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Research Article

Antimicrobial Potential of Green Synthesized Gold Nano-Particles Against Human Pathogens

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Abstract

Green synthesis is a novel approach for the biogenesis of nano-particles- tiny structures having enormous applications. In the current study, fungal strains were explored for their ability to biosynthesize both intra- and extracellular gold nano-particles. Out of twelve, four fungal strains namely SSG-2, SSG-5, SSG-8 and SSG-11 were selected on the basis of development of varied colors in cell free supernatant and fungal beads upon exposure with tetra-chloroauric acid. Crystallographic characterization of biomass associated, and cell free nano-particles were carried out by X-Ray Diffraction (XRD) and UV-Visible spectroscopy. A wide variation in crystallite size for intracellular and extracellular nano-particles has been observed. Further these intra- and extracellular nano-particles exhibited intense antimicrobial activities against human pathogens.

Keywords: Green Synthesis; Nano-Particles; XRD (X-Ray Diffraction); Antimicrobial; Tetra-Chloroauric Acid

Introduction

Emerging infectious diseases and increasing resistance in pathogenic microbes towards antibiotics is of serious concern in biomedical and pharmaceutical fields. Antibiotic resistance profiles of these pathogens have led to fear about emergence and re-emergence of multidrug-resistance (MDR) [1]. Further MDR bacterial infections have lead significant increase in mortality, morbidity and serious health problems. Due to increased incidences of drug resistance among pathogenic microbes, the development, modification and search for the new antimicrobials with bactericidal potential has become inevitable [2]. Advancement in nanoscience and nanotechnology has brought to fore the nano-sized organic and inorganic particles having promising antimicrobial properties [3]. Nanotechnology is of tremendous importance due to its ability to change the chemical, physical and optical properties of metals and modulate these to nano size. Noble metals nano-particles like gold, silver, platinum and palladium etc. have been extensively used due to their unique electronic, mechanical, optical, chemical and magnetic properties [4-8]. These metallic nano-particles exhibited large surface area to volume and are of varied shapes and sizes. Gold nano-particles have been widely exploited in therapeutics for targeted drug delivery in cancer, diabetes mellitus, cardiovascular diseases etc. and also in various other fields like optoelectronics, biosensors, bioremediation technology [9]. Further gold nano-particles have been reported to possess both antibacterial and antifungal activities [10,11]. Although several physical and chemical methods have been used for the synthesis of metallic nano-particles but green synthesized nano-particles involving microbes are eco-friendly, quite effective and less toxic [12]. Many biological systems have ability to uptake and reduce the metals ions into highly stable nano-particles. Several bacteria, fungi and actinomycetes has been reported as "bio-nano factories" for the synthesis of metallic nano-particles [13-17]. Fungi have been reported as potential source for biosynthesis of nano-particles due to its tolerance and inherent ability to produce higher concentrations of proteins which aids in bioreduction of metals to less toxic form [18]. Mukherjee., *et al*. reported bioreduction of AuCl4- and formation of extracellular gold nano-particles by fungus *Verticillium* sp. and *Fusarium oxysporum* [19,20]. Bioreduction of gold by cell free supernatant of *Trichoderma viride* [21] and *Botrytis cinerea* [22] confirms that the Au-reducing proteins were secreted in the extracellular space. Although extensive research has been carried out for the synthesis of gold nano-particles, but fungal biogenesis of gold nano-particles is still confined. Owing to continuous demand

for nano-particles having antimicrobial potential, the present study was aimed towards biosynthesis of fungal intra- and extracellular gold nano-particles along with their antimicrobial profile against human pathogens. Present study reported biosynthesis of nano-particles considerably in less time and nano-particles were found quite stable without any capping or stabilizing agent for long period of time. In future these nanostructures having antimicrobial activity can be incorporated into active food packages to prevent the food spoilage.

Methodology

Screening of fungal strains for gold nano-particles biosynthesis

Different fungal strains collected from contaminated agar medium were inoculated over Potato Dextrose Agar (PDA) plates and incubated at 28^{O}C at 72 h. Isolated fungal cultures were repeatedly sub-cultured over fresh PDA plates to ensure the axenic nature of the isolates. After purification, individual strains were screened for biosynthesis of gold nano-particles on exposure to tetra-chloroauric acid. For this, actively growing cultures from the periphery were inoculated in 100 ml of Potato Dextrose Broth (PDB) and incubated at 28^{O}C for 72 h at 120 rpm followed by washing of fungal beads with sterile distilled water at regular interval of 4 h with continuous shaking to remove the traces of the medium. Washed fungal beads were exposed to 100 µl of tetra-chloroauric acid (50 µM) and incubated at 28^{O}C at 120 rpm till appearance of blue/red color which served as visible inference for formation of intracellular gold nano-particles. For extracellular, biomass free supernatant was incubated with tetra-chloroauric acid (50 µM) and change in color was observed. A control was also maintained to ensure the biological origin of gold salt reduction and nano-particles biosynthesis. Positive isolates were further subjected to viability test after exposure to tetra-chloroauric acid. Fungal beads were inoculated over fresh PDA plates and incubated at 28^{O}C for 72 h. Plates were regularly observed for fungal growth.

Morphological characterization of the isolates

Morphological characterization of the fungal strains was studied on PDA plates by observing the colonial morphology and microscopic features were examined after staining with lactose phenol cotton blue dye.

Crystallographic characterization of the nano-particles

Crystallographic characterization of the nano-particles (colored fungal beads and biomass free supernatant) along with control (fungal beads without tetra-chloroauric acid) was carried out by X-Ray diffraction (XRD) and UV-Visible spectroscopy. For intracellular nano-particles, fungal beads were filtered through coarse filter paper and dried in an oven at 150^{O}C. Dried samples were powdered using pestle mortar and were analyzed by X-Ray Diffractometer. For extracellular nano-particles, filtrate was air dried and analyzed [23,24]. Extracellular samples were also analyzed by UV-Visible spectroscopy. Samples were prepared by adding 100 µl of tetra-chloroauric acid to the biomass free supernatant followed by incubation at 28^{O}C till reduction of pale yellow color to violet. A spectral scan was recorded over wide spectral range on UV-Visible spectrophotometer (SHIMADZU UV-1800) to evaluate the Plasmon resonance bands associated with each metal particle formation [25].

Antimicrobial activity of gold nano-particles

The synthesized fungal gold nano-particles (both intraceullar and extracellular) were evaluated for their antimicrobial activity against bacteria (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella*) on Muller Hinton agar plates and fungi (*Aspergillus* and *Penicillium*) on Sabouraud agar plates by agar well diffusion method. Bacterial cultures were grown overnight in broth medium and inoculums were prepared by diluting with 0.85% NaCl followed by swabbing over the plates. Similarly, fungal cultures were diluted and swabbed over Sabouraud agar plates. Wells (5 mm) were prepared with sterile cork borer and 50 µl of respective intra- and extracellular samples were added into the wells. Plates were incubated at 37^{O}C for bacteria and 28^{O}C for fungi followed by observation of clear zone around the wells. Antimicrobial activity was assayed by measuring the diameter of clear zone formed around the wells.

Results and Discussion

Screening of fungal strains for gold nano-particles biosynthesis

Twelve different fungal strains collected from contaminated medium were purified by repeated sub-culturing on fresh PDA plates. Further these purified isolates were screened for reduction of tetra-chloroauric acid and biosynthesis of gold nano-particles. Out of twelve, only four strains (SSG-2, SSG-5, SSG-8 and SSG-11) were found positive for nano-particles biosynthesis by visible appearance of different colors. A wide range of color i.e. pink in SSG-2, violet in SSG-5, multi blue in SSG-8 and light violet in SSG-11 has been observed in fungal beads after incubation of 1 h with tetra-chloroauric acid which indicated the intracellular nano-particles biosynthesis while change in color of biomass free supernatant i.e. extracellular nano-particles was observed after incubation of 5 min. No color development has been observed in control incubated along which indicated the biological origin of reduction and

biosynthesis (Figure1). Similarly, intracellular and extracellular bioreduction of gold by fungi *Aspergillus fumigatus* and *A. flavus* has been reported [24] An extracellular biogenesis of gold Nano-particles has been reported for *Fusarium* sp. by Sawle., *et al.* [26]. All the isolates were found viable on PDA plates with appearance of extending mycelium followed by sporulation upon exposure with gold salt (Figure 2).

Figure 1: Visual inference of intracellular and extracellular gold Nano-particle biosynthesis by fungal isolates SSG-2, SSG-5, SSG-8 and SSG-11 along with control upon exposure with tetra-chloroauric acid.

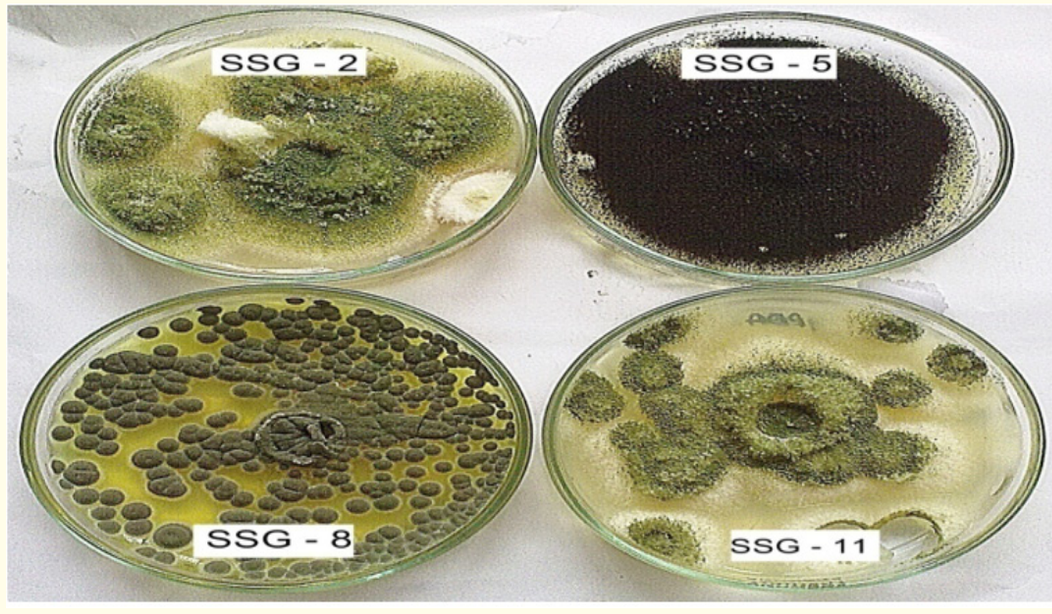

Figure 2: Figure shows the viability of SSG-2, SSG-5, SSG-8 and SSG-11 on PDA plate after exposure with tetra-chloroauric acid.

Morphological characterization of fungal isolates

All the positive isolates were characterized on the basis their colony morphology i.e. mycelial growth and sporulation on PDA plates and along with microscopic examination (Figure 3) as given in Table 1

Strain	Morphological Observation	Microscopic Examination
SSG-2	Green coloured powdery growth	Aseptate, multinucleate conidiosphores resembling a brush like head
SSG-5	Black coloured spores having aerial hyphae	Aseptate sprongiophores having coenocytic condition with black coloured sporangiospores
SSG-8	Greyish green coloured sporulating not powdery growth	Aseptate, multinucleate long stalked conidiosphores
SSG-11	Yellowish green coloured powdery growth	Multinucleate green coloured sporangiophores having green sporangia with green spores

Table 1: Morphological and microscopic characterization of selected fungal isolates.

Crystallographic characterization of Au Nano-particles

XRD (X-Ray Diffraction) Analysis

X-ray diffraction patterns of the intracellular and extracellular samples were recorded using a Panalytical's X'Pert Pro Powder X-ray diffractometer with Cu Kα radiation (λ = 1.541 Å) in the 2θ range 20° - 80° at generator tension 45 kV and generator current 40 mA. Broadening in the recorded XRD pattern indicated the formation of metallic gold nano-particles in both extracellular and intracellular synthesis. From the diffraction line width of recorded diffractogram, average crystallite size of both intracellular and extracellular nano-particles was calculated using Debye-Scherrer equation [27].

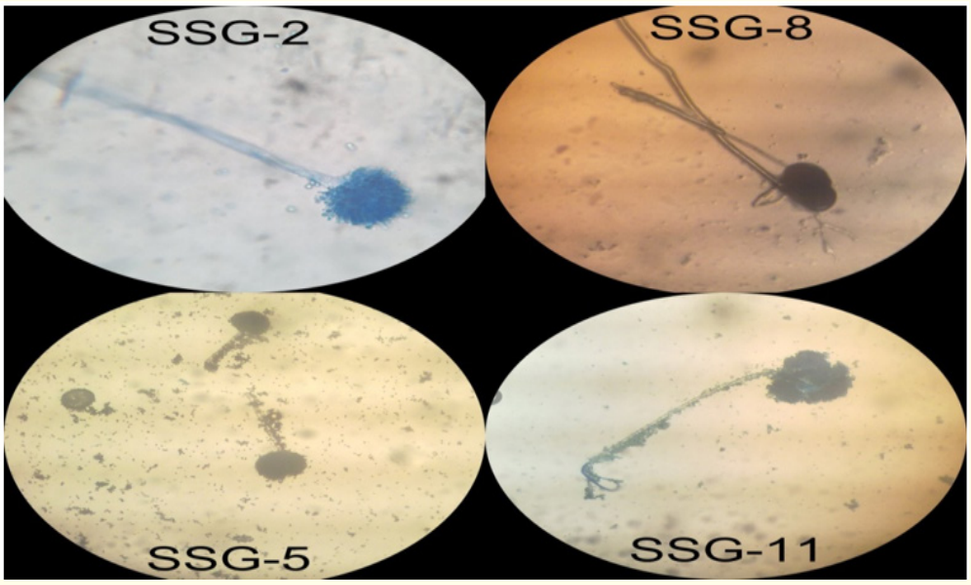

Figure 3: Microscopic examination of positive fungal strains.

$$D = \frac{0.89\lambda}{\beta \cos\theta}$$

Where, D is the average crystallite size, λ is incident X-ray wavelength, β is the full width at half maximum (FWHM) of X-ray reflection in terms of 2θ expressed in radians and θ is the position of the diffraction peak in the diffractogram

In XRD pattern of SSG-2, peaks at 2θ angles: 38.11^{o}, 44.04^{o} and 64.7^{o} with Bragg reflection corresponding to (111), (200) and (220) were observed in biomass associated nano-particles whereas in biomass free supernatant peaks at 2θ angles: 37.86^{o}, 48.88^{o}, 67.38^{o} and 72.46^{o} with Bragg reflection corresponding to (111), (200), (220) and (311) were obtained (Figure 4). A wide variation in crystallite size of intracellular (7.63 nm) and extracellular (25.38 nm) nano-particles were observed. Similarly, for strain SSG-8, peaks at 2θ angles: 38.22^{o}, 44.41^{o} and 64.64^{o} with Bragg

reflection corresponding to (111), (200) and (220) and peaks at 2θ angles: 38.19^{O}, 44.31^{O} and 72.30^{O} corresponding to (111), (200) and (220) with crystallite size of 10.95 nm and 36.45 nm were obtained for intracellular and extracellular nano-particles respectively. Peaks at 2θ angles: 38.56^{O}, 44.56^{O} and 72.25^{O} with Bragg reflection corresponding to (111), (200) and (220) were observed in biomass free supernatant of SSG-5 and peaks at 2θ angles: 41.78^{O}, 48.84^{O}, 62.44^{O} and 72.58^{O} with Bragg reflection corresponding to (111), (200), (220) and (311) were observed in XRD pattern of SSG-11 (Figure 4). Extracellular nano-particles having crystallite size of 25.05 nm and 73.21 nm were synthesized by strain SSG-5 and SSG-11 respectively.

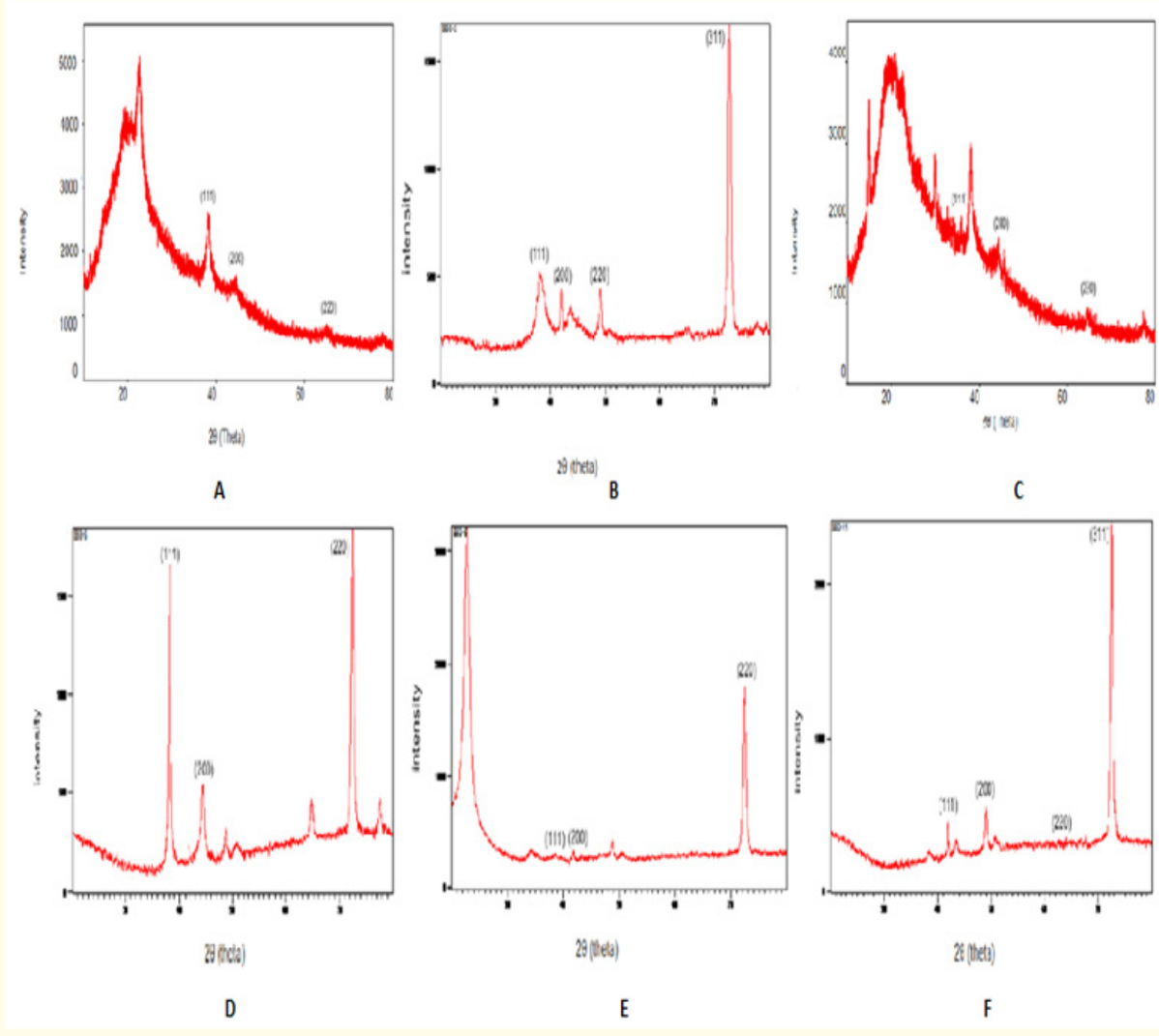

Figure 4: XRD analysis of intracellular and extracellular Nano-particles: (A) SSG-2 (intracellular) (B) SSG-2 (extracellular) (C) SSG-8 (intracellular) (D) SSG-8 (extracellular) (E) SSG-5 (extracellular) (F) SSG-11 (extracellular).

UV Visible Analysis

Extracellular nano-particles synthesized by SSG-2, SSG-5, SSG-8 and SSG-11 strains were characterized by UV-Visible spectroscopy and absorption maxima was determined by analyzing the spectral scan between 200-800 nm. Metallic nano-particles have free electron abundance and these moves through conduction and valence band which is responsible for surface Plasmon resonance absorption band [28]. A characteristic surface plasmon resonance (SPR) band corresponding to SSG-2, SSG-5, SSG-8 and SSG-11 was observed in visible range at 557 nm, 563 nm, 594 nm, 556 nm respectively (Figure 5). The presence of broad plasmon resonance indicated the formation of gold nano-particles in the samples. A strong resonance band at 545 nm was observed for *Fusarium oxysporum* by Mukherjee and co-workers [20].

Fungal biomass (SSG-2)			Supernatant (SSG-2)		
Pos. [°2Th.]	**FWHM [°2Th.]**	**d-spacing [Å]**	**Pos. [°2Th.]**	**FWHM [°2Th.]**	**d-spacing [Å]**
38.11	1.09	2.360	37.8664	0.3346	2.37405
44.04	1.13	2.055	48.8809	0.1338	1.86176
64.7	2.3	1.440	67.3896	0.2676	1.38850
			72.4631	0.1338	1.30327
Fungal biomass (SSG-8)			**Supernatant (SSG-8)**		
Pos. [°2Th.]	**FWHM [°2Th.]**	**d-spacing [Å]**	**Pos. [°2Th.]**	**FWHM [°2Th.]**	**d-spacing [Å]**
38.22	0.76	2.3530	38.1974	0.2342	2.35619
44.41	1.40	2.038	44.3133	0.2342	2.04417
64.64	2.3	1.441	72.3027	0.3672	1.30577
Supernatant (SSG-5)			**Supernatant (SSG-11)**		
Pos. [°2Th.]	**FWHM [°2Th.]**	**d-spacing [Å]**	**Pos. [°2Th.]**	**FWHM [°2Th.]**	**d-spacing [Å]**
38.5635	0.3346	2.33466	41.7885	0.1171	2.16164
44.5662	0.2007	2.03315	48.8442	0.1506	1.86462
72.2538	0.1224	1.30653	62.4437	0.2007	1.48728
			72.5879	0.2040	1.30134

Table 2: Crystallographic characterization of biomass (SSG-2 and SSG-8) and supernatant (SSG-2, SSG-5, SSG-8 and SSG-11) associated gold Nano-particles.

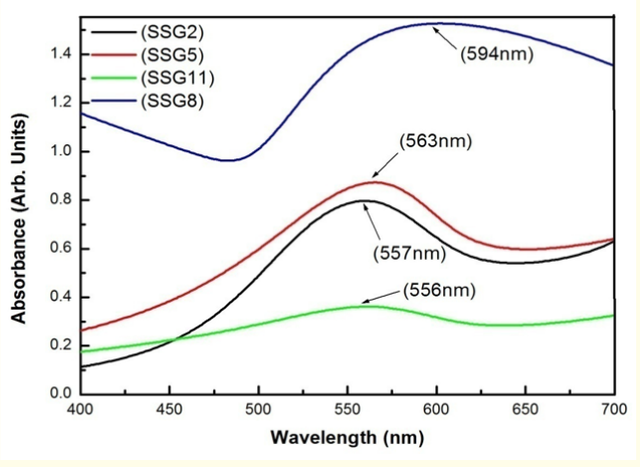

Figure 5: Spectral scan of strains SSG-2, SSG-5, SSG-8 and SSG-11 showing SPR at 557 nm, 563 nm, 594 nm and 556 nm respectively.

Antimicrobial activity

The antimicrobial activity of gold nano-particles was examined against human pathogens i.e. *E. coli, S. aureus, K. pneumoniae, Salmonella, Aspergillus* and *Pencillium* by agar well diffusion method. The synthesized gold nano-particles were found highly effective against both bacteria and fungi. A clear zone of inhibition of growth around wells was observed for both intracellular and extracellular nano-particles except SSG-8 extracellular nano-particles which were found ineffective against fungi. Among all, SSG-11 nano-particles were found quite inhibitory in nature except for *K. pneumoniae* and *S. aureus* where maximum inhibition was observed for SSG-8 (extracellular) and SSG-5 (intracellular) respectively (Figure 6). Although exact mechanism for antimicrobial action of nano-particles has not been well understood but different mechanisms have been proposed by several researchers. Gold nano-particles attach to the cell by electrostatic interaction and disrupt integrity by creating holes and changing membrane potential. Nano-particles also bind with DNA and inhibit binding of tRNA with ribosomal subunit thus affecting the transcriptional and translational events [29-31].

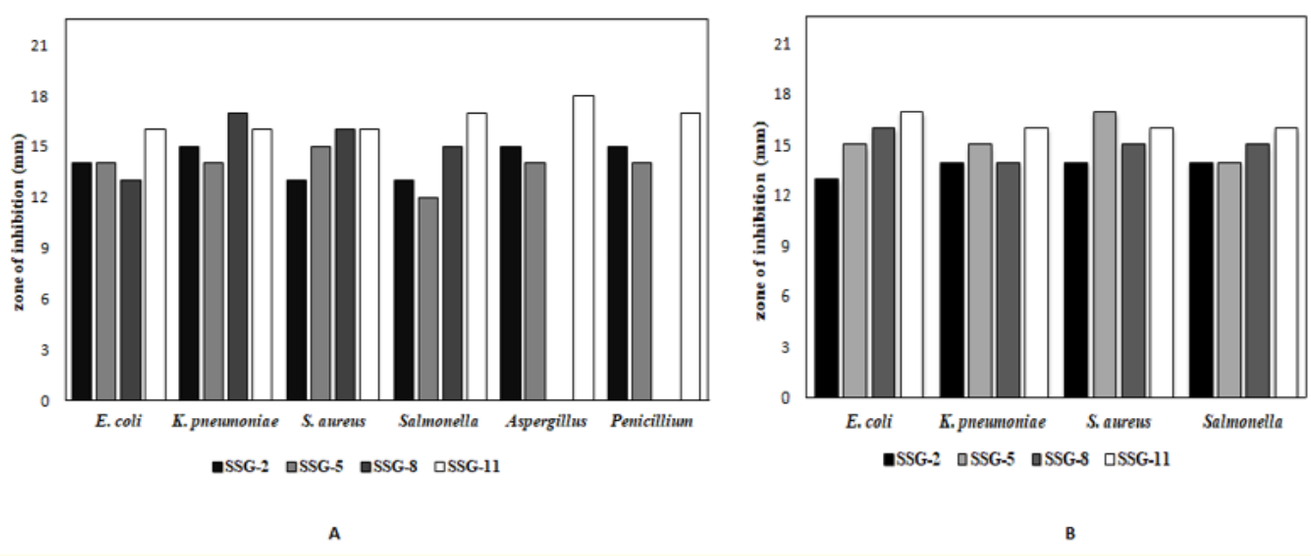

Figure 6: Bar graph showing zone of inhibition against human pathogens for extracellular (A) and intracellular (B) Nano-particles.

Conclusion

Nanotechnology is gaining increased interest as it finds vast application in various fields as biotechnology, pharmaceutical, biomedical, environment, food industry etc. A quite eco-friendly and rapid methodology has been developed by exploring fungal species for the biogenic synthesis of gold nano-particles. Further these nano-particles as antimicrobials can find immense applications in different fields like biomedical and pharmaceuticals, textiles, cosmetics and paints, food processing and packaging etc.

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