Silver decorated myconanoparticles control growth and biofilm formation in uropathogenic *E. coli*

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Research Article

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Abstract

Nanotechnology involves the synthesis of nanoparticles that has been used in therapeutic application for treating diseases. In this present study we have adopted the synthesis of myconanoparticles from the extracellular extract of endophytic fungi *Penicillium sclerotiorum* (PsNps) and validated for its antibacterial potential against antibiotic resistant uropathogenic *E. coli* and ATCC (25922) strain of *E. coli*. Endophytic fungi were isolated from the healthy leaves of *Tamarindus indica*. The genomic DNA from endophytic fungi was isolated and ITS region was amplified by polymerase chain reaction (PCR) using universal fungal primers ITS1 and ITS4and sequenced for the identification of endophytic fungal isolates. *Penicillium sclerotiorum* extract were used for the synthesis silver nanoparticles (PsNps) and were characterized by UV-Vis spectroscopy, Fourier transform- infrared spectroscopy (FTIR), Zeta potential, FE-SEM and Energy- Dispersive X-ray analysis (EDAX). Antibacterial activity of PsNps was tested against the antibiotic resistant uropathogenic *E. coli* and ATCC (25922) strain of *E. coli*. Further experiments were carried out to explore the potential of PsNps in regulating the CTX-M-15 gene. The antimicrobial activity showed that the PsNps inhibited growth, biofilm formation in both the strains of *E. coli*. The expression of gene encoding CTX-M-15 was down regulated in resistant strain of uropathogenic *E. coli.* Our results suggest that the PsNps could be used as an alternative source for the antibiotics. Thus, further studies can be conducted to prove the *in vivo* potential of PsNps and can be formulated for commercialization.

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

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Authors' contributions

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

Introduction

Endophytic fungi are the endosymbionts that are present in healthy tissue of plants. Endophytic organism plays an important role in higher plants by inducing disease resistance. Endophytes are rich in producing large number of active secondary metabolites which help its host to combat the microbial diseases. Previous literature support that isolated endophytic fungi from medicinal plants showed antifungal, antibacterial, antimicrobial properties against different pathogens (1). Secondary metabolites have a broad array of applications in various fields especially in pharma industry. Endophytic microorganisms are great source of natural and novel bioactive compounds that can be used to meet pharmaceutical, medical and industrial demand. Under optimum conditions endophytes deliver large number of secondary metabolites which are extremely effective against detrimental pathogens such as MDR microbes (2, 3).

Material Size ranging in nanometer is known as nanoparticles. Because of their large surface area, nanoparticles are more reactive against bacterial cells and causing cell death by one or several mechanisms. The recent interest in material stabilization is the development of efficient biological synthesis of nanoparticles for medicinal applications (4). AgNPs had unique thermal, optical, electrical chemical and physical properties due to the high proportion of high energy surface atoms (5). Silver is highly toxic towards the microbial cells and thus can be utilized as an antimicrobial agent (6). In recent times, more attention has been paid towards the biosynthesis of AgNPs by microorganisms due to targeted drug delivery and antimicrobial agent (7). Microorganisms are considered as a potential source for the stabilization and reduction of AgNPs, AuNPs, CdNPs and ZnNPs. Different microorganisms have been exploited as a reduction and stabilizing agent for biosynthesis of silver nanoparticles. Biological method of NP synthesis is more efficient than physical and chemical methods due to less toxicity (8).

In this study, endophytic fungus, *Penicillium sclerotiorum* was isolated from leaves of *Tamarindus indica*. Tamarind is a tropical fruit which is traditionally used in food. In Ayurveda, the tamarind leaves, stems, oils, seed extract are used to control many health problems such as inflammation, pathophysiological disorders and immunological disorders. In Unani and Siddha, Tamarind is traditionally used in all forms due to their active substances. Tamarind plays a potential role as anti-inflammatory, antidiabetic, anticancer agents, antimicrobial, anti-venomic, antioxidant, antimalarial, cardio protective, anti- asthmatic and playing a potential role in the treatment or prevention of obesity and other chronic diseases (9). Fruits, leaves and seeds of tamarind tree are natural sources of antioxidants is related to phenolic compounds such ascatenin, epicatechin, glucose, mucilage, pectin, uronic acid, procyanidin B2, tartaric acid, arabinose, xylose, galactose, triterpene (10).

This current study concentrated on synthesis of myconaoparticles using endophytic fungal extract of *Penicillium sclerotiorum* (PsNps). The synthesized PsNps were biophysically confirmed by using various characterization methods. The antimicrobial activity of PsNps was studied by performing MIC, MBC and Biofilm assays. Upon treatment of bacterial strain with PsNps, the antibiotic resistance gene CTX-M-15 gene expression were analyzed by using Polymerase chain reaction. CTX-M-15 is an ESBL (Extended Spectrum Beta-Lactamase) enzymes produced by Gram negative bacteria that can break penicillin and make them ineffective. ESBL group of enzymes causes various infections such as urinary tract infections and other life-threatening diseases. CTX-M-15 was first discovered in India that mostly active against cefotaxime and then identified in Turkey, France, Romania, and UK (11).CTX-M-15 is produced by multidrug-resistant UTI pathogens of *E. coli* and *P. aeruginosa* from hospitals in Nigeria, is a type of ESBLs enzymes produced by MDR pathogens by continuous mutation which led to resistance towards (11).

Materials And Methods

The *E. coli* strains such as ATCC (25922) and clinical isolate of biofilm forming, uropathogenic, Multi drug resistant (MDR) strains of *E. coli* were isolated from Tagore Medical College and Hospital, Chennai after proper ethical approval from BSACIST (Ref. no. BSAU: REG-OFF: 2016/02SLS).

Isolation and identification of endophytic fungi from *Tamarindus indica*

Healthy leaves of *Tamarindus indica* were collected from B.S. Abdur Rahman Crescent Institute of Science & Technology, Vandalur, Chennai. The leaves were washed several times under running tap water to avoid the contamination. Leaves were cut into small pieces (1mm) and surface sterilized with distilled water and followed by sequential rinsing in 70% ethanol for 1 min, 1% Sodium hypochlorite for 1min, 90% ethanol for 5 min, finally rinsed with sterile distilled water for 2-3times and then allowed to dry under sterile conditions in laminar air flow. The cut surface of the segments were placed in petri dishes containing PDA supplemented with Ampicillin (1mg/lit) in a laminar air flow chamber and incubated at 28^{o}C for 4-7 days. Plates were periodically observed for growth of endophytic fungi. After 7 days,

endophytic fungi were growing out from the explants was sub-cultured in new PDA plates to obtain pure culture (12- 15).

Identification of the endophytic fungi isolated from *Tamarindus indica*

1g of fresh fungal mycelium carefully cleaned with sterile water and ground into fine paste in a mortar and pestle. The fungal DNA was isolated using CTAB method (16). Further identification of endophytic fungi was carried out through amplification of fungal DNA using universal ITS primers ITS1 (5´TCC GTA GGT GAA CCTTGC GG 3´) and ITS4 (5´TCC TCC GCT TAT TGA TAT GC 3´). Polymerase chain reaction was performed as prescribed previously (17). The amplified PCR product was used for sequencing and further utilized for construction of phylogenetic tree using MEGA-X, based on Maximum Likelihood algorithm and, endophytic fungal sequence was submitted to NCBI Genbank (12- 15).

Biosynthesis of myconnaoparticles loaded with metabolites of Endophytic fungus *Penicillium sclerotiorum*

Extracellular extract of endophytic fungi *Penicillium sclerotiorum*, isolated from leaves of *Tamarindus indica* was used for biosynthesis of myconanoparticles (PsNps) (18). Equal volume of endophytic fungal extract and 1 mM $AgNO_3$ solution was mixed with gentle swirling and incubated in dark for 48h at room temperature. The colour of the solution changed from colourless to brown confirmed the synthesis of myconanoparticles (12-16, 18).

Physio-chemical characterization of green silver nanoparticles

The synthesis of silver mediated myconanoparticles was confirmed by measuring the absorbance using UV-Visible spectroscopy (300nm-700nm), with a double beam spectrophotometer (Jasco V-730 spectrophotometer). FT-IR spectrum was measured between 400- 4000cm-1 to identify the potential chemical groups present in PsNps. This helps us to identify the possible biomolecules, which could play the role in reduction, stabilization and capping during synthesis of myconanoparticles.(Perkin Elmer Spectrum100 FT-IR) (20-23). Colloidal nanoparticle solution was used to identify the hydrodynamic size (Z average), polydispersity index (PDI), and surface charge (zeta potential) of the synthesized nanoparticles using particle size analyser combined with zeta analyser (Malvern Instruments Ltd, UK). Particle size analysis was performed at the scattering angle of 90°, medium viscosity 0.895 mPa·s, count rate of 210 kCPS, at 25°C. FESEM combined with EDAX was used to study the morphology and elemental composition of nanoparticles (SIGMA HV – Carl Zeiss with Bruker Quantax 200 – Z10 EDS Detector) (24-27).

Antibacterial assay of myconanoparticles

The efficacy of silver nanoparticles was evaluated for its antibacterial and antibiofilm activity by assessing minimum inhibition concentration, minimum bactericidal concentration, growth and antibiofilm effect. Further the study was extended to validate the expression of antibiotic resistant gene

CTX-M-15 upon treatment with synthesized nanoparticles. The minimum inhibitory concentration was performed in 96 well microtiter plate (Ranjani et al., 2020a-k). The growth of the tested organism was followed by treating with synthesized silver nanoparticles and ampicillin treatment and compared with control upto 24hours of incubation (28-32).

The gene expression of CTX-M-15 was analyzed by amplifying the treated and control DNA from strains with CTX-M-15 primers (31).

Results

Isolation and identification of Endophytic fungi isolated from *Tamarindus indica*

The leaves of *Tamarindus indica* was used as explant and endophytic fungi was isolated as described in the methods. After sequencing of the selected strain, phylogenic tree was constructed using Maximum Likelihood algorithm by aligning closely related sequences and as per sequence similarity and clustering of the phylogenetic tree, the sequence was identified as *Penicillium sclerotiorum* and deposited in NCBI Genbank with accession number MK942602. *Penicillium* is one of the widely distributed fungi in environment (Fig.1a).

Penicillium sclerotiorum mediated synthesis of silver nanoparticles (PsNps) and Biophysical characterization

Pure cell free extract of *Penicillium sclerotiorum* was used for the synthesis of silver nanoparticles and the colour change was noted from pale while to brown colour which indicates the synthesis of *Penicillium sclerotiorum* mediated synthesis of PsNps (Inlet fig.1 b,c,d). The UV-visible absorption spectrum was taken between 200-800nm, which showed the SPR peak around 400nm which confirmed the synthesis of *Penicillium sclerotiorum* mediated synthesis of silver nanoparticles (PsNps) (Fig.1 e).

FTIR spectrum of PsNps was taken to observe the possible biomolecules involved in the reduction and capping to achieve the synthesis of stable PsNps. The Peak wavelength (cm^{-1}) of 3146, 2902, 2107, 1992, 1887, 1610, 1311, 1078, 985, 627 correspond to functional groups of carboxylic acid (O-H stretching), N-H stretching amine salt and alcohol (O-H stretching), isothiocyanate N=C=S stretching, aromatic compound (C-H bending), aromatic compoundα,β-unsaturated ketone (C=C stretching), sulfone (S=O stretching), primary alcohol (C-O stretching), alkene (C=C bending), halo compound (C-Br stretching) respectively (Fig.1 f) (Table 1).

The DLS technique was utilized to identify the size of nanoparticles based on Brownian movement and it was observed as 331.2 nm and the negative charge-20.6 mV confirms the stability of the nanoparticles synthesized using *Penicillium sclerotiorum* extract (Fig.2 a, b).

From the field emission microscopic image at 25.00 KX magnification at 1µm scale, 50.00 KX magnification at 200nm scale and 100.00KX magnification at 100nm scale, the nanoparticles were

observed as different shaped and different within nanometer in range (Fig.3 a,b,c). The elemental composition reveals the presence of Ag by showing peak at 3KeV. Apart from silver other elements such as C, O, Mg, Si, S, Ca, Al and Cl were present in the nanoparticles (Fig.3 d). The percentage weight of each element was found as 35.37%, 29.49%, 18.32%, 6.95%, 6.91%, 1.58%, 0.82%, 0.56% for Ag, O, C, Ca, Cl, Si, S, Al respectively. The percentage of atom was found as 7.87%, 44.23%, 36.61%, 4.16%, 4.68%, 1.35%, 0.61%, 0.5% for Ag, O, C, Ca, Cl, Si, S, Al respectively (Fig.3 e). These elements may derive from the secondary metabolites of fungal extract, which act synergistically in imparting efficient antibacterial activity.

Antibacterial potential of PsNps

Many pathogens are developing resistance towards routine antibiotics. Hence, there is a pressing need of the hour to find alternative solution by synthesizing nanoparticles using *Penicillium sclerotiorum* extract. As *Penicillium* is a very important genus used for the mass production of valuable products such as penicillin. *Penicillium sclerotiorum* was reported to produce antimicrobial secondary metabolites (Petit et al., 2009) which was utilized in this study to synthesize silver nanoparticles and validated against *E. coli* ATCC (25922) and urinary tract infection causing antibiotic resistant *E. coli*. The minimum inhibitory concentration is the minimum concentration of PsNps which inhibit the growth of test organism. The visual turbidity of *E. coli* ATCC (25922) and UTI causing clinical pathogen was observed and the wells with invisible growth was considered as MIC. The MIC of *E. coli* ATCC (25922) and UTI causing MDR pathogen was calculated as 0.75 µg/ml and 6.25 µg/ml respectively. The decrease in the growth rate of bacterium at their MIC concentration was represented graphically in fig. 4 a. The minimum bactericidal concentration is the lowest concentration of PsNps which completely kills the test organism. The MBC of *E. coli* ATCC (25922) and UTI causing MDR pathogen was calculated as 6.25µg/ml and 12.5µg/ml respectively (Table 2). This study focused on assay to validate the antibiofilm effect of PsNps on biofilm forming UTI causing *E. coli*. From our experiments it was observed that upon treatment with 12.5 µg/ml of PsNps, the biofilm formation was reduced by 87% and 85% for *E. coli* ATCC (25922) and UTI causing MDR *E. coli* pathogen (Fig.4 b). In addition, this research work focused on studying the amplification of CTX-M-15 gene upon treatment with PsNps and ampicillin. From the results it was observed that the expression of CTX-M-15 gene was found in control and ampicillin treated strain, however the expression of CTX-M-15 gene in PsNps treatment was suppressed (Fig.4 c).

Discussion

Endophytic fungi produce plethora of secondary metabolites, which has potent antibacterial activity. Among that *Penicillium* species are reported to produce pharmaceutically important secondary metabolites. They are reported to have antibacterial, antifungal, lowering cholesterol levels and immunosuppressant properties. *Penicillium* species produce important secondary metabolites such as compactins, mycophenolic acid, kojic acid, viridicatol, quinolines, diketopiperazines, alkaloids, quinazolins etc (33). *Penicillium sclerotiorum* was reported to produce azophilones sclerotiorin, pencolide, isochromophilone, pipergalone. Pipergalone reported to have potent antibacterial activity against

Staphylococcus aureus, Pseudomonas aeruginosa, and *Candida albicans*. Sclerotiorin was reported to block RAS signalling pathway, inhibits lipase, hence patent utilized for the production of acne creams and antiobesity biscuits, aldose reductase enzymes, which plays an important role in diabetes complications such as neuropathy, nephropathy. Sclerotiorin has antibacterial activity against *Escherichia coli*, *Lysteria monocytogenes*, *Klebisiella pneumoniae, Bacillus subtilis, Salmonella typhimurium* (34). Thus, the effect of these pharmacologically important, bioactive secondary metabolites can be harnessed for the synthesis of silver nanoparticles to offer a potent antibacterial activity against UTI causing *E. coli* (35).

The UV-visible absorption spectrum showed the SPR peak around 400nm The SPR depicts the size, shape and agglomeration of PsNps. When light wave passed into colloidal solution, collective oscillation of electrons presents in silver nanoparticles trigger the free electrons to form SPR absorption spectrum. There were literature supporting the fact that 320–580 nm is characteristic λmax for silver nanoparticles, where the absorption spectrum of nanoparticles mainly depends on particle size, environment, dielectric constant (36). In addition, phytoreduction and phytocapping of silver nanoparticles was also achieved by secondary metabolites such as flavonoids, terpenoids, saponins, alkaloids and other compounds such as proteins, enzymes which were present in the *Penicillium sclerotiorum* extract (37). It was observed that broad spectral bands at 3146 cm−1 are characteristic to the O-H stretching of hydroxyl group of polyphenols and N-H stretching vibrations in primary and secondary amines of amino acids, peptides and proteins. There were previous reports supporting that protein molecules involve in nanoparticle synthesis by interacting with their amide bond. Thus, protein capped silver nanoparticles will help to maintain the stability of particles without agglomeration (38).

Based on DLS, the size of PsNps was 331.2 nm, which was measured due to Brownian movement of PsNps in colloidal solution and zeta value was -20.6 mV confirms the stability of the nanoparticles synthesized using *Penicillium sclerotiorum* extract. FESEM image of PsNps help us to observe the morphology, size and shape of the PsNps. All the physiochemical and other instrumental analysis gives the information on morphology, size and shape of nanoparticles, which supports in providing effective antibacterial activity through its mode of action via several mechanism.

The MIC of *E. coli* ATCC (25922) and UTI causing MDR pathogen was calculated as 0.75 µg/ml and 6.25 µg/ml respectively. The minimum bactericidal concentration is the lowest concentration of PsNps which completely kills the test organism. The MBC of *E. coli* ATCC (25922) and UTI causing MDR pathogen was calculated as 6.25µg/ml and 12.5µg/ml respectively (Table 2). The growth of *E. coli* ATCC (25922) and UTI causing MDR pathogen was observed up to 24 hours of time period on treatment with 12.5 µg/ml of PsNps and it was observed that growth rate was decreased by 90% and 82% respectively when compared with the control, whereas ampicillin treatment could not control the growth of UTI causing MDR pathogen (Fig.4 a). These results confirmed that PsNps has potent bacteriostatic and bactericidal activity against the tested organism. Silver nanoparticles showed antibacterial activity in liquid medium because of good dispersion ability of silver ions without any aggregation. Due to larger surface area of silver nanoparticles, a greater number of bacterial cells will interact with silver ions which ultimately become toxic to bacterial cells and cause cell death. There were several reports on mechanism of silver

nanoparticles and its action on bacterial cell. First the silver ions of silver nanoparticles inactivate the bacterial cell by damaging the cell membrane, protein inactivation, lipid peroxidation, creates pits on the cell membrane, followed by the disintegration of membrane integrity, disturb the transport chain and causes leakage of cell membrane (39). It is reported that silver ions have higher affinity towards Sulphur and phosphorous. When the silver ion comes in contact with cell membrane it interacts with Sulphur containing protein of cell membrane and when it enters inside the cell silver ions interact with Sulphur containing protein and phosphorous containing DNA (40). This interaction induces drastic changes in the cellular metabolism, cellular respiration and decrease in the production of ATP, which ultimately leads to cell death (38). Thus, silver ions along with phytochemicals enhances the bactericidal activity of silver nanoparticles by coordinating with several biochemical and molecular mechanisms.

Biofilm is the major causative agent for several deadly diseases. UTI causing uropathogenic *E. coli* accounts for 80% of UTI infections in human. Biofilm formation causes resistance to antimicrobial agents and make it very difficult to penetrate inside the biofilm. From our experiments it was observed that upon treatment with 12.5 µg/ml of PsNps, the biofilm formation was reduced by 87% and 85% for *E. coli* ATCC (25922) and UTI causing MDR *E. coli* pathogen (Fig.4 b). This shows that PsNPs have potent antibiofilm activity against biofilm causing pathogens. Silver nanoparticles interact physically and chemically with the biofilm and effectively evade and stop the synthesis of extra polysaccharides, which is responsible for the formation of biofilm (41). There are reports supporting our fact that silver nanoparticles inhibit the transcription of biofilm associated genes. There were previous report showed that exposure of silver nanoparticles results in intemperance of proton motive force (42). The biocidal silver ions induce DNA assortment as a defense mechanism to protect the bacterial cell from toxic environment but simultaneously nullify its replication ability subsequently reduce the bacterial population, which in turn reduces the biofilm formation. The ROS production inside the cell also increases the toxicity inside the cell by inhibiting the enzymatic action, which pave the way for cell death (43).

CTX-M-15 is the antibiotic resistant gene which produces enzymes which cleave the beta lactam ring of antibiotics before its action. CTX-M-15 gene is predominant in south India, which was found to be present in most of the antibiotic resistant organisms isolated from clinical samples. From the amplification study it was found that the presence of CTX-M-15 gene was suppressed (Fig.4 c). This shows that PsNp directly targets the DNA thereby reduces the ability of transcription and translation of the cell. Ultimately replication of the cell would have stopped without multiplication of bacterial cell, thereby decreasing the whole population in nanoparticles environment (32, 40).

Conclusion

In this research work systemic methodology was adopted to synthesize myconanoparticle using *Penicillium sclerotiorum,* to explicate the superior antibacterial and anti-biofilm effects against biofilm forming, multi drug resistant UTI causing *E. coli*. PsNps showed excellent bacteriostatic, bactericidal and antibiofilm activity against UTI causing *E. coli*. Further validation on commercial production of PsNps,

can be formulated as nanogels and vaginal wash, which could be used to prevent the urinary tract infection caused by uropathogenic organisms.

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Tables

Table:1 The major peaks and the functional group present in PsNps synthesized using *Penicillium sclerotiorum,* endophytic fungus isolated from *Tamarindus indica*

Table 2: Minimum inhibitory concentration and Minimum bactericidal concentration of PsNps against *E. coli* ATCC (25922) and Uropathogenic, MDR *E. coli*

Strains	Concentartion of PsNps synthesized using *Penicillium sclerotiorum*	
	Minimum inhibitory concentration (µg/ml)	Minimum bactericidal concentration (µg/ml)
E. coli ATCC (25922)	0.75	6.25
Uropathogenic, MDR *E. coli*	6.25	12.5

Figures

S.No	Peak wavelength	Functional group
1.	3146	Carboxylic acid (O-H stretching)
2.	2902	Alcohol (O-H stretching) N-H stretching amine salt
3.	2107	Isothiocyanate (N=C=S stretching)
4.	1992	Aromatic compound (C-H bending)
5.	1887	Aromatic compound
6.	1610	α, β-unsaturated ketone (C=C stretching)
7.	1311	Sulfone (S=O stretching)
8.	1078	Primary alcohol (C-0 stretching)
9.	985	Alkene (C=C bending)
10.	627	Halo compound (C-Br stretching)

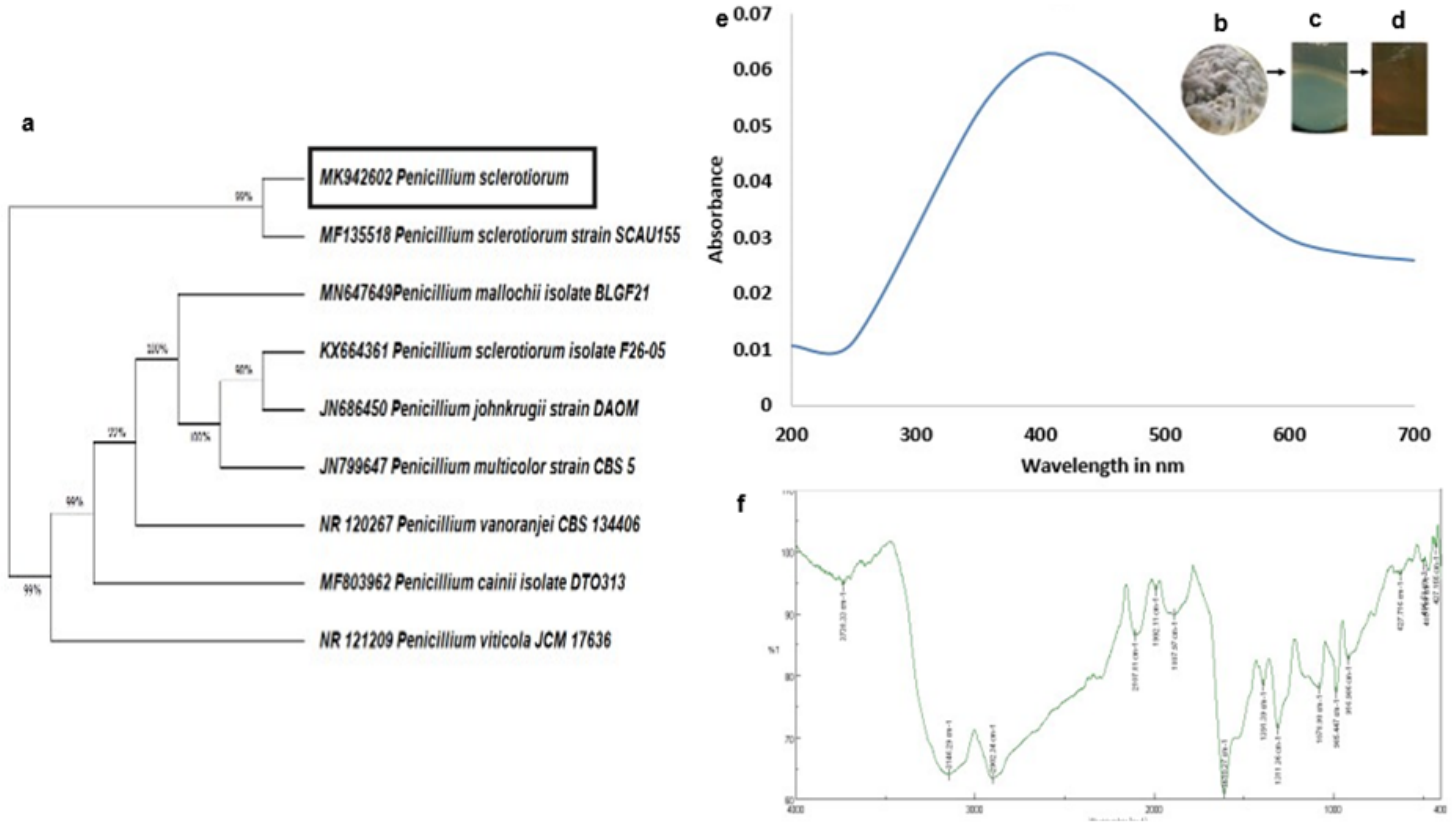

Figure 1

a. Phylogenetic relationship of Endophytic fungus Penicillium sclerotiorum with fungal isolates retrieved from NCBI GenBank using MEGA-X, Maximum Likelihood method. Inlet fig. b. Endophytic fungus Penicillium sclerotiorum. Inlet fig. c. Penicillium sclerotiorum cell free aqueous extract. Inlet fig. d. Visual

colour change during PsNps synthesis. e. UV-Visible absorption spectrum of Penicillium sclerotiorum mediated myconanoparticle synthesis (PsNps). f. FTIR footprints showing the wavelength, which corresponds to functional group present in Penicillium sclerotiorum extract mediated PsNps.

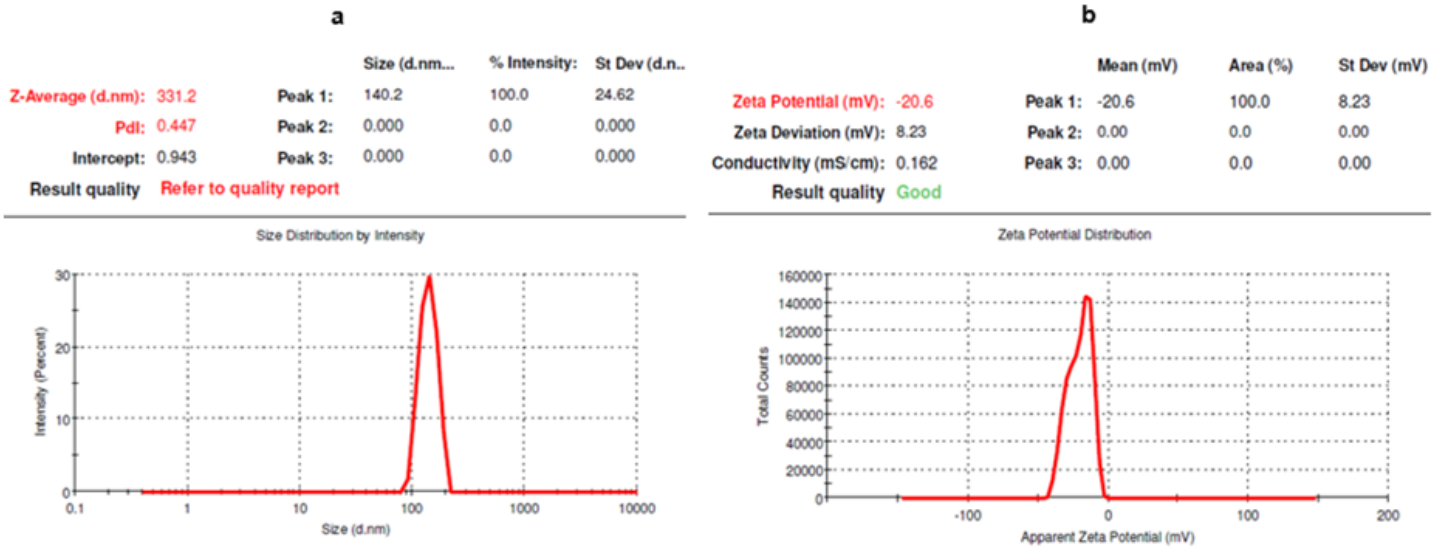

Figure 2

a. Particle size distribution Penicillium sclerotiorum extract mediated PsNps, observed through Brownian movement. b. Zeta potential value of Penicillium sclerotiorum extract mediated PsNps, by measuring the zeta values.

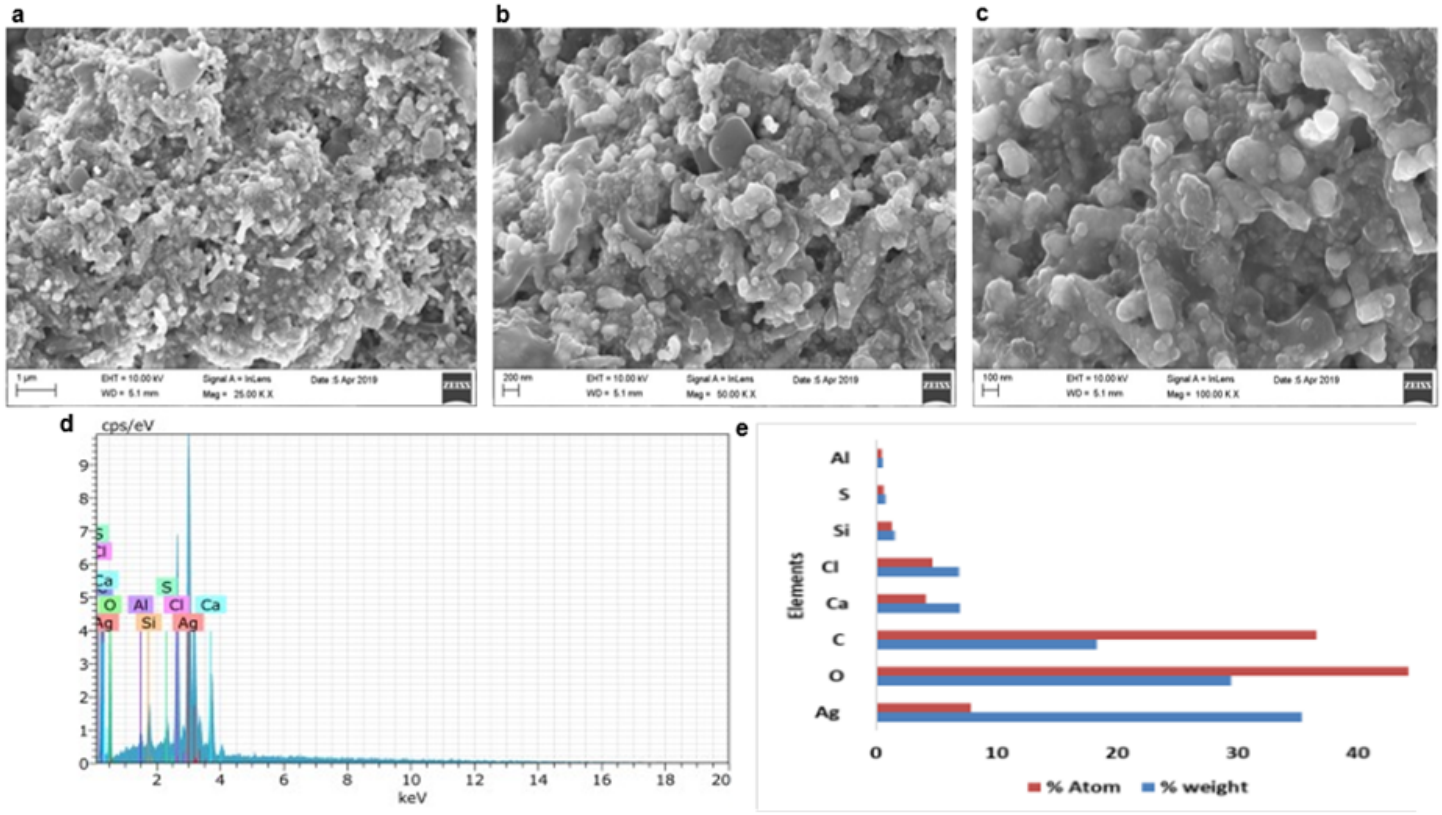

Figure 3

a. FESEM image of PsNps at 25.00 KX magnification at 1µm scale. b. FESEM image of PsNps at 50.00 KX magnification at 200nm scale. c. FESEM image of PsNps at 100.00 KX magnification at 100nm scale. d. EDAX spectrum of PsNps, which represent the elemental composition of PsNps. e. Graphical representation of elemental composition of PsNps obtained through EDAX spectrum

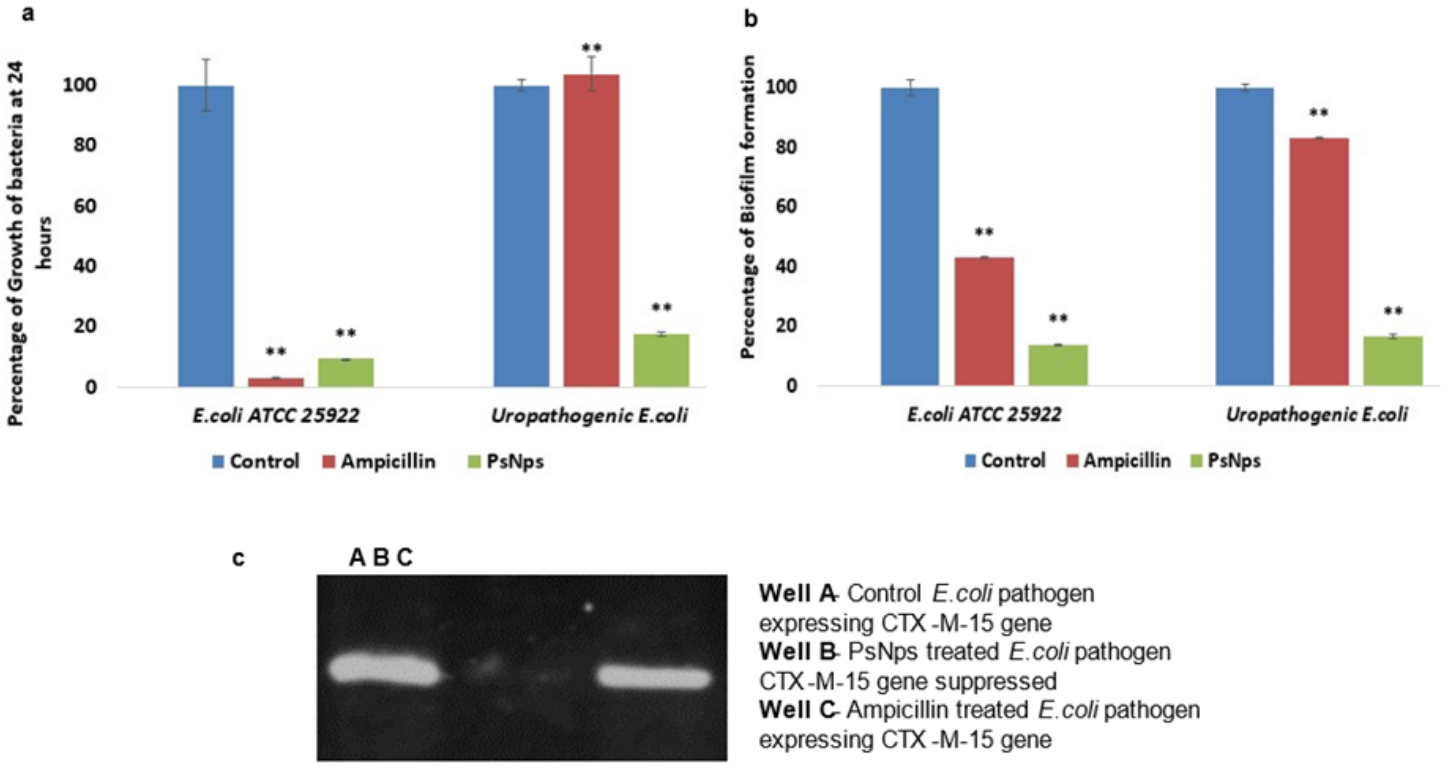

Figure 4

a. Percentage of bacterial growth kinetics after 24 hours, treated with PsNps when compared to control and ampicillin treated strains. b. Percentage of biofilm formation in PsNps treated strains when compared to control and ampicillin treated strains. Error bars were representing means ± standard errors. ** denotes t-test ($P < 0.01$). c. Gel image depicts the expression of CTX-M-15 gene: Well A. Uropathogenic E. coli expressing CTX-M-15 gene. Well B. Upon treatment with PsNps, expression of CTX-M-15 gene was controlled. Well C. Upon treatment with Ampicillin Uropathogenic E. coli expressing CTX-M-15 gene was shown.