

Green Nanoparticles Synthesized From Endophytic Fungi (*Colletotrichum Gloeosporioides*) Break Antibiotic Resistance in Pathogenic *E. Coli*

Mohamed Juvad N

BS Abdul Rahman University: B S Abdul Rahman Crescent Institute of Science & Technology

Ranjani S

BS Abdul Rahman Institute of Science and Technology: B S Abdul Rahman Crescent Institute of Science & Technology

S Hemalatha (✉ hemalatha.sls@bsauniv.ac.in)

B S Abdul Rahman Crescent Institute of Science & Technology <https://orcid.org/0000-0002-8150-7721>

Research Article

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Abstract

Green Nanotechnology synthesizes nanoparticles from a myriad of perspectives or sources including plants and microscopic structures as well as biologically active compounds. Endophytic fungi are organisms that really can mimic and produce secondary metabolites similar to their hosts. In this study, we focus on the Endophytic fungal mediated silver nanoparticles (CgAgNPs) synthesized by using aqueous extract of endophyte *Colletotrichum gloeosporioides*. Synthesized nanoparticles were characterized using a UV-Vis spectrophotometer and SEM. To assess the efficacy of CgAgNPs, antimicrobial assays were performed against *Escherichia coli* ATCC 25922 and antibiotic resistant pathogenic clinical strains of *E. coli*. In order to find the interaction ability of fungal metabolites with CTX-M-15 and AmpC gene products *In silico* inspections were done through Molecular docking. Lipinski screening and ADMET analysis were performed for fungal metabolites to screen the lead compounds and drug ability to analyze the pharmacokinetic properties.

1. Introduction

Nanotechnology is an integrative approach in the branches of science, engineering and technology which has numerous numbers of utilization in all the fields. It entails manipulating matter on a nano scale to conceive a new and modified product that can be used in various ways. To stress on one important property of the nanoparticles is that they have high surface to volume ratio which could help in bring about the change and thus making it use for many antimicrobial applications [1]. Biotechnology is a field of science which is the utilizes the biological processes for the benefit of mankind in industrial and other purposes, it encompasses especially the use of genetic manipulation of microorganisms for the production of antibiotics, hormones and many other useful products [2]. Nano biotechnology is another logical field that is arising at the convergence of materials research, nano sciences, and molecular biotechnology. This exceptionally interdisciplinary field is connected to the physical and synthetic properties of natural and inorganic nanoparticles, alongside different aspects of sub-atomic science, Genetic Engineering and protein engineering, and immunology [3].

Nanoparticles are primarily synthesized using two approaches: physical (top down) and chemical (bottom up). The top down or physical method entails converting bulk material into nano-scale fragments. It encompasses techniques such as laser ablation, chemical vapour deposition, and electro deposition. The disadvantage of these methods is that they do not allow for the creation of nanoparticles of a specific size or structure. Bottom-up approach, on the other hand, entails creating nanoparticles from a small structure, such as atoms, to a larger structure, i.e. from the bottom. This method is typically preferred because it provides greater precision in synthesizing these particles. Hydrothermal synthesis, sonochemical methods, and radiolytic methods are examples of this approach.

Technological advancement has resulted in the discovery of alternative methods for synthesizing nanoparticles, one of which is the biological method of synthesis. Bacteria, fungi, and plants are some of the biological sources of nanoparticle production. Green nanotechnology is the utilization of like plants,

plant-based materials, microbes, organisms, and secondary metabolites as a reducing material during the synthesis of nanoparticles [4]. Nanoparticles can be engineered to perform multiple functions, such as antibacterial, antifungal, and anticancer drugs. Silver Nanoparticles have been shown to be effective against fungal species and other prokaryotes even at very low concentrations. In the development of safer, less expensive, and more environmentally friendly technologies, nanomaterial synthesis is in high demand. Thus, biogenic synthesis of silver nanoparticles has picked the interest of research groups due to their unique properties such as size and shape, ease of production, easily scaled up for large scale, environmentally friendly, and strong physico-chemical properties and optical properties [5, 6]. Silver nanoparticles are used to replace antibacterial agents in wastewater treatment, the textiles, medical and food packaging, and other applications. Silver nanoparticles are also involved in counteracting adhesive substances, inhibiting the formation of biofilms [7].

Endophytes are endosymbionts that live within plants for at least part of their lives without causing deadly disease. Endophytes are mandated for plant growth and development because they obtain chemical energy from the atmosphere, soil, water, and organic matter. Endophytic fungi are essential for nutrient uptake. Thus, plants and endophytes have a symbiotic relationship in which both the plant and the endophytes benefited through their symbiotic relationship through protective mechanisms and plant growth [6]. Endophyte-mediated nanoparticle synthesis is a novel research area that combines biology and nanotechnology. On the planet, there are approximately 300,000 plant species, each with its own set of endophytic organisms. The unique properties of endophytes can be harnessed to produce AgNPs, which can be used in a variety of applications. Many studies can be carried out to examine the world of endophytes and see how the synthesis methods and applications differs [8]. *Colletotrichum gloeosporioides* is a plant pathogen that is found all over the world. For optimal development, it requires a temperature of 25–28°C and a pH of 5.8–6.5. This organism is dormant during the dry season and transforms into dynamic stages when exposed to favourable natural conditions and when it changes it will act as pathogen [9]. Secondary metabolites which are obtained from endophytic fungi are consistently considered as rich sources and have normally active molecules and these molecules have good antimicrobials, drugs and herbicidal properties. Therefore, the portrayal of secondary metabolites found in different *Colletotrichum* species could assist with revealing insight into the identification of this family of endophytes and to additionally it clarifies the role of the metabolites in the pathogenicity of these organisms [10].

The aim of the study is to accomplish and synergise the antimicrobial application of CgAgNPs. Plasmid-encoded CTX-M sort extended range Beta- lactamases (ESBLs), which have been broadly revealed all through the most recent years, are generally found and connected with *Escherichia coli* [11, 12]. CTX-M-15, found in India since 2001 and is currently perceived as the most appropriated CTX-M compound [13]. It differs from CTX-M-3 by one amino corrosive substitution at position 240 (Asp-240 Gly), which appears to present increased ceftazidime reactant movement. [14]. Since the last part of the 1970s, amp C beta-lactamases have been perceived as one of the goes between of antimicrobial opposition in Gram negative microscopic organisms. These enzymes are cephalosporinases, which can hydrolyze all beta-lactams to some degree. There are two types of amp C beta- lactamases: plasmid-mediated ampC and

chromosomal or inducible ampC [15]. The CTX-M pandemic is caused by *E. coli* delivering CTXM catalyst, which spread throughout the world as a result of the onset of UTIs. CTXM-15, which was discovered in 2001, is the most commonly used catalyst. CTXM is in charge of diseases picked up at emergency rooms. CTX-M beta-lactamases hydrolyze ceftriaxone, cefotaxime, and, most notably, ceftazidime. CTXM-15 ESBLs were discovered in India and Japan in *K. pneumoniae*, *E. coli*, and *Enterobacter aerogenes*. Southern India has a higher prevalence of CTXM-15 type ESBLs. In the current study, CTXM-15 encoding clinical *E. coli* strains were used to reduce the antibacterial impact of endophytic fungal compound-based silver nanoparticles. [16].

This research work focuses on the green synthesis of endophytic fungal extract mediated silver nanoparticles CgAgNPs from the aqueous extract of the endophyte *Colletotrichum gloeosporioides* and evaluated for its antibacterial efficacy in *Escherichia coli* ATCC 25922 and its pathogenic strains. *In silico* analysis was done to check the efficiency of secondary metabolites of endophyte against the gene of interest which are CTX-M-15 and AmpC of *Escherichia coli* using Molecular docking and fungal lead compounds were screened using Lipinski Rule of five and pharmacokinetic properties was checked using Swiss ADME.

2. Materials And Methods

2.1. Preparation of fungal Aqueous extract, phytochemical screening and silver nanoparticle synthesis

The endophytic fungi was grown on the Potato Dextrose Broth (PDB) and the grown fungal mycelium was transferred to the autoclaved distilled water to extract the secondary metabolites from the fungal mycelium. Phytochemical Analysis was performed for the aqueous extract to check for the presence of phenols, alkaloids, carotenoids, flavonoids, saponins, anthocyanins, tannins and steroids [17].

Nanoparticles were synthesized by utilizing 1mM AgNO₃ solution. in the ratio of 1: 5 and then they are incubated in the dark for 2 days with periodic monitoring for the prominent colour change. Then the mixture was centrifuged at 13.4 rpm for 10 minutes and the supernatant was discarded, and the pellets were collected and air dried in hot air oven for a period of time and once they are dried, nanoparticles were dissolved in 10% DMSO and ultrasonicated for period of 10 minutes to attain uniform distribution of nanoparticles in the colloidal solution.

2.3. Antibacterial Activity of endophytic fungal silver nanoparticles (CgAgNPs) :

Escherichia coli ATCC 25922 multi drug resistant strains (MDR) of *E. coli* were obtained from Tagore Medical College (Ref. no. BSAU: REG-OFF: 2016/02SLS).

2.3.1. Agar Well diffusion method :

The pathogenic and drug-resistant organisms (EC) *Escherichia coli* ATCC 25922 and other pathogenic strains were inoculated in LB broth and was grown on a rotating shaker at 37°C for the time period to achieve its log phase. Fresh cultures were streaked over LB agar plates according to the Mcfarland standard. Various concentrations of CgAgNPs (25 µg/ml and 50 µg/ml) were loaded into 6 mm wells. Ampicillin was used as a standard, and 10% DMSO was used as a negative control (both at 25 µg/ml concentration). After loading the plates was incubated for 24 hours at 37°C to observe the zone of clearance and to record for the same.

2.3.2. Assessment of Minimum Inhibitory Concentration and growth rate:

The bacteriostatic action of CgAgNps was determined. The 96-well plate was filled with 100 µl of LB broth, which was then serially diluted with 100 µl of CgAgNps. 2 µl of the bacterial strains were inoculated and kept at rest for 24 hours in a shaking incubator at 100 rpm at 37°C. After 24 hours of incubation the well with least concentration of CgAgNps without visual turbidity was recorded as MIC of the respective test organism. Bacterial growth was measured using a plate reader (Perkin Elmer Inspire, Multimode reader) at 600 nm. The bacteriostatic concentration of CgAgNps was determined by observing the A600, plotting the time kill curve, and calculating the percentage of growth inhibition.

2.3.3. Minimum Bactericidal Concentration :

The purpose of this experiment was to determine the minimum bactericidal concentration, which is a complementary measure assay for the minimum inhibitory concentration. MBC was used to determine the minimal CgAgNps concentration needed to kill the organism. The same microtiter plate was examined after 24 hours, and the wells with no visible growth were chosen for CgAgNps-treated strains of *Escherichia coli*, and 2 µl from the chosen wells were inoculated on LB agar plates. Then, the plates were incubated overnight at 37°C [6, 1].

2.4. *In silico* Studies:

2.4.1. Selection of Ligands:

Endophytic fungi metabolites were chosen, and their 3D structures were obtained in SDF format from PubChem [10].

2.4.2. Protein Preparation:

Protein 3D structures were retrieved from RCSB PDB. In the pdb format, it is represented as 4HBT (CTX-M-15 ESBL) and 2BLS (AmpC Beta-Lactamase). This is then processed further by the AutoDock 4.2 software. The unwanted water molecules are then removed in the protein preparation section, followed by the hetatm removal and the addition of hydrogens (polar only), and finally the Kollman charges are added and found to be -1.134 for 4HBT and 17.067 for 2BLS.

2.4.3. Ligand Preparation:

The 3D structures of ligands were obtained from PubChem in sdf format and converted to pdb format by Open Babel discipline to facilitate molecular docking analysis. Following that, the active binding sites in the protein were identified using tools such as CASTp (Computed Atlas Of Surface Topography of Proteins), which aids in setting up the grid parameters and defining the binding site. The grid box was set at 60 along with the number of points in the three-dimensional axes, and the spacing angstrom was kept at 0.425 Å for 4HBT and 0.625 Å for 2BLS. The grid box is then centered to allow for easier molecular docking.

2.4.4. Molecular Docking of Targets against Ligands :

Following the preparation of the protein and ligand, AutoDock 4.2 was used to perform molecular docking analysis. Docking estimations were performed utilizing the Lamarckian Genetic calculation. The parameters of the Genetic Algorithm (GA) were set to be the default which is 10 runs. Ten runs were carried out, with the best binding energies being ranked first. The docked results were saved as a (dpf) file. Discovery Studio Visualizer was used to visualize the resulting conformations. Binding site residues were examined to determine the best docked poses.

2.4.5. *In Silico* Analysis

2.4.5.1. Lipinski Screening of Ligands:

The Lipinski rule of five, is utilized to assess the drug- likeness and drugability of a secondary metabolite of *Colletotrichum gloeosporioides* [18]. Utilizing this thumb rule of five, any naturally or synthetically dynamic compound can be tried for its oral development. The ligands in this examination were partitioned into five classifications which are mass, hydrogen acceptor, hydrogen contributor, lipophilicity and molar refractivity.

2.4.5.2. ADMET Analysis

Prior to being considered as a drug competitor, any natural or synthetically unique compound ought to go through pharmacokinetic testing. Bio availability, intestinal retention, blood-frontal cortex boundary vulnerability, drug-similarity, poisonousness, and an assortment of different factors should all be assessed. The Swiss ADME online device was utilized to assess the ligands in our examination. This is essentially to help future examinations concerning the meanings of our ligands. Water dissolvability was resolved utilizing the ESOL Topological method, and lipophilicity was also resolved [19].

3. Results And Discussion

3.1. Phytochemical Analysis for the fungal aqueous extract:

Phytochemical analysis for the fungal aqueous extract is performed to determine the secondary metabolites. The Endophytic Fungal extract contains phenols and alkaloids in the extract. Different therapeutic plants contain phenolic compounds and flavonoids that are used in therapeutics also as

nutrition consumption. The presence of dynamic phytochemical constituents, for example, phenolic mixtures and alkaloids is the prevalent justification of a secondary metabolites to show its potential therapeutic action. Phenolic and alkaloid compounds are notable antioxidants. According to reports, a large number of alkaloids and phenolics can control the growth of bacteria and viruses [20, 21]. The presence of these secondary metabolites in the endophytic fungal extract may function as a reducing and capping agent [22] Table 1.

Table 1
Phytochemical Analysis of the *Colletotrichum gleosporioides* endophytic fungal aqueous extract

S.No	Phytochemical Test for Fungal extract	Positive or Negative
1.	Tannins	Negative
2.	Phenols	Positive
3.	Saponins	Negative
4.	Carotenoids	Negative
5.	Flavonoids	Negative
6.	Anthocyanins	Negative
7.	Alkaloids	Positive
8.	Steroids	Negative

3.2. Synthesis and Characterization of nanoparticles:

The endophytic fungal aqueous extract was mixed with silver nitrate solution and incubated in dark. At first the colour was in light brown in colour, upon incubation it changed into dark brown colour which is a primary indication of nanoparticle synthesis. Reduction of silver nitrate to silver nanoparticles was noticed outwardly with change in shade of the colour was in response to change in size and this is due to surface plasmon resonance of the silver nanoparticles [23]. These outcomes acquired associate the discoveries of previous works which clearly suggests that the secondary metabolites of the endophytic fungi are reason CgAgNPs. To confirm the synthesis of CgAgNPs UV-Visible characterization was employed by scanning in the 200-800nm range. The peak was observed around 430nm, which is due to SPR of CgAgNps and confirms the synthesis of nanoparticles (Fig. 1a). In the previous study states that the nanoparticles that are synthesized is in the absorbance of 420 nm and they also observed a small peak around 300 nm which could encode for other substances like proteins and upon its unique size and shape they can be used for many biological applications like antibacterial activity [23]. The nanoparticle SPR revealed a band design that could be related to particle shape, size, distribution, and aggregation.

Furthermore, the closeness of the valence and conduction bands indicates that the electron displacement in the reaction mixture was unusually high, as indicated by the change in nanoparticle band pattern. The combined variation of electron shift on the outside of silver nanoparticles may allow access to a UV-Vis band of surface plasmon resonance [24]. Our findings were consistent with previous research by Ahmad, Ubaid and Hemalatha and Huang [25, 26, 27], who discovered that incubating biological materials with silver nitrate solution results in the formation of silver nanoparticles with diameters ranging from 300 to 700 nm.

SEM analysis is used to inspect the surface morphology, size, and shape of CgAgNPs. As a result, the size of the CgAgNps synthesized ranges from 59 to 81nm, with the average size of CgAgNPs being 70nm and shape is seeming to be spherical in shape (Fig. 1b). From this we can infer that the secondary metabolite from the endophytic fungal extract helps in dictating the size and shape of the nanoparticles. Previous reports suggests that compounds like proteins to low molecular weight compounds like secondary metabolites that are alkaloids, polyphenols, saponins, steroids and tanins and also like polysaccharides helps in the green synthesis of the nanoparticles [28].

3.3. Antibacterial activity of CgAgNPs :

The antibacterial action of CgAgNPs was tested against the different strains of *Escherichia coli* (*Escherichia coli* ATCC 25922, MDR strains- *EC* 16, *EC* 13, *EC* 36 and *EC* 3T) (Fig. 2). The results of the Agar Well diffusion method were observed by measuring the zone of inhibition. According to the findings, CgAgNps showed effective antibacterial activity Table 2. For the strain *Escherichia coli* ATCC 25922 it is found that our CgAgNps at the concentration of 25 µg/ml and 50 µg/ml the zone of clearance is found to be 14 mm and 15 mm respectively and it is also found that the stain is sensitive to antibiotics which is used and showed zone of clearance of 16 mm for Ampicillin at the concentration of 25 µg/ml. Similarly, for *EC* 16 it is found that our CgAgNps showed the zone of clearance of 12 mm and 15 mm, for *EC* 13 the zone of clearance is found to be 13 mm and 14 mm, for *EC* 36 the zone of clearance is found to be 13 mm and 15 mm and *EC* 3T the zone of clearance is found to be 12 mm and 13 mm at the concentration of 25 µg/ml and 50 µg/ml respectively.

Table 2

The Zone of clearance observed for different concentrations of CgAgNPs for the different pathogenic strains of *E. coli*

S.No	Strains	A AgNO ₃	B 25µg/ml of EFAgNPs	C 50µg/ml of EFAgNPs	D DMSO	E Extract	Z Ampicillin
1.	<i>E. coli</i> ATCC 25922	Nil	14 mm	15 mm	Nil	Nil	16 mm
2.	<i>E. coli</i> 16	Nil	12 mm	15 mm	Nil	Nil	Nil
3.	<i>E. coli</i> 13	Nil	13 mm	14 mm	Nil	Nil	Nil
4.	<i>E. coli</i> 36	Nil	13 mm	15 mm	Nil	Nil	Nil
5.	<i>E. coli</i> 3T	Nil	12 mm	13 mm	Nil	Nil	Nil

3.4. Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) value for CgAgNPs of *Escherichia coli* ATCC 25299 is 6.25µg/ml, *Escherichia coli* 16 the MIC value is found to be 25µg/ml, *Escherichia coli* 13 the MIC value is found to be 6.25µg/ml, *Escherichia coli* 36 the MIC value is found to be 6.25µg/ml and *Escherichia coli* 3T the MIC value is found to be 3.125µg/ml. Previous studies reported that the growth rate is reduced when there is use of silver nanoparticles against *E. coli* at various concentrations. Similarly, the current study also supports to the previous finding through proving the antibacterial activity of CgAgNPs when treated with *E. coli* strains with different concentration. This result emphasis more on concentration dependent which agrees with the previous studies [23, 29]. MIC values differ from one strain to another.

From the growth curve (Fig. 3), it can be inferred that upon treatment with CgAgNPs, the percentage of growth was reduced considerably when compared with control. It was noted that, when compared to control the percentage of growth was reduced to 93.62 %, 61.59%, 36.61 %, 51.49%, 87.81% for *Escherichia coli* ATCC 25299, *EC 16*, *EC 13*, *EC 36*, *EC 3T* respectively at their sub-MIC concentration. The synergistic effect of secondary metabolites and silver interacts more efficiently with our targets and thus inhibiting the growth. This could be possibly because of the positively charged silver ions of silver nanoparticles interact with the opposite charge of cell membrane of the bacterial cell where it ends up with the disintegration of the cell and leading to cell death [30]. Apart, the other mechanism is adherence of nanoparticles to the cell membrane where the size and shape of the nanoparticle plays a major role in cell death. Our nanoparticles were compatible with the previous findings and the CgAgNPs size is less than 100 nm and play a major role in antibacterial activity. It is also said that the nanoparticles can also interact with the macromolecules inside the cell such as enzymes, Proteins, DNA and RNA and disrupts the cell machinery thus killing the cell and its doubling [31]. Further silver nanoparticles upset the dehydrogenase of respiratory chain and raise the ROS levels inside the cell. ROS level causes DNA harm

and lipid peroxidation. A ROS creation forestall the development of the cell and its replication and at the end reducing the bacterial growth [32, 33, 34].

3.5. Minimum Bactericidal Concentration (MBC)

MBC is the least concentration of CgAgNps required to kill the bacteria (showed no growth on the agar plate) which is a complement assay to MIC. *Escherichia coli* ATCC 25299 has an MBC of 12.5 µg/ml, *EC* 16 of 50 µg/ml, *EC* 13 of 12.5 µg/ml, *EC* 36 of 12.5 µg/ml, and

EC 3T of 6.25 µg/ml. The results of MIC and MBC are summarized in Table 3.

Table 3
MIC and MBC of pathogenic *E. coli* strains on treatment with CgAgNPs

S.No	Strains	MIC (µg/ml)	MBC (µg/ml)
1.	<i>E.coli</i> ATCC 25922	6.25	12.5
2.	<i>E.coli</i> 16	25	50
3.	<i>E.coli</i> 13	6.25	12.5
4.	<i>E.coli</i> 36	6.25	12.5
5.	<i>E.coli</i> 3T	3.125	6.25

As it is known that the whole bacterial cell size is in microns and since our nanoparticles that is been synthesized can easily penetrate and enter the cell as the silver ions are small the cell membrane and interact with macromolecules, causing the cell's entire machinery to malfunction by inhibiting replication, transcription, and translation. The cell cycle will be terminated, preventing it from doubling. According to reports, silver ions cause 'pits' due to proton motive force. As a result of the disorganized membrane components, cell wall structures changes. This increases membrane permeability, which leads to cell death [35].

5.6. *In silico* Analysis

5.6.1. AutoDock

Molecular docking was performed to find the binding affinity of the secondary metabolites that is been produced by the fungi with target protein using AutoDock 4.2 software. The binding energies was measured in (kcal/mol) and ligand binding sites (amino acids residues) was seen after performing molecular docking of our compounds with target proteins. The results show that ligand binding energies are promising and that they can bind with proteins. Gleosporene and moncerin was in the ligand binding

studies at cut off range of -6 kcal/mol for CTX-M-15 gene **Table 4** and the AmpC gene Table 5 of *E. coli*. Apart from gleosporene and moncerin, colletoric acid showed good binding energy for our target proteins. The least binding energy is observed in Colletotricole A in the case of CTX-M-15 gene – 4HBT whereas in the colletoric acid and Colletotricole A showed least binding energies for AmpC gene – 2BLS.

Table 4 Binding energies of secondary metabolites of *C. gleosporiodes* with CTX-M-15 of *E. coli* (PDB: 4HBT)

S.No	Ligands	Binding Energies (kcal/mol)	Ligand Binding Residue sites	Number of H-Bonds
1.	Colletopiperazine	-5.83	SER70,SER237,SER130,THR216, THR235	5
2.	Colletoric acid	-6.95	PRO268,TYR240,GLY241,ASN132, ASN104,SER70,SER237,SER130	8
3.	Colletotrichine A	-5.58	SER130,THR235	2
4.	Colletotricole A	-4.63	SER130,THR235,LYS234	3
5.	Colletotricone A	-4.83	GLY241,ALA270,SER272	3
6.	Colletotricone B	-5.38	ASN132,SER70,SER130	3
7.	Fusaperazine C	-5.44	ASN104,ASN132,THR235	3
8.	Fusaperazine E	-6.07	ALA219	1
9.	Fusarentin-6,7-dimethyl ether	-5.89	ASN132,THR216,SER70,SER237	4
10.	Fusarentin 6-methyl ether	-6.34	SER70,SER237ASN104,ASN170	4
11.	Gleosporene	-6.57	SER130,ASN132	2
12.	Monocerin	-6.98	SER70,SER237ASN132,ASN170	4

Table 5

Binding energies of secondary metabolites of *C. gleosporioides* with AmpC of *E. coli* (PDB: 2BLS)

S.No	Ligands	Binding Energies (kcal/mol)	Ligand Binding Residue sites	Number of H-Bonds
1.	Colletopiperazine	-5.83	SER70,SER237,SER130,THR216, THR235	5
2.	Colletoric acid	-6.95	PRO268,TYR240,GLY241,ASN132, ASN104,SER70,SER237,SER130	8
3.	Colletotrichine A	-5.58	SER130,THR235	2
4.	Colletotricole A	-4.63	SER130,THR235,LYS234	3
5.	Colletotricone A	-4.83	GLY241,ALA270,SER272	3
6.	Colletotricone B	-5.38	ASN132,SER70,SER130	3
7.	Fusaperazine C	-5.44	ASN104,ASN132,THR235	3
8.	Fusaperazine E	-6.07	ALA219	1
9.	Fusarentin-6,7-dimethyl ether	-5.89	ASN132,THR216,SER70,SER237	4
10.	Fusarentin 6-methyl ether	-6.34	SER70,SER237ASN104,ASN170	4
11.	Gleosporene	-6.57	SER130,ASN132	2
12.	Monocerin	-6.98	SER70,SER237ASN132,ASN170	4

Thus, the common ligands of choices are gleosporene and monocerin with efficient binding energy and it is given priority for the study. The best conformation poses were visualized using Discovery studio Visualizer after molecular docking (Fig. 4).

5.6.2 Lipinski Screening of Ligands

The Lipinski rule of five helps with distinguish drug-like and non-drug-like particles. It predicts a high probability of drug-likeness or not and if any one of the two or more rules are violated the particular molecular is not considered for a lead compound. As per the standards for Lipinski Screening rule are 1. molecular mass less than 500 dalton, 2. Lipophilicity as LogP which should be less than 5, 3. less than 5 hydrogen donors, 4. less than 10 hydrogen acceptors, 5. Molar refractivity should be in the range of 40 to 130. Except for colletoric acid (violation), all of the other molecules are drug-like and meet all five criteria Table 6.

Table 6
Lipinski Screening of secondary metabolites of *C. gleosporioides*

S.No	Ligands	Binding Energies (kcal/mol)	Ligand Binding Residue sites	Number of H-Bonds
1.	Colletopiperazine	-5.31	ASN152,SER64,TYR150,ASN343	4
2.	Colletoric acid	-4.85	ASN343,ASN152,ASN289	3
3.	Colletotrichine A	-5.67	LYS197,LYS50,GLU196,TRP201	4
4.	Colletotricole A	-4.22	ARG148,ARG296	2
5.	Colletotricone A	-5.80	ARG148,THR262,MET265,ASP264	4
6.	Colletotricone B	-5.81	ASP264,SER282,HIS314	3
7.	Fusaperazine C	-6.53	SER64,GLU272,THR316,ARG148	4
8.	Fusaperazine E	-6.49	ASN152,SER212	2
9.	Fusarentin-6,7-dimethyl ether	-6.22	LYS67,SER64,ASN346,ARG349, ARG343	5
10.	Fusarentin 6-methyl ether	-6.36	ARG148,THR262,GLU272	3
11.	Gleosporene	-6.19	ALA318,LYS67,ASN152,GLN120	4
12.	Monocerin	-6.05	LYS67,ASN152	2

5.6.3 ADMET Analysis

Any natural or synthetically unique compound ought to be exposed to pharmacokinetic testing prior to being considered as a drug or the lead compound. Bio availability, intestinal retention, blood-frontal cortex boundary vulnerability, drug-similarity, harmfulness, and various different components are checked. Pharmacokinetic analysis utilizing Swiss ADME has yielded critical outcomes for the entirety of the compounds, which will help in future analysis while performing *in vitro* studies. With the exception of colletoric acid, which is poorly soluble in water and remaining compounds are water soluble. Colletotricone A had the least lipophilicity value when compared with the other compounds Table 7.

Table 7
Swiss ADME analysis of secondary metabolites of *C. gleosporioides*

S.No	Ligands	Mass	H-Donor	H-Acceptor	Log P	Molar Refractivity
1.	Colleto-piperazine	308	2	6	0.173	79.18
2.	Colletoric acid	524	4	10	4.799	136.1
3.	Colletotrichine A	250	2	3	1.989	69.98
4.	Colletotricole A	215	1	4	0.918	53.19
5.	Colletotricone A	252	2	4	1.024	67.86
6.	Colletotricone B	252	2	4	1.024	67.86
7.	Fusaperazine C	318	2	6	0.850	85.80
8.	Fusaperazine E	360	0	5	2.99	102.18
9.	Fusarentin-6,7-dimethyl ether	310	2	6	2.042	79.65
10.	Fusarentin 6-methyl ether	296	3	6	1.73	74.76
11.	Gleosporene	326	1	5	3.26	85.78
12.	Monocerin	308	1	6	2.578	77.55
S.No	Ligands	Water solubility	Gi absorption	Bbb Permanent	P-Gp substrate	Lipophilicity
1.	Colleto-piperazine	-2.34 (soluble)	High	No	No	2.34
2.	Colletoric acid	-6.99 (poorly soluble)	Low	No	No	2.68
3.	Colletotrichine A	-2.17 (soluble)	High	Yes	No	2.35
4.	Colletotricole A	-1.85 (soluble)	High	No	No	2.14
5.	Colletotricone A	-2.20 (soluble)	High	No	No	1.72
6.	Colletotricone B	-2.20 (soluble)	High	No	No	1.95
7.	Fusaperazine C	-2.67 (soluble)	High	No	No	2.85
8.	Fusaperazine E	-4.08 (Moderately soluble)	High	Yes	No	3.52
9.	Fusarentin-6,7-dimethyl ether	-3.46 (soluble)	High	No	No	2.85

S.No	Ligands	Mass	H-Donor	H-Acceptor	Log P	Molar Refractivity
10.	Fusarentin 6-methyl ether	-3.25 (soluble)	High	No	No	2.27
11.	Gleosporene	-3.67 (soluble)	High	Yes	No	3.15
12.	Monocerin	-3.51 (soluble)	High	Yes	No	2.99

4. Conclusion

This research shows that CgAgNps can be synthesized using a green approach, which is a low-cost, environmentally friendly method that uses endophytic fungal extract. The current study focused on synthesizing silver nanoparticles from endophytic fungal extract and characterizing the nanoparticles. Various techniques, such as UV-Vis spectroscopy for determining the Surface Plasmon Resonance of nanoparticles and SEM for morphology, size, and shape of CgAgNPs.

The antimicrobial study of silver nanoparticles was carried out using the agar well diffusion method, MIC, and MBC, and it demonstrated a good antibacterial effect against various strains of *Escherichia coli*. Endophytic fungal nanoparticles synthesized using endophytic fungal extract could be used as the best antimicrobial agent against multidrug resistant bacteria in order to overcome the negative effects of antibiotic resistance in *Escherichia coli*, according to a recent study. If any further developed and validated, endophytic fungal silver nanoparticles CgAgNPs will have a significant impact in pharmaceutical and medical applications. Besides that, *in silico* examination was utilized to research the restorative capability of different ligands to restrain the proteins of beta-lactamases created by the CTX-M-15 quality and the AmpC gene. Overall, the outcomes showed that the secondary metabolites can possibly tie to proteins of intrigue and hinder their turn of events. These mixtures will be concentrated further in cells and the model system for their pharmacodynamics and kinetics properties.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest

Availability of data and material

Data will be available on request

Code availability

Not Applicable

Ethics approval

Not Applicable

Consent to participate

Not Applicable

Consent for publication

All authors read and approved the manuscript for publication

Authors' contributions

SH conceived and designed research. MJN, SR conducted experiments and analyzed the data. All authors wrote the manuscript. All authors read and approved the manuscript.

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Figures

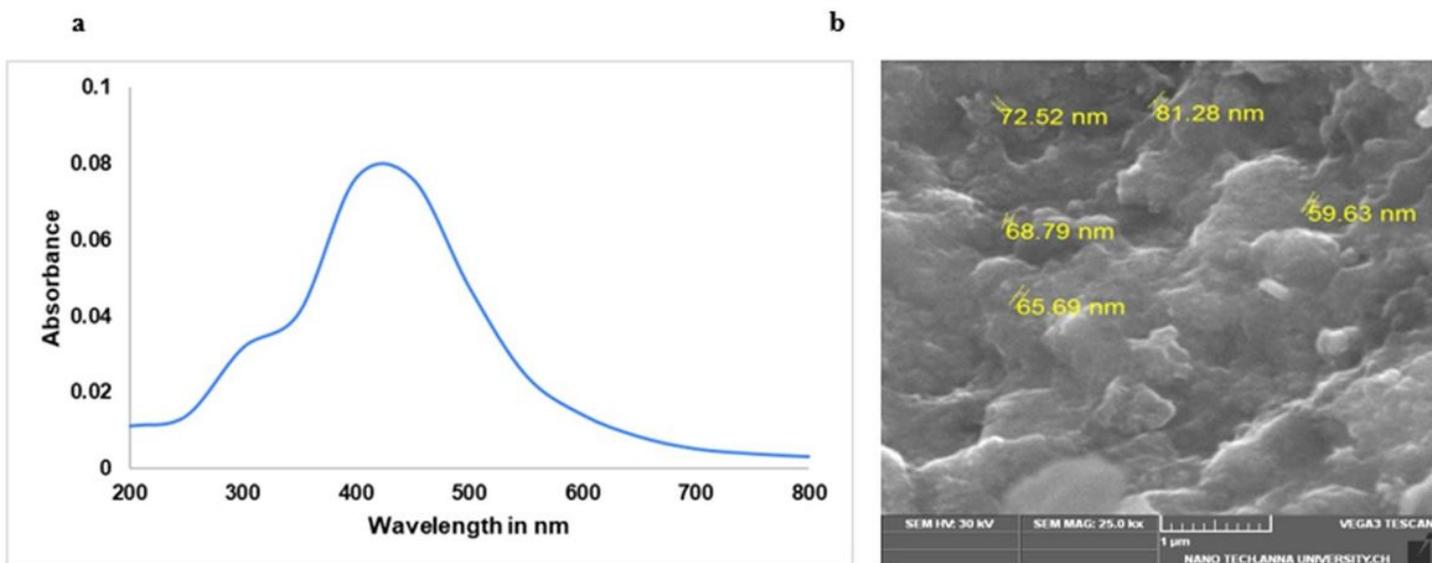


Figure 1

a) UV-Vis spectra of CgAgNPs b) SEM images of synthesized CgAgNPs showcasing its size, shape and morphology.

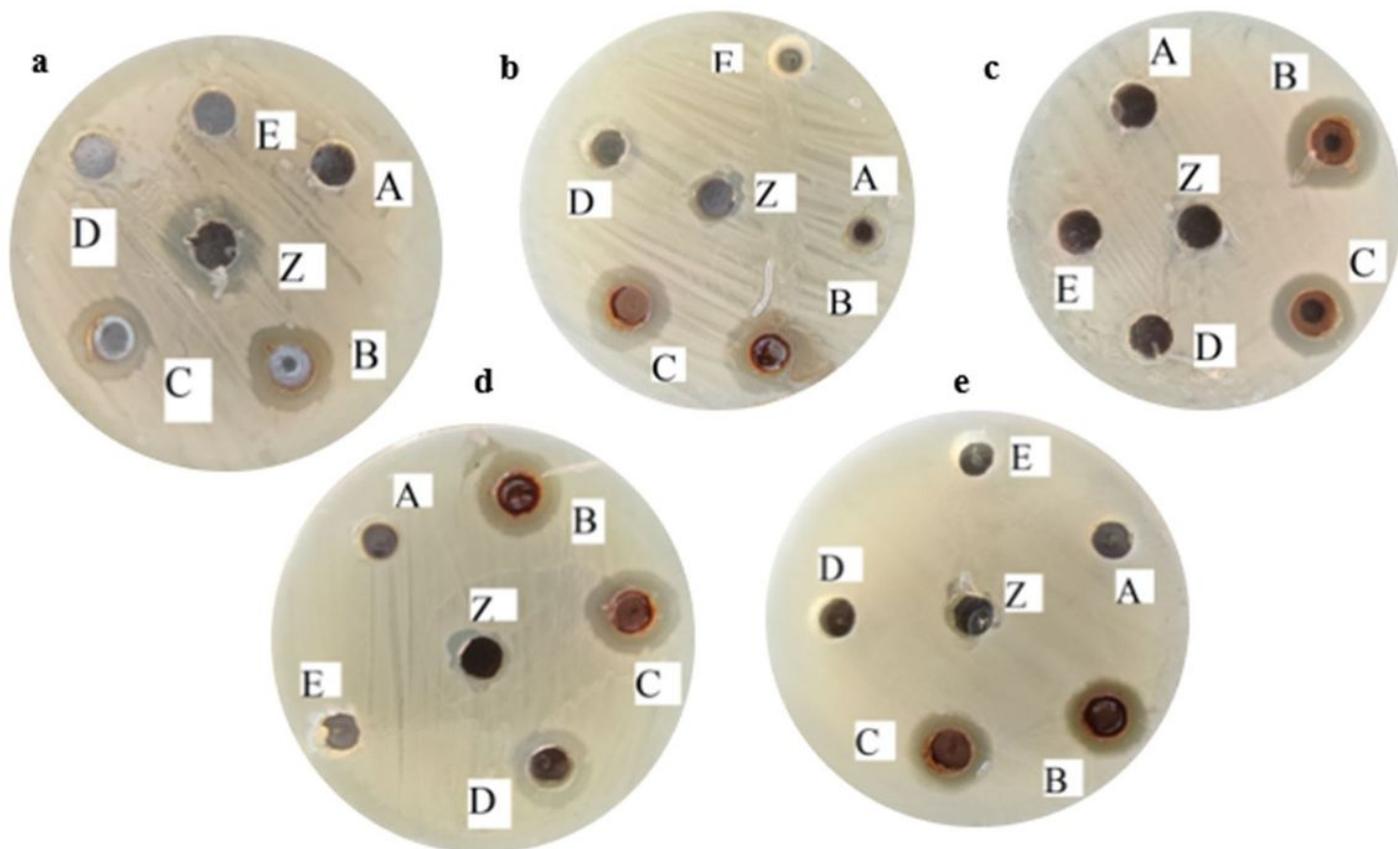


Figure 2

Antibacterial activity was screened by Agar-well Diffusion method against control strain a. *E. coli* ATCC 25922 b. *E. coli* 16 c. *E. coli* 13 d. *E. coli* 36 e. *E. coli* 3T

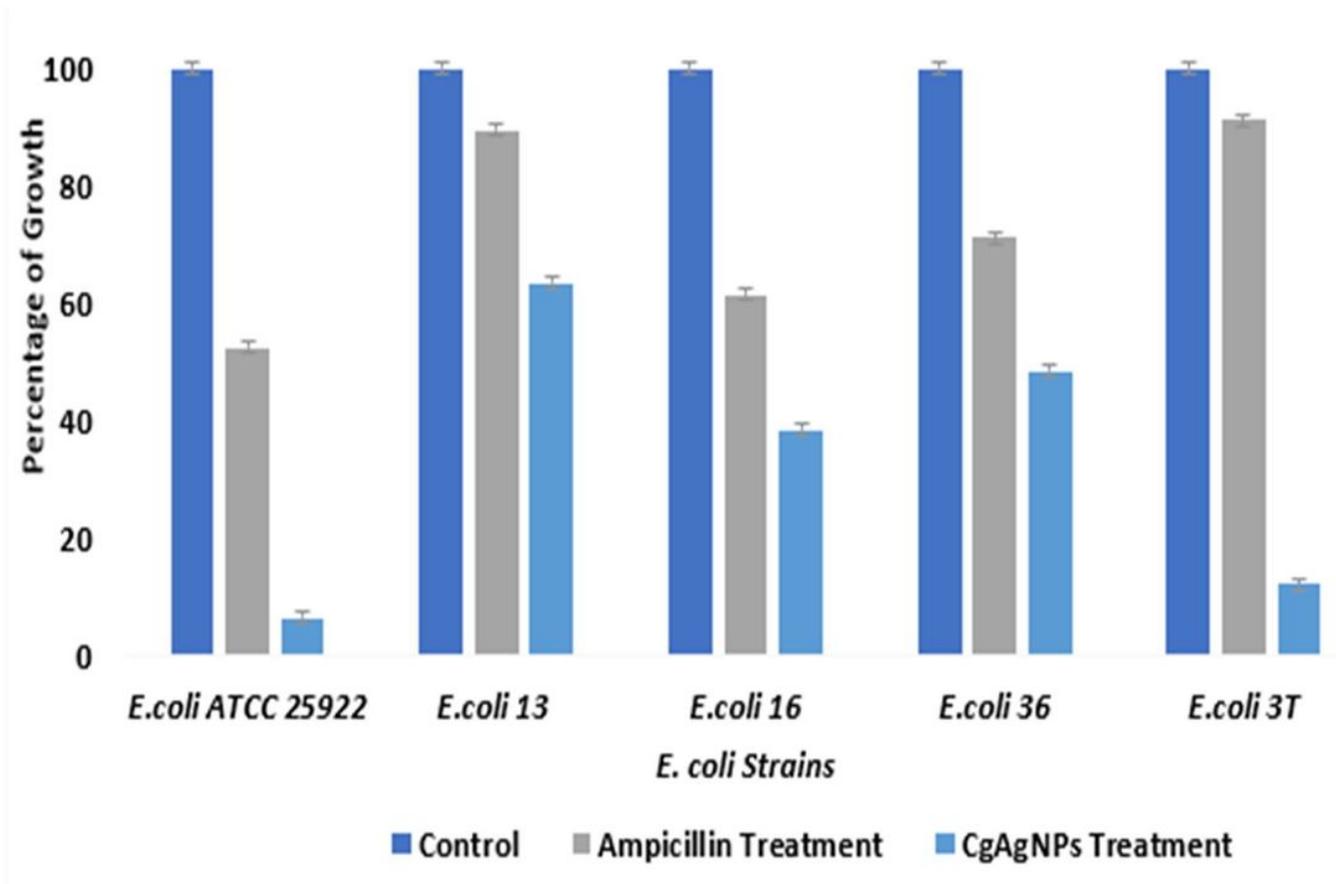


Figure 3

Growth curve of *E. coli* strains after 24 hours of treatment with CgAgNPs when compared to control and ampicillin treated strains

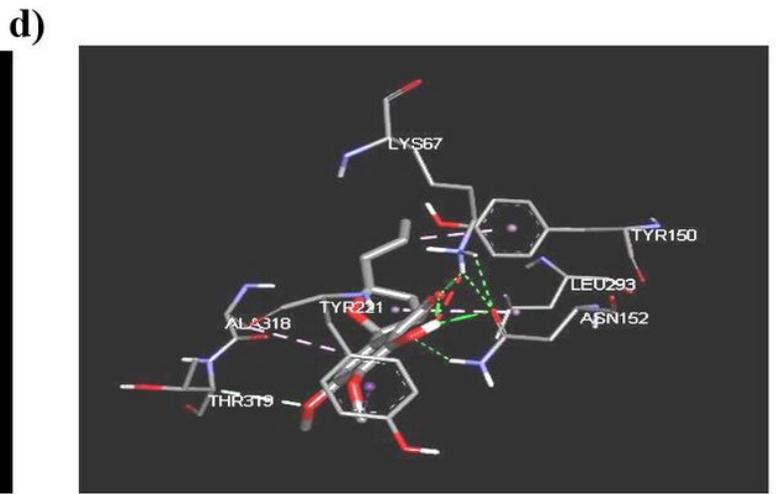
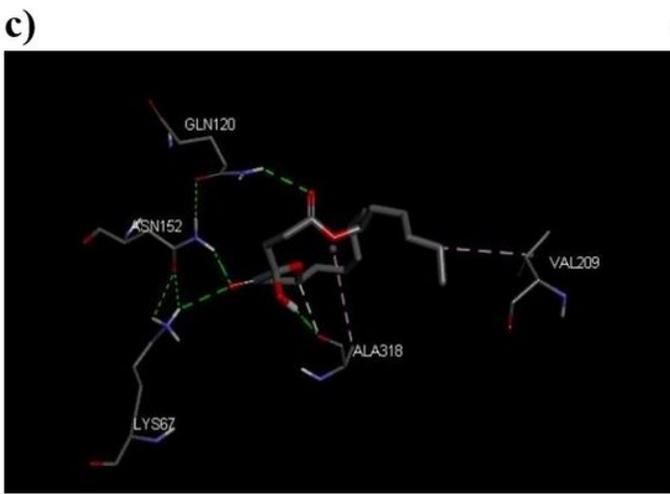
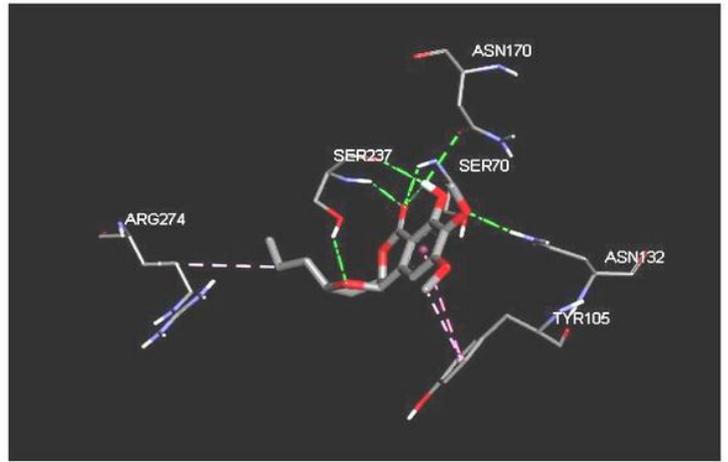
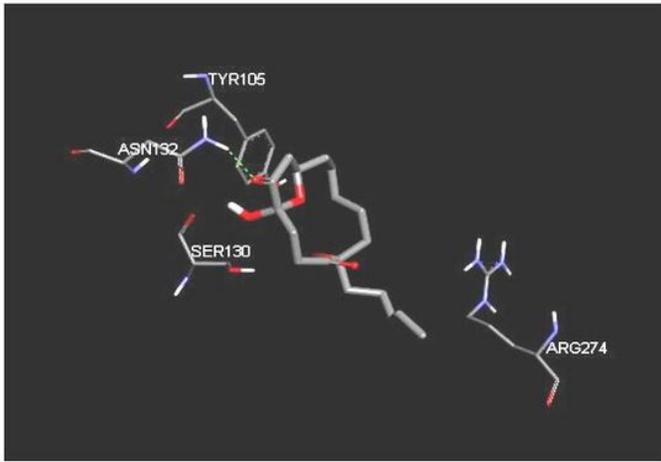


Figure 4

Best conformation poses for the dockings that have been performed b) 4HBT- Gleosporene b) 4HBT- Monocerin c) 2BLS- Gleosporene, d) 2BLS- Monocerin