (Puri S C 2005), and the anticancer lead compounds podophyllotoxin from *Phialocephala fortinii* (Eyberger 2006) fueled further research on endophytic fungi to discover many other important known and novel anticancer compounds that could be reliable, economical, and environmentally friendly.

Jatropha heynei was collected from extremely stressed locations, specifically dry districts of Karnataka, India. In the present study, the bioactivities and in-silico drug-likeness prediction of chemicals isolated from the endophytic fungus *Alternaria alternata* of *J.heynei* were examined. The ongoing desire to uncover new natural scaffolds with biological activity from endophytic fungi in medicinal plants inspired us to perform the present research, and the results of this endophytic fungus have valuable therapeutic potential for a wide range of illness situations. In addition, molecular docking was utilised to examine the binding affinities of spectrum fractions with the target proteins and their interaction, resulting in the identification of potent antibacterial and anticancer compounds.

Materials And Methods

Collection of plant sample and isolation of endophytic fungi

Root, leaf, and fruit samples of healthy J.heynei plants were collected from their native habitat in Surammanahalli village, Chitradurga district, Karnataka, India (14°6262"N and 76°60'85"E). Based on their morphological characters, the plant species was recognised. (Gamble 1934). The collected plant samples were rinsed in running water and treated to surface disinfection (0.1% hydrogen peroxide for 1 minute, 70% ethanol for 1 minute, and 2.5% sodium hypochlorite for 2 minutes) and the Successful surface disinfection was tested by briefly pressing plant sample fragments onto agar and incubation of imprints (Unterseher M and Schnittler M 2009). The samples were segmented into 1-cm pieces and placed on potato dextrose agar (PDA) or malt extract agar (MEA) media (PDA and MEA, Himedia laboratory, Mumbai) supplemented with ciprofloxacin 250 mg L^{-1} (Ciprodac, CADILA Pharmaceuticals limited) and water agar methods and incubated for 5–7 days at 232 °C under 12/12 h light/nUV (Nischitha R and Shivanna M B 2020). After 5–7 days of

incubation, endophytic fungi were isolated and identified according to their morphological characteristics using standard reference materials. (Subramanian 1983). After obtaining pure cultures on PDA, they were transferred to PDA slants for storage at 4 °C. The morphotypes of anamorphic fungal endophytes incapable of producing reproductive propagules (Nischitha R and Shivanna M B 2020).

Molecular characterization of endophytic fungi

Molecular characterisation and isolation of fungal genomic DNA using a modified Sodium Dodecyl Sulfate (SDS) DNA extraction technique (Aamir S et al. 2015). The internal transcribed spacer (ITS) region was amplified using the following primers: ITS1 5.8S: 5- TCC GTA GGT GAA CCT CGG and ITS4 5.8S: 5- TCC TCC GCT TAT TGA TAT GC -3. The PCR reaction mixture contained 1 L of diluted genomic DNA and amplicon (Taq polymerase). Initial denaturation was performed at 94 °C for 5 minutes, then 30 cycles of amplification, denaturation (94 °C, 1 minute), annealing (50 °C, 30 seconds), and elongation (72 °C, 1 minute) were performed, with the final elongation performed at 72 °C for 7 minutes. After the reaction, the ITS region amplification was confirmed by electrophoresis, PCR products were purified, and amplified DNA fragments were sequenced using Sanger's method. Multiple sequence alignment was used to execute a BLAST search on the obtained ITS sequence at the National Center for Biotechnology Information (NCBI) database (CLUSTAL W). The research sequence was submitted to Genbank, and a phylogenetic tree was created (RAxML GUI v.2.0.0.0 software) using maximum likelihood analysis with 1,000 bootstrap replications using the GTRGAMMA+I model proposed by jModelTest v.2.1.10 (Darriba et al. 2012).

Preparation of endophytic fungus crude extract

The endophytic fungus *A.alternata* was inoculated into potato dextrose broth (600 ml PDB, Himedia laboratory, Mumbai) placed within a 1000 ml Erlenmeyer flask and kept at 272 °C in the dark for 9–12 days with periodic shaking (Gagana et al. 2020). To separate culture filtrate (CF) and mycelial mat, the culture was filtered through Whatman filter paper No. 1. The CF was extracted with ethyl acetate (Himedia Laboratory, Mumbai) in a separating funnel (solvent–solvent extraction), and the extract was kept at 4 degrees Celsius.

In vitro antibacterial activity

Agar well diffusion method

The crude extracts of the endophytic fungus and the host plant were evaluated for their potential antibacterial activity by agar well diffusion method (Nischitha R and Shivanna M B 2021) against ten clinical and pathogenic bacterial strains obtained from Institute of Microbial Technology (IMTECH) in Chandigarh, India. Gram-positive bacteria-*Staphylococcus aureus* (MTCC 902), *Enterococcus faecalis* (MTCC 439) and Gram-negative bacteria- *Escherichia coli* (MTCC 1559), *Salmonella enterica* (MTCC-738), *Salmonella typhi* (MTCC-734), *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604) and *Klebsiella pneumoniae* (MTCC-7028). Levoflaxacin (Cipla, 500 mg) used as positive control and DMSO (Sigma, United States) used as the negative control. The cethylacetate extract of *A.alternata* dissolved in DMSO (10 $mgml^{-1}$) and diluted to 100, 50, and 25% concentrations. Using a sterile cork borer, 0.5 mm wells were drilled into the solidified medium, 20 µl of extract was added to each well, and the plates were incubated at 37 °C for 24

hours. Plates were examined for the zone of inhibition (ZI, mm), and ZI was quantified to assess the antibacterial activity.

MTT Cytotoxicity assay *in-vitro*

The cytotoxic impact of endophytic fungal extract was assessed by the 3-(4, 5-dimethylthiazole-2yl)-2, 5-diphenyltetrazolium bromide (MTT) cell proliferation test (Alley M C et al. 1986) on MCF-7- Human Breast cancer cell line (NCCS, Pune) and A549- Human lung cancer cell line (NCCS, Pune) (NCCS, Pune). In order to create a working solution (1%v/v), the extract was dissolved in Dulbecco's Eagle (DMEM) (#AL007A, Himedia) medium. A 96-well plate containing 200 µl of cell suspension at the desired cell density (20,000 cells per well) was incubated overnight without the test agent. Different quantities of fungal extract 31.25, 62.5, 125, 250, and 500 gml^{-1} were added to the cell suspension and incubated for 24 hours at 37°C in a 5% CO2 atmosphere on a microtiter plate containing the cell suspension. Curcumin served as a positive control, while DMSO served as a negative control. After the incubation period, 100 µl of solubilization solution (DMSO) and the MTT reagent were added. Using an ELISA reader, the absorbance of each well was recorded at 570 nm (Tecan infinite, Austria). The IC_{50} value was determined by using a linear regression equation i.e., Y = mx+c

Here, Y = 50, M and C values were derived from the viability graph.

According to the formula, absorbance values from the ELISA reading were translated to % cell inhibition: Cell inhibition = (Abs. value of control-Abs. value of sample)/Abs value of control x 100%.

Chemo profiling of endophytic fungal extract using QTOF-HRLCMS

HR-LCMS technique (G4220B pump, G4226A auto sampler, G1316C, and a diode array detector) was used to examine the mycochemical composition and quantitative estimation of the endophytic fungi *A. alternata* extract that exhibits antibacterial and anticancer activity (DAD). A gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min made up the elution solvent. The gradient system's starting composition was 95% A: 5% B, and it progressed to 5% A: 95% B in 50 minutes before returning in 10 minutes and remaining at that composition for 5 minutes. The ESI positive ionisation mode was used for the MS analysis. The following MS source parameters were used: capillary voltage of 3500 V, gas temperature of 250 0C, drying gas flow of 13 L/min, sheath gas temperature of 300, sheath gas flow of 11, nebulizing gas pressure of 35 (psig), fragment or of 175 V, skimmer of 65 V, octopole RF peak of 750 V, and mass range of m/z 50-1000. There was a 40,000 FWHM resolution. The conformation was organised using the Metlin database. Based on previously documented research, the chemicals that were discovered in large quantities had their antibacterial and anticancer properties further examined.

Insilico molecular docking

Certain spectrum compounds observed in the OHR-LCMS analysis of the endophytic *A. alternata* ethylacetate fractions and their chemical structures were generated using Chemdraw and afterwards employed as ligands. Using Open Babel, the SD files were converted to their respective 3D structures and then saved as pdb files (O'Boyle et al. 2011). The ligands were screened using the Lipinski rule (Lipinski et al. 2010). For

docking stimulation, only those ligands that were capable of meeting these variations without default were utilised. The Auto dock software was utilised to eliminate water molecules, incorporate gasteiger charges, and merge non-polar hydrogens. The 3D crystal structures of bacterial proteins such as **5IUU**-*Pseudomonas syringae*, **2JXU**-*Klebsiella pneumoniae*, and **5Y63**-*Enterococcus faecalis* as well as human cancer related apoptotic regulator protein Bcl-2 **(1G5M)** were retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank (PDB). Using Auto Dock4.2 software, the protein structures were cleaned, water molecules were removed, Kollman charges were computed, and polar hydrogen was added. (Huey et al. 2007). Auto Dock Vina was utilised to conduct docking simulations, creating nine conformations of the ligand in association with the receptor, which were then rated according to their binding energy (Trott et al. 2010). The docking technique was guided by genetic algorithm (GA) parameters of 200 (population size), 70 (generations), and 3 (number of solution). Between the protein ligands, the bond energies hydrogen bond (Hb), van der Waals (VdW), and electrostatic interaction were discovered. Discovery Studio Visualizer was used to display the resulting conformations (Biovia et al. 2017).

Statistical analysis

The experiments were carried out in triplicate, and the results were expressed as the mean standard deviation. Graph Pad Prism 7 and PAST were utilised for the statistical analysis (Paul et al. 2020). The mean of the samples was compared using Duncan's multiple range test ($P \leq 0.05$) (Nischitha and Shivanna M B 2022).

Results And Discussion

Charcterization of endophytic fungus

The endophytic fungus *A. alternata* was isolated from *J. heynei* leaf tissue. On the PDA medium, endophytic fungal growth was predominant. Based on various physical characteristics such as colony colour, mycelia, hyphal pattern, and sporulation, *A. alternata* was identified as the endophytic fungus. The fungal isolate was identified using morphological and molecular analysis. The ITS rDNA sequencing of endophytic fungus indicated a 100 percent sequence similarity with *A. alternata* by BLAST similarity analysis and NCBI homology identification. The sequence was submitted to NCBI GenBank with the accession number OP288199 based on the results of the BLAST search conducted for the present investigation. From the BLSAT search, sequences with close similarity were recovered, and multiple sequence alignments were done using CLUSTAL W. Further, the maximum likelihood analysis using RAxML and the GTRGAMMA + I model as indicated by jModelTest v.2.1.10 reveals the closest relationship between newly produced sequences and *A. alternata* with 97% bootstrap support (Fig. 1). It has been demonstrated that the phylogenetic tree construction indicates a link between the reference and type strains and the isolated strain (Kaur et al. 2020).

In-vitro **antibacterial potential of** A. alternata **crude extract**

Table 1 summarises the *in-vitro* antibacterial activity of the endophytic fungus *A. alternata* extract against clinical bacterial strains. At concentration of 10 $mgml^{-1}$, the extract exhibited antibacterial activity against all tested bacteria and exhibited remarkable high activity against two gram-negative bacterial strains; *P. syringae*

and *K. pneumoniae* with zones of inhibition of 19.66 ± 0.57 and 14.66 ± 0.57 mm, respectively (Fig. 2). In addition, the extract exhibited significant antibacterial activity against the gram-positive bacteria *E. feacalis*, with an inhibition zone of 14 ± 0 mm. The inhibitory effect of extract against tested pathogenic bacterial strains may be attributable to the action of antimicrobial compounds that inhibit the bacterial enzyme DNA gyrase, a crucial enzyme for DNA replication, or inhibit the cell wall (peptidoglycan biosynthesis) or protein synthesis in bacteria (Kapoor et al. 2017).

Table 1
In-vitro antibacterial activity of ethylacetate extract of *A.alternata*

Test organisms	**Zone of inhibition in mm/conc. %**			
	100%	**50%**	**25%**	**Standard**
Esherichia coli	11.33 ± 0.57	8.66 ± 0.57	5.6 ± 0.58	25 ± 1
Psuedomonas syringae	19.66 ± 0.57	11.33 ± 0.57	8.66 ± 0.57	26.33 ± 0.57
Salmonella typhi	12.33 ± 0.57	9.66 ± 0.57	7 ± 1	24 ± 1
Klebsiella pneumoniae	14.66 ± 0.57	10.66 ± 0.57	7.66 ± 0.57	26.33 ± 0.57
Staphylococcus aureus	12 ± 1	9.66 ± 0.57	6.33 ± 0.57	23.66 ± 0.57
Xanthomonas campestris	12.33 ± 0.57	9.66 ± 0.57	6 ± 0	26.66 ± 0.57
Psuedomonas aeruginosa	13.33 ± 0.57	10.33 ± 0.57	7 ± 0	25 ± 0
Enterococcus faecalis	14 ± 0	11 ± 0	6.33 ± 0.57	23.33 ± 0.57
Note:				
Values are mean ± SE of three replicates,				
Values are mean inhibition zone of three replicates (n = 3) crude extracts were dissolved at 10 $mgml^{-1}$ of DMSO				
Each well received 20 µl/well				

In-vitro **cytotoxic effect of** A. alternata **crude extract**

As demonstrated in Table 2, the cytotoxic activity of extract against A549 lung carcinoma and MCF-7 breast cancer cells was evaluated using the MTT test. The extract was non-toxic to the MCF-7 cell line, but it was cytotoxic to the A549 cell line at various doses, with an IC_{50} value of 393.5 gml^{-1} after 24 hours of treatment. As demonstrated in Fig. 3, the proportion of viable cells reduced dramatically after 24 hours of treatment with extract at increasing concentrations. After extract treatment, the morphology of the cells was examined under a light microscope. At greater concentrations, the cytotoxic action of crude extract generated cell shrinkage, cell rounding, and a reduction in the number of viable cells, all of which are indicative of apoptosis (Karthik et al. 2014). The cytotoxic action of extract is attributable to the presence of anticancer and antiinflammatory chemicals (mentioned in Table 3) in the extract. Several studies demonstrated that anti-inflammatory agents

have role in increased apoptosis and resistance of conventional treatments and thereby decreasing the proliferation and metastasis resulting in the identification of predicted candidates for cancer therapy (De Groot et al. 2007).

Table 2
In-vitro cytotoxic activity of ethylacetate extract of *A.alternata* against MCF-7 and A549 cell lines

Sl.No.	Sample Concentration (μgml^{-1})	Anticancer cell lines					
		MCF-7			**A549**		
		Cytotoxicity (%)	**IC_{50} (μgml^{-1})**	**Cytotoxicity of curcumin at 10 µM (%)**	**Cytotoxicity (%)**	**IC_{50} (μgml^{-1})**	**Cytotoxicity of curcumin at 10 µM (%)**
1	31.25	4.52 ± 0.01	NA	51.17 ± 0.03	11.19 ± 0.03	393.52	52.41 ± 0.01
2	62.5	9.28 ± 0.01			19.35 ± 0.01		
3	125	23.16 ± 00			31.9 ± 00		
4	250	31.32 ± 0.06			40.36 ± 0.01		
5	500	40.59 ± 0.01			56.79 ± 0.01		

Table 3
Metabolite profiling of crude extract of *A. alternata* by QTOF-HRLCMS analysis

Sl. No	Compound	Molecular formula / weight RT(min)	mz Cloud Best Match	Properties	References
1	Chloral hydrate	C_2 H_3 Cl_3 O/ 163.9171; 22.199	164.9243	Sedative	Fong et al. 2021
2	Glycophymoline	C_{16} H_{14} N_2 O/ 250.1098; 6.624	251.117	Anticancer and antimalarial	Teja et al. 2021
3	Tryptanthrine	C_{15} H_8 N_2 O_2; 248.0599; 7.339	271.0489	Antiviral	Narkhede et al. 2021
4	Levofuraltadone	C_{13} H_{16} N_4 O_6/ 324.1088; 8.589	325.116	antibiotic	Janssens P G and De Muynck A 1977
5	5-O-Feruloylquinic acid	C_{17} H_{20} O_9/ 368.1104; 1.034	368.1107	Antioxidant, antifungal	Matei et al. 2012
6	Kaempferol 3-O-β-D-galactoside	C_{21} H_{20} O_{11}/ 448.0991; 6.252	447.0918	Antiinflammatory and antimicrobial	Orhan et al. 2007
7	Salfredin B11	C_{13} H_{12} O_4/ 232.0725; 7.6	277.0707	anti-protein tyrosine phosphatase 1B activity	Liu et al. 2019
8	Avenalumic acid	C_{11} H_{10} O_3/ 190.0624; 7.914	189.0551	Antioxidant	Decker et al. 2002
9	Columbianetin	C_{14} H_{14} O_4/ 246.0886; 8.536	245.0814	Antiinflammatory	Jeong et al. 2009
10	Okanin	C_{15} H_{12} O_6/ 288.0632; 8.856	287.0558	Antioxidant	Kabanda et al. 2021
11	Gambiriin A3	C_{30} H_{28} O_{12}; 580.1573; 9.113	579.1505	Antioxidant	Kassim et al. 2011
12	2-Hydroxychrysophanol	C_{15} H_{10} O_5/ 270.0526; 9.161	270.0528	anticancer	Cichewicz et al. 2004
13	9,10-Dihydro-2,3,5,7-Phenanthrenetetrol	C_{14} H_{12} O_4/ 244.0735; 9.178	243.0662	Antidiabetic	Zhao et al. 2019
14	Kigelinone	C_{14} H_{10} O_5/ 258.0525; 9.65	257.0453	Antimicrobial	Binutu et al., 1996
15	Isoathyriol	C_{14} H_{10} O_6/ 274.0477; 10.07	273.0404	Antioxidant, Antiinflammatory, anticancer	Umoh et al. 2021

Sl. No	Compound	Molecular formula / weight RT(min)	mz Cloud Best Match	Properties	References
16	Pinobanksin	$C_{15}H_{12}O_5$/ 272.0685; 12.423	271.0612	Antioxidant	Zheng et al. 2018

Characterization of metabolites in Alternaria alternata extract

The high sensitivity of the QTOF-HRLCMS enabled the detection of a large number of compounds. Positive and negative ionisation modes each discovered a total of 37 compounds. Compounds included those with conspicuous peaks and high retention values in addition to those with insignificant peaks, which were represented by other compounds. According to available database searches, some of compounds with high peaks possessed unknown activity. These could be identified as new compounds that may be associated with the antibacterial and cytotoxic properties demonstrated in this investigation. In addition, these compounds must be identified based on their chemical profiling and related actions. In contrast, based on the literature search, a number of well-established bioactive compounds with documented properties have been identified. 14 compounds out of 37 in *A.alternata* were found in the positive ionization mode and 23 compounds were detected in the negative ionization mode; and the principal compounds with their biological properties are shown in Table 3. The important bioactive compounds detected in the extract were include Levofuraltadone, Kigelinone, 2-Hydroxychrysophanol, Isoathyriol, Columbianetin, Glycophymoline, Kaempferol 3-O-β-D-galactoside, 5-O-Feruloylquinic acid, Avenalumic acid, Okanin, Gambiriin A3, Pinobanksin, Chloral hydrate and Tryptanthrine. Among the above compounds Levofuraltadone was an antibiotic used to treat African trypanosomiasis (Janssens P G and De Muynck A 1977) and also it was used to treat chagas disease caused by bacteria *Trypanosoma cruizi* (Aronson P R 1962). While, compound Kigelinone is a well known metabolite has broad spectrum of antibacterial and antifungal property reported in previous studies (Binutu et al. 1996). Further, compound 2-Hydroxychrysophanol has a potential cytotoxic effect on breast, colon and lung cancer cell lines (Cichewicz et al. 2004). While, compounds such as Columbianetin (a coumarin derivative), Glycophymoline, Kaempferol 3-O-β-D- galactoside, and Isoathyriol were reported for their antiiflammatory and cytotoxic properties (Jeong et al. 2009; Teja et al. 2021; Orhan et al. 2007; Umoh et al. 2021). The present study also document the certain compounds such as 5-O-Feruloylquinic acid, Avenalumic acid, Okanin, Gambiriin A3 and Pinobanksin were associated with antioxidant property (Matei et al. 2012; Decker et al. 2002; Kabanda et al. 2021; Kassim et al. 2011; Zheng et al. 2018). Further, compound tryptanthrine has antiviral property (Narkhede et al. 2020) and Chloral hydrate was used as sedative or analgisic agent (Fong et al. 2021).

Insilico molecular docking analysis

In-vitro confirmation of antibacterial and anticancer activity of fungal extrcat was performed, as well as a molecular docking investigation of the mechanism of antibacterial activity and apoptosis in cancer cells at the protein level. Docking small molecule compounds into the binding site of a receptor and calculating the binding affinity of the complex is an essential step in structure-based drug design (Seeliger D and de Groot B

L 2010). The extract showed maximun inhibitory activity against *P. syringae* and *K. pneumoniae* and *E. feacalis* so that we have selected respective bacterial target proteins **(5IUU, 2JXU, 5Y63)** for docking study further, the cytotoxicity is based on mechanism of apoptosis and we have have selected apoptotic regulator protein **(1G5M)** for our docking study. In the present investigation, spectrum compounds were submitted to the Lipinski rule of five in order to evaluate their potential as pharmaceuticals. The rule was used to determine the mass, number of hydrogen bond donors, number of hydrogen bond acceptors, log P (octanol-water partition coefficient), and molar refractivity of both Schiff bases, which are listed in Table 4. To explore the antibacterial, anticancer potential of the spectral compounds, docking analysis was supported by selective pharmacological targets; antibacterial targets such as a tellurite resistance protein (2JXU) from *K.pneumoniae*, Indole-3-acetaldehyde Dehydrogenase (5IUU) from *P.syringae*, and Alkyl hydroperoxide reductase (5Y63) from *E. feacalis* and 1G5M as anticancer target protein. Results of the docking study demonstrated that spectral compounds Kigelinone, Levofuraltadone, 2-Hydroxychrysophanol and Isoathyriol have significant binding modes, with the best binding energy score with their respective antibacterial and anticancer target proteins. Among the tested ligand molecules kigelinone established high binding affinity with antibacterial target proteins 2JXU (-7.3), 5IUU (-8.1) and 5Y63 (-6.0). (Figs. 4 and 5; Table 5). Additionally, Kigelinone binding with **2JXU** protein active site established H-bonds with single amino acid residue ASP, for protein **5IUU**, amino acid residues are LYS, ASN, GLY, PHE followed by for the protein **5Y63** amino acids are ASN and GLU. While, levofuraltadone binding to 2JXU produced hydrogen bonds with ALA, with 5IUU protein TRP, ASN, GLY and for 5Y63 protein hydrogen bonds established with TRP and SER. Further, anticancer ligand molecule 2-hydroxychrsophanol interacted with **1G5M** protein and established hydrogen bonds with GLN, TRP, ASP amino acid residues. It is believed that the existence of amino acid residues, conventional hydrogen bonds, alkyl bonds, and carbon-hydrogen bonds are essential for the efficient interaction of ligand with target protein (Nayab et al. 2020).

Table 4
Druglikeness prediction by **"Lipinski rule of 5"**

Parametrs	Ligand molecules				Default conditions for druglikeness
	Levofuraltadone	Kigelinone	2-hydroxychrysophanol	Isoathyriol	
Mass	324.29	258.23	270.24	274.23	< 500 Dalton
Hydrogen bond donar	0	2	3	3	< 5
Hydrogen bond acceptor	10	5	5	6	< 10
Log P	0.69	1.88	3.24	2.09	< 5
Molar Refractivity	86.79	64.97	70.78	72.55	40–130

Table 5
Molecular docking analysis spectral compounds showing estimated binding affinity and interacting residues in the binding sites of 2JXU, 5IUU, 5Y63, and 1G5M.

Proteins	Ligand molecules	Binding affinity	Hydrogen bond-interacting amino acid residues
2JXU	Levofuraltadone	-6.4	ALA
	Kigelinone	**-7.3**	ASP
	Levpoflaxcin	-7.2	LYS
5IUU	Levofuraltadone	-7.2	TRP, ASN, GLY
	Kigelinone	**-8.1**	LYS, ASN, GLY, PHE
	Levpoflaxcin	-8.0	SER, ASN
5Y63	Levofuraltadone	-5.6	TRP, SER
	Kigelinone	**-6.0**	ASN, GLU
	Levpoflaxcin	-6.4	TRP
1G5M	2-hydroxychrysophanol	**-7.4**	GLN, TRP, ASP
	Isoathyriol	-6.8	ALA, TRP
	curcumin	-6.7	HIS, TRP, TYR

Conclusion

The study demonstrates the presence of antibacterial, anticancer, antiviral, anti-inflammatory, and analgesic chemicals in the extract of the endophyte fungi *A. alternata* and also provides comprehensive evidence with molecular docking analysis of spectral compounds against target proteins. Further research is required before these bioactive compounds can be utilised for the benefit of humanity.

Declarations

Author contributions

AGB performed the investigation, methodology and experimental analysis and writing the original draft; MBS; review and editing of the manuscript; finalized the manuscript. All authors read and approved the final manuscript.

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Code availability

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Conflict of interest

The authors declare that there are no conflicts of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Figures

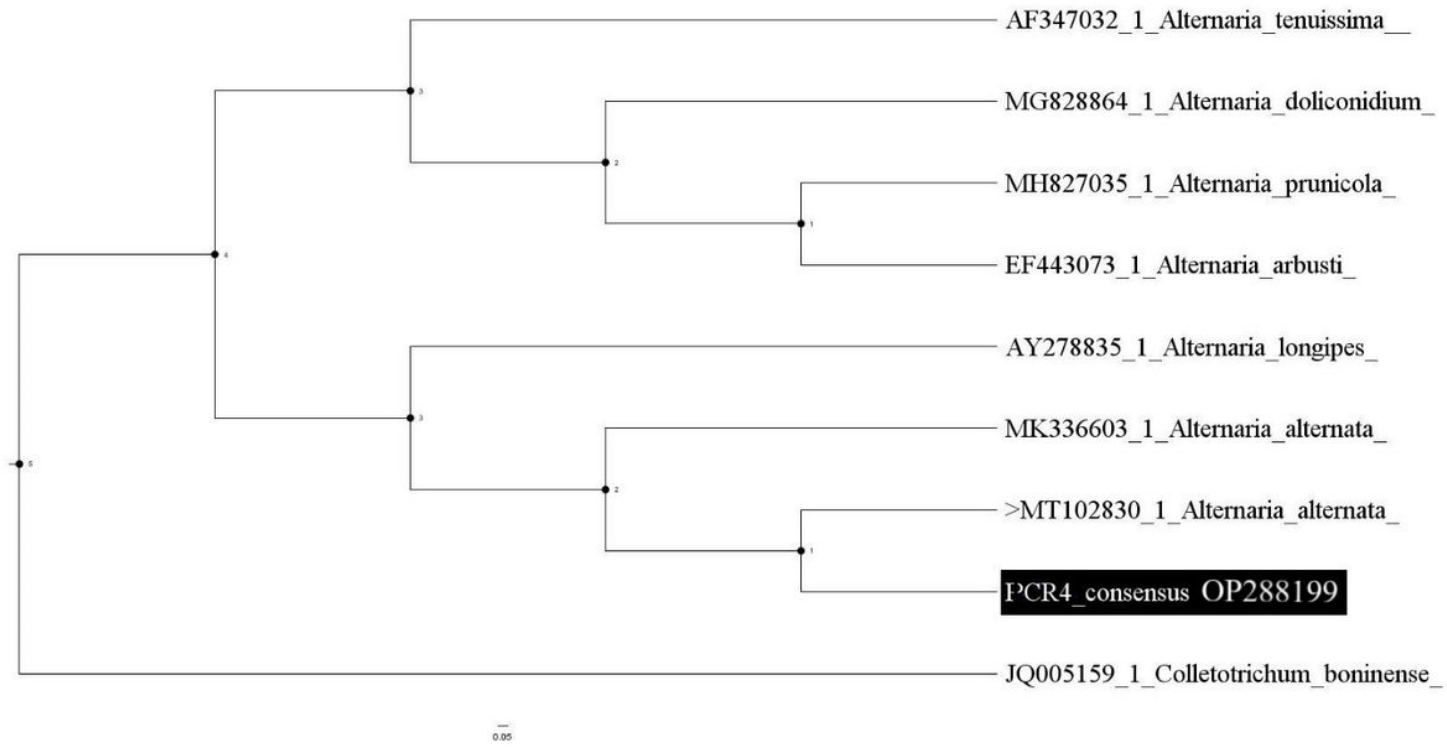

Figure 1

Phylogenetic tree of *Alternaria alternata* generated by maximum likelihood analysis of ITS 1 and 4 sequences using RaxML (GTRGAMMA+I model)

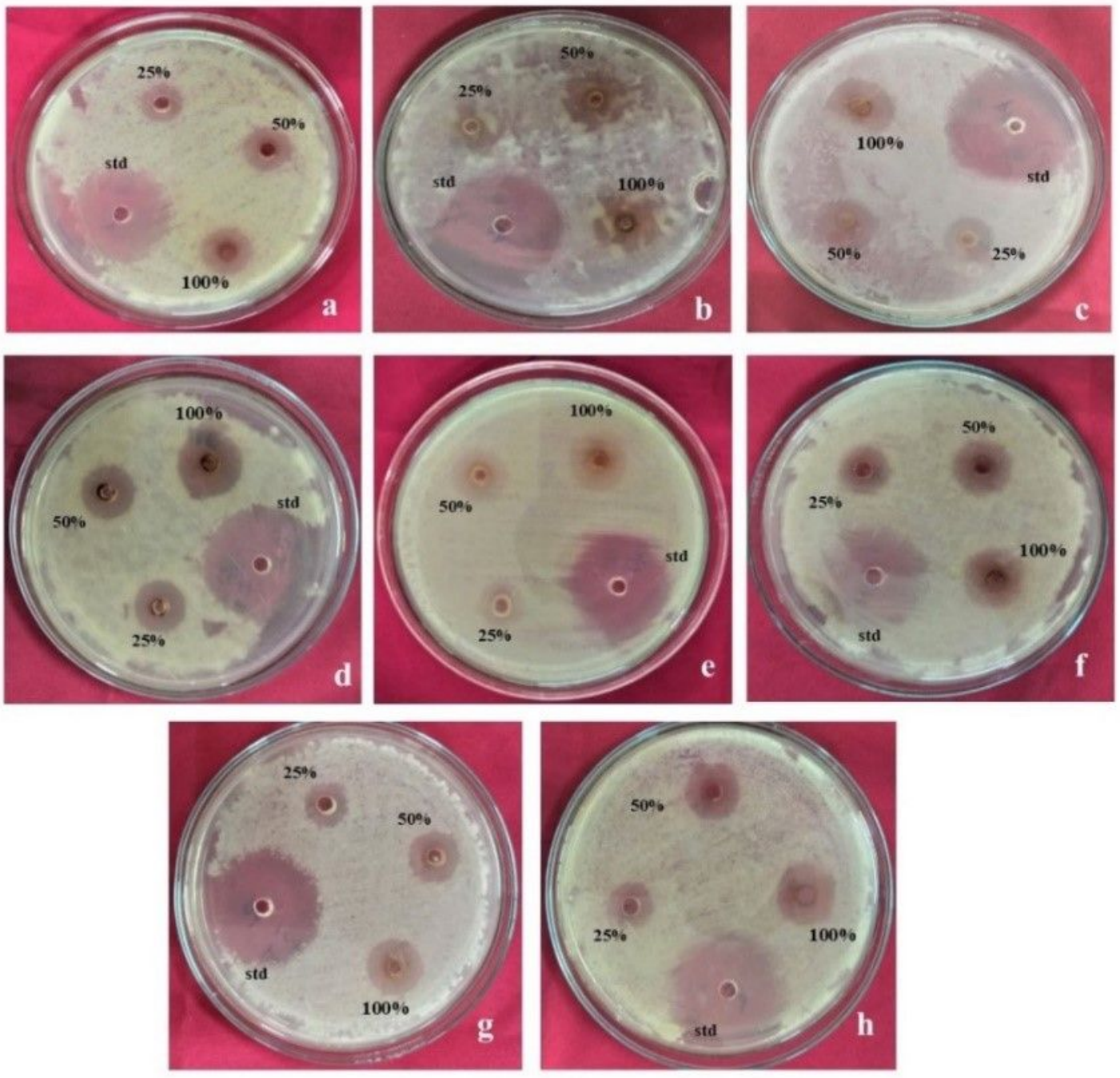

Figure 2

Antibacterial inhibitory effect of extract against (a) *Esherichia coli* (b) *Enterococcus faecalis* (c) *Klebsiella pneumoniae* (d) *Psuedomonas aeruginosa* (e) *Psuedomonas syringae* (f) *Staphylococcus aureus* (g) *Xanthomonas campestris*

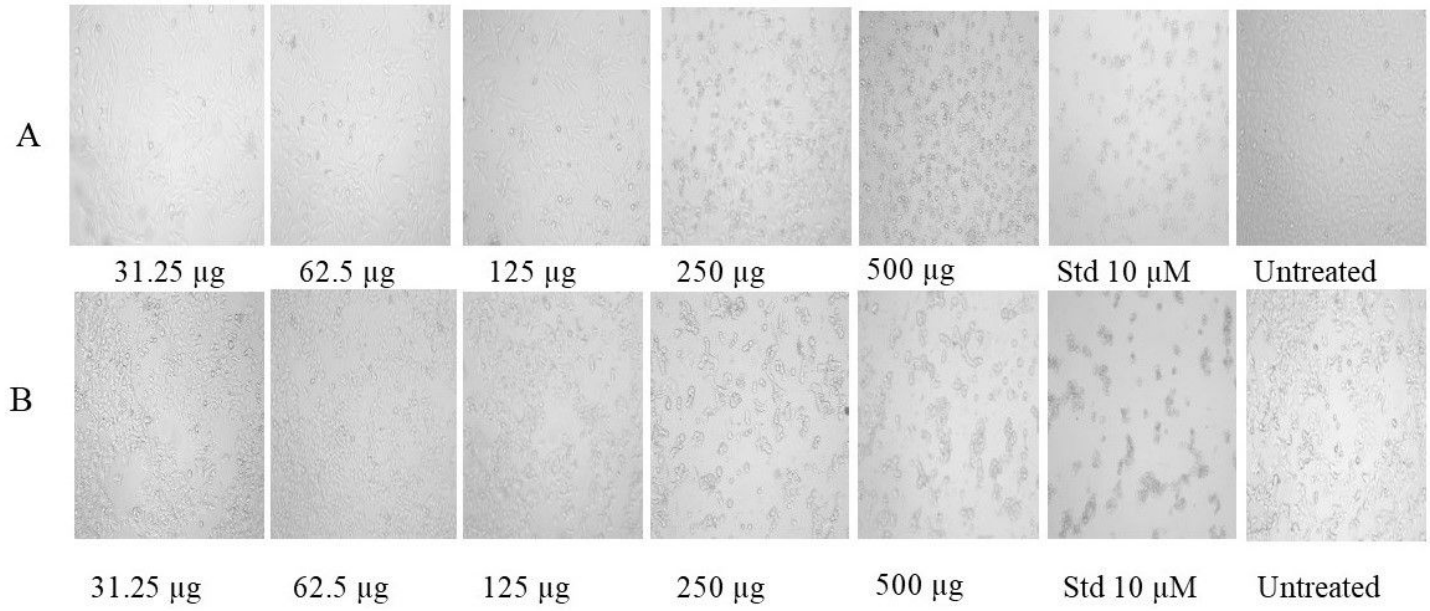

Figure 3

Morphological changes induced by different concentration of extract in (A) A549 (B) MCF-7 cell lines.

Figure. 4. The 2D and 3D views of ligand and protein interactions. (A) Kigelinone (B) levofuraltadone (C) levoflaxacin with protein **5Y63**, and (D) Kigelinone (E) levofuraltadone (F) levoflaxacin with **2JXU**.

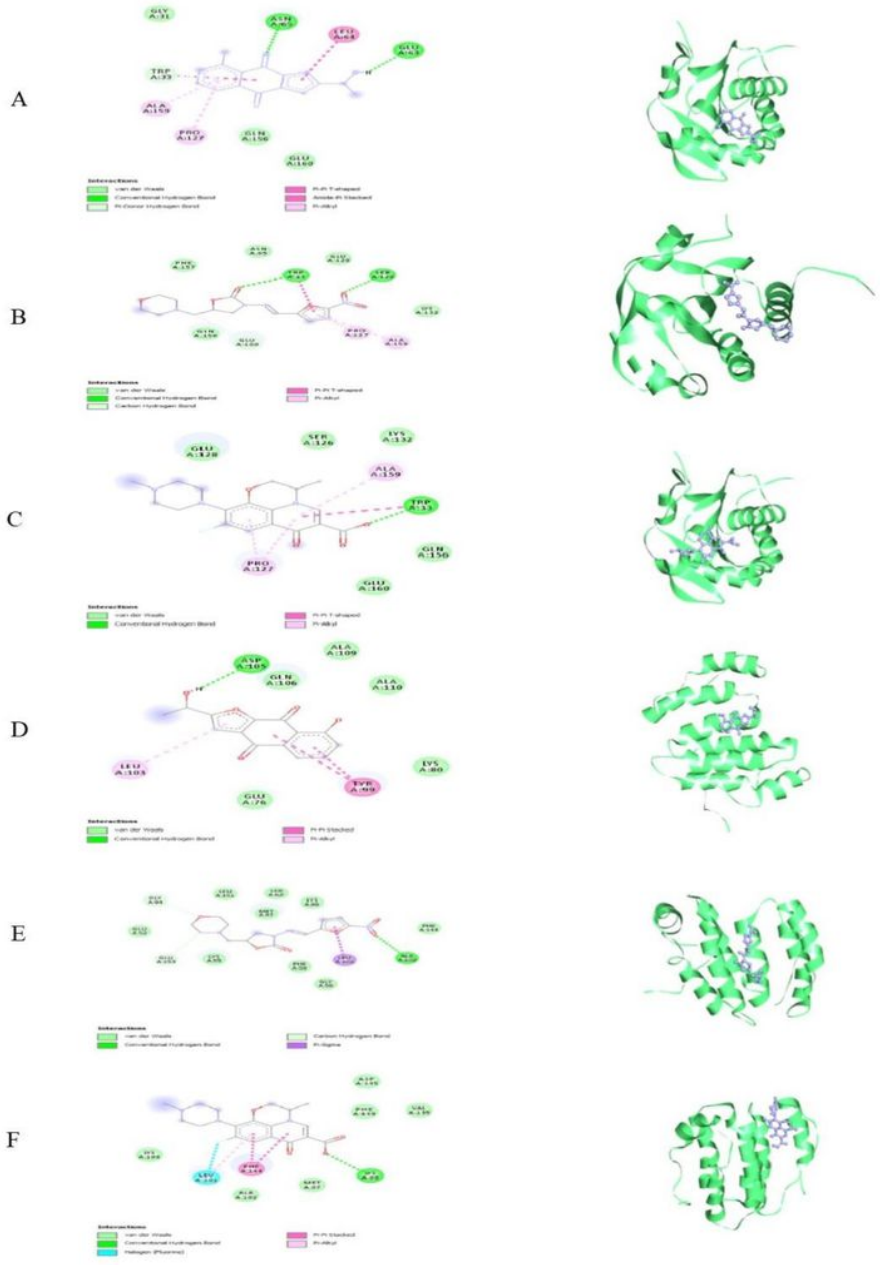

Figure 4

See image above for figure legend.

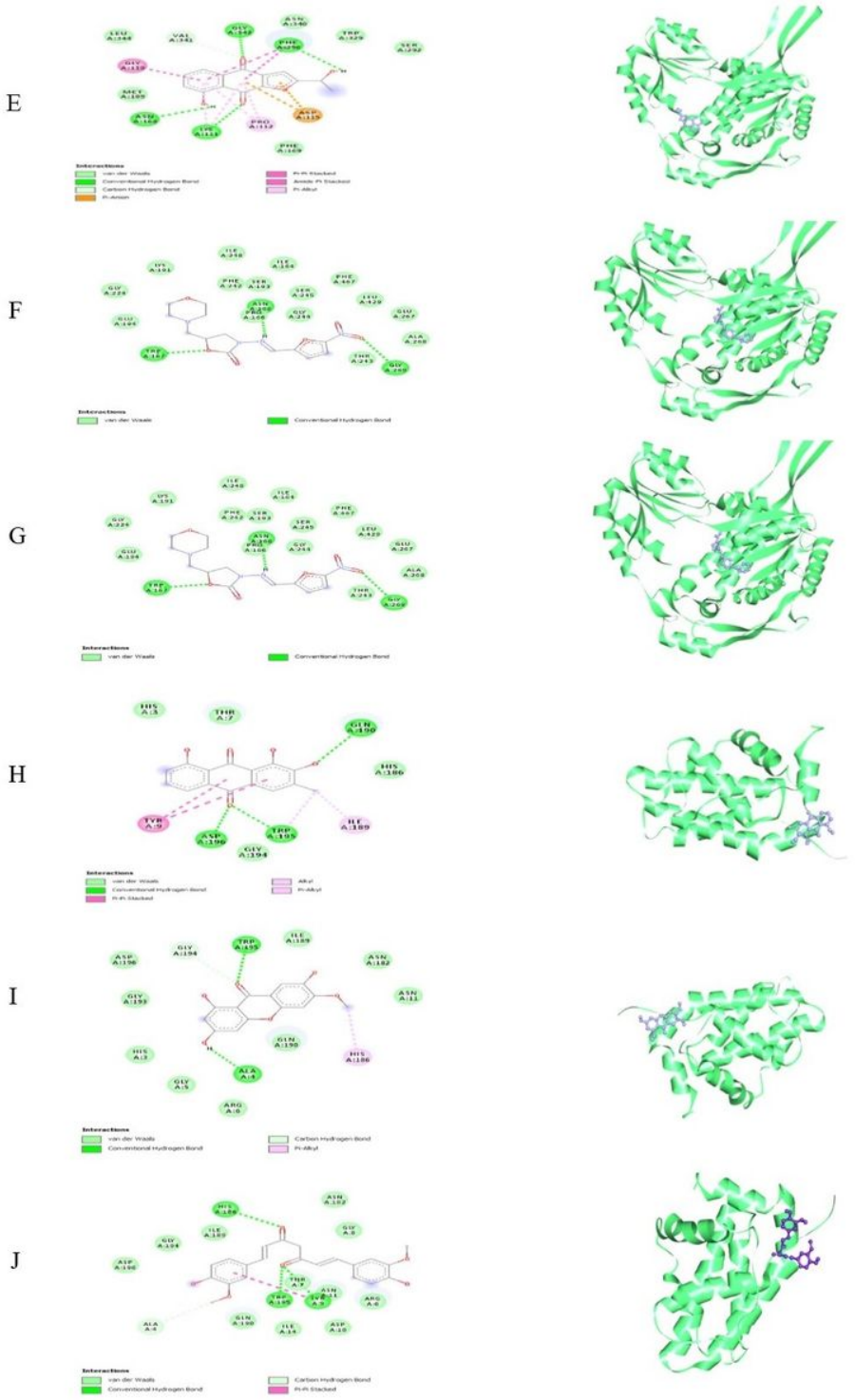

Figure 5

The 2D and 3D views of ligand and protein interactions. (A) Kigelinone (B) levofuraltadone (C) levoflaxacin with protein **5IUU**, and (D) 2-hydroxychrysophanol (E) Isoathyriol (F) curcumin with **1G5M**.