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BIOSYNTHESIS OF SILVER NANOPARTICLES USING LATEX OF *SYANDENIUM GRANTII* HOOK F AND ITS ASSESSMENT OF ANTIBACTERIAL ACTIVITIES

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We report hereby a green synthesis method employing latex of *Syandenium grantii* Hook f to biosynthesize silver nanoparticles in presence of sunlight irradiation. Silver nanoparticles with antibacterial potencies are of great interest in the biomedical field. Biogenic silver nanoparticles were biosynthesized by using latex of *S. grantii* and were characterized in Fourier Transform Infra Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM). In antibacterial studies, Kirby-Bauer disk diffusion susceptibility test was used to measure efficacy of biogenic silver nanoparticles against clinical isolates of Gram positive and Gram negative bacteria strains available from hospital like *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli* and *Pseudomonas aeruginosa* in terms of zone inhibition. Biogenic silver nanoparticles have possessing different desirable physicochemical properties owing in polydispersity, presence of biomolecules capping on the surface of silver nanoparticles and its particle size was found to be nanoscale below 50nm. Various peaks in FTIR denote the different functional groups present in the latex of *Syandenium grantii* acts as capping agent. From SEM result, it has shown the agglomerates of biogenic silver nanoparticles. TEM has displayed the different dimensional images of biogenic silver nanoparticles with particle size distribution ranging from 14-35 nm. Antibacterial activity was measured. Efficacy of biogenic silver nanoparticles against bacterial strains was found in ascending order of *P. aeruginosa* > *S. aureus* > *E. coli* > *S. pyogens*. A green approach for development of silver nanoparticles using latex of *S. grantii* appears an ease, economic, ecofriendly and expressive biosynthesis with aid of sunlight irradiation.

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1. Introduction

Traditionally, chemical reduction is the most groundwork synthesis of metallic nanoparticles frequently but toxic chemicals are utilized to involve in most of the synthesis protocols. A green approach to synthesize metallic nanoparticles is environmental protective, ecofriendly, cost effective and simple method. This is called as "green chemistry". Novel methods of ideally synthesizing nanoparticles are thus being formed at neutral pH, ambient temperatures, low costs and environmentally friendly fashion.

Syandenium grantii Hook f is a medicinal plant which is popularly grown in the southern Brazil and belongs to family Euphorbiaceae. Its latex has been traditionally used to treat use tumours, peptic ulcers gastritis and inflammation [1, 2].Isolated phytochemicals such as friedelin, 3β-friedelinol, euphol and lanosterol [2] were found in *S. grantii*. Latex of *S. grantii* showed more active cytotoxic to the mice with B16F10 melanoma cells than steroid citrostadienol while euphol is lacked of it [3]. Latex of *S. grantii*has been reported for its different pharmacological properties such irritant [4], antiangiogenic [5], immunomodulatory efficacy of lectin [6], fibrinolytic

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associated with glycoprotein [7]. Tigliane, senadenol, phorbol type diterpenoids [8, 9], triterpenoids [10] and anthocyanins [11] have been isolated from *S. grantii*. In our previous studies, the lectin was isolated from *S. grantii* and was used to differentiate erythrocyte agglutination pattern [12] and to detect surface carbohydrates of RBC [13] in normal and cancer patients.

We report hereby a green synthesis method employing latex of *Syandenium grantii* Hook to biosynthesize silver nanoparticles with the aid of bright sunlight irradiation. Biogenic silver nanoparticles with antibacterial are of great interest in the biomedical field.

2. Experimental

2.1 Latex extraction from *Syandenium grantii*

The plant of *S. grantii* was available in the local area of Malkapur, Karad as shown in Fig.1.

The latex was extracted through slit in the stem bark of *S. grantii* longitudinally; the pure latex was collected in a white plastic bottle which was to be protected from light. The extract was concentrated as a stock extract and stored in a refrigerator (4°C) until use. To prepare the diluted latex as a working solution, 1ml of latex was dispersed in 9ml of distilled water and then it was filtered.

Fig.1: The plant of Syandenium grantii

2.2 Green synthesis

2ml of diluted latex of *S. grantii* was transferred to 48ml of 0.1M silver nitrate solution in a measuring cylinder and stirred the reaction mixture with a glass of rod. The reaction mixture in measuring cylinder was exposed to sunlight and a change in colour was observed from pale white to reddish brown within a few minutes. Biogenic silver nanoparticles were centrifuged to remove any excess of extract and unreduced silver ions solution and stored in a suitable container.

2.3 Physicochemical characterization

Silver nanoparticles were biosynthesized by using latex of *S.grantii* and were characterized in Fourier Transform Infra Red Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Transmission Electron Microscopy (TEM).

2.3.1 Fourier Transform Infra Red spectroscopy (FTIR)

FTIR analysis of the dried silver nanoparticles was carried out through using Nicklet 380 Thermo, US Fourier Transform Infrared Spectrometer.

2.3.2 Scanning Electron Microscopy (SEM)

Each of the colloidal solution containing biogenic silver nanoparticles was centrifuged at 4,000 rpm for 15 min and the pellets were discarded and the supernatants were again centrifuged at 5,000 rpm for 30 min. Supernatants were discarded and the final pellets were dissolved in 0.1 ml of deionized water. The pellet was carefully placed on a glass cover slip followed by air-drying. The cover slip itself was screened under scanning electron microscopy (SEM) analysis.

2.3.3 Transmission Electron Microscopy (TEM)

The size and of the biogenic synthesized silver nanoparticles were recorded by using transmission electron microscope (Model: Philips CM200). The sample was dropped onto carbon-coated copper TEM grids.

2.4 Antibacterial activity

In antibacterial studies, standard agar well diffusion method as described in Clinical and Laboratory Institute [14] was used to measure efficacy of biogenic silver nanoparticles against clinical isolates of Gram positive and Gram negative bacteria strains available from Krishna hospital, Karad like *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli* and *Pseudomonas aeruginosa* in terms of zone inhibition.

The pure cultures of bacteria were sub-cultured on Mueller Hinton Agar. Each strain was swabbed uniformly onto the individual plates using sterile cotton swabs. Wells of 8 mm diameter were made on nutrient agar plates using sterile micropipette tips. Using a micropipette, 20µl of nanoparticles solution was poured onto each well on all plates. After incubation at 37°C for 24 hrs, the diameter of zone inhibition was measured in millimeter, and was recorded as mean ± SD.

3. Results and Discussion

3.1. Fourier Transform Infra Red spectroscopy (FTIR)

In Fig. 2, FTIR spectrum of phytosynthesized silver nanoparticles showed the strong three IR bands and a weak band.IR absorption bands (3428.81cm^{-1}), and (2075.69 cm^{-1}) are strongly related to the amide-I linkage. Absorption peak (1637.15cm^{-1}) in the infrared region of the electromagnetic spectrum exhibits the binding of amide linkage with silver nanoparticles which may be assigned to the carbonyl stretch in proteins and clearly indicates the presence of protein as capping agent for silver nanoparticles. Proteins have stronger affinity to bind silver nanoparticles which increases the stability of synthesized nanoparticles [15]. Various peaks in FTIR denote the different functional groups present in the latex of *S. grantii* acts as capping agent.

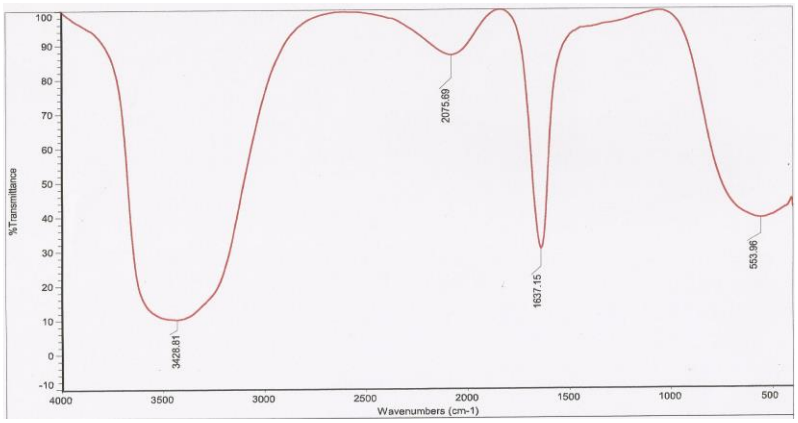

Fig. 2: FTIR image of biogenic silver nanoparticles

3.2. Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM)

Biogenic silver nanoparticles as polycrystalline structure were revealed as shown in Fig.3. From SEM result, it has shown the agglomerates of biogenic silver nanoparticles. It was shown that relatively spherical and uniform AgNPs were formed with diameter of 106.67 to 147.27 nm. Due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the silver nanoparticles appear larger as a result of the aggregation of the smaller ones.

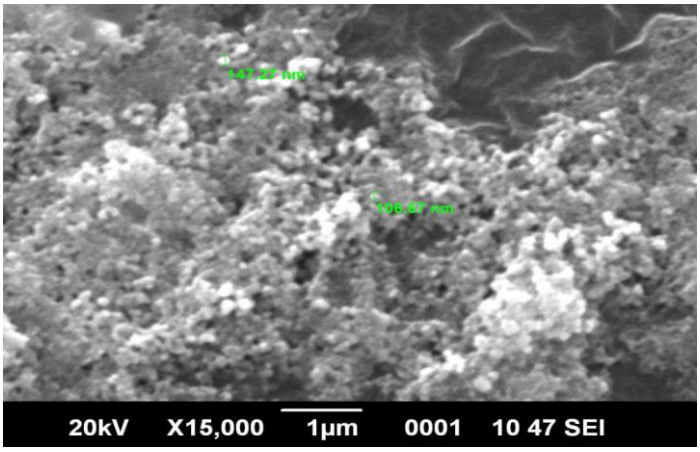

Fig. 3: SEM image of biogenic silver nanoparticles

3.3. Transmission Electron Microscopy (TEM)

Morphology of biogenic silver nanoparticles of *S. grantii* was investigated by TEM in Fig 4. TEM has displayed the different dimension of images of biogenic silver nanoparticles with particle size distribution spherical triangle, truncated triangles, and decahedral morphologies in range of 14-35 nm.

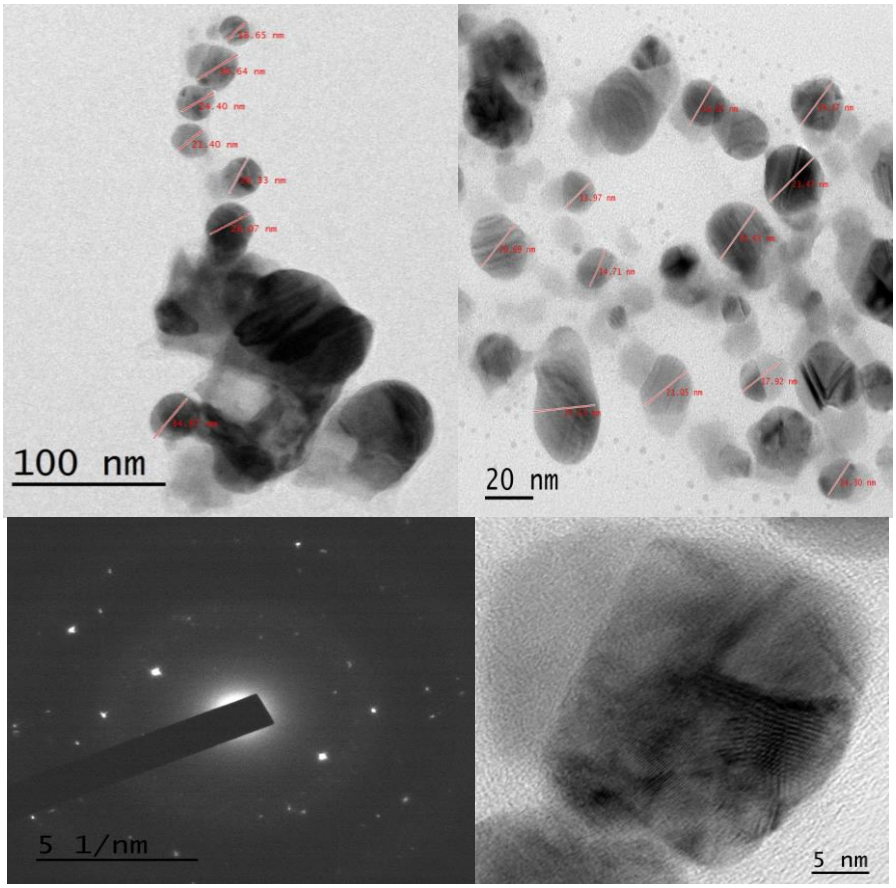

Fig. 4: TEM image of biogenic silver nanoparticles

3.4 Antibacterial activity

50µl of biogenic silver nanoparticles was placed in the agar well diffusion. After incubation of 24hours it was observed the zone of inhibition in the culture media. Antibacterial activity was measured the efficacy of biogenic silver nanoparticles against bacterial strains was found in ascending order of *P. aeruginosa*>*S. aureus* >*E. coli*>*S. pyogens* as shown in following Table 1 and Fig. 5.

Table 1: Antibacterial activities of biogenic silver nanoparticles by S. grantii

Bacterial strains	**ZI (mm)**	**ZI (mm)**	**ZI (mm)**	**ZI (mm)**	**Mean ± SD**
E. coli	13	13	14	14	13.5 ± 0.57
P. aeruginosa	22	23	22	23	22.5 ± 0.57
S. aureus	17	15	17	16	16.25 ± 0.95
S. pyogens	12	12	13	13	12.5 ± 0.57

ZI-Zone of Inhibition, E .coli - Escherichia coli, S. aureus - Staphylococcus aureus, S. pyogens- Streptococcus pyogens, P. aeruginosa - Pseudomonas aeruginosa

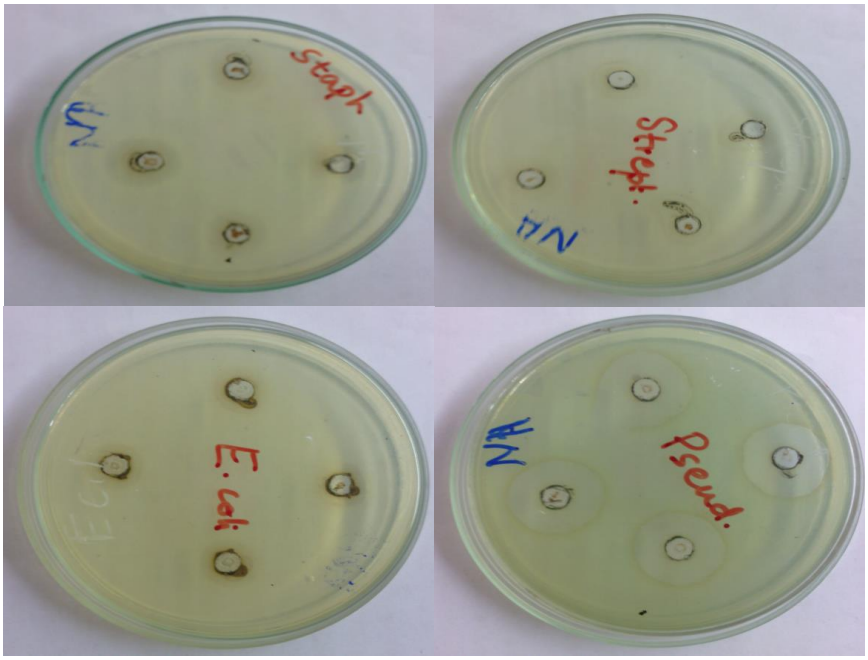

Fig. 5: Antibacterial activities of biogenic silver nanoparticles

Generally Gram negative pathogens are more vulnerable to biogenic silver nanoparticles than that of Gram positive pathogens. Due to physiological difference in their cell wall membranes, Gram positive bacteria strains showed more resistance perhaps due to their pepticoglycan layer compared with Gram negative bacteria strains having only external lipopolysaccharide structure [16].

Some researchers have reported that the positive charge on the silver nanoparticles is responsible for its antimicrobial activity through the electrostatic attraction to negative charged cell membrane of microorganism [17]. However, results in our previous study reported that there is neither positive nor negative charge on silver nanoparticles through atomic absorption spectroscopy in our previous study. It indicates that silver nanoparticles have zero valence which was reduced from Ag^{+} to Ag^{0} in the biosynthesis reaction. Therefore our previous study [18] is not in agreement with the possible mechanism of antimicrobial activity due to presence of positive charge on silver nanoparticles. Therefore the exact mechanism of the inhibitory effects of silver ions on microorganisms is yet to be known and need to be explored in further research studies. Perhaps we may have the same opinion for the accumulated silver nanoparticles in the bacterial membrane leading to increase the permeability, resulting in cell death due to leakage [16].

But surprisingly, it is observed here that biogenic silver nanoparticles of *S. grantii* have shown effective against *S. aureus,* Gram positive pathogen which was isolated from clinical samples. Perhaps, biogenic silver nanoparticles may contain some potent antimicrobial phytochemicals of *S. grantii* responsible to be effective against *S. aureus.* From these results, it is interestingly to propose in the further studies that biogenic silver nanoparticles of *S. grantii* may work effectively against multidrug resistant *Staphylococcus aureus*.

4. Conclusion

Biogenic silver nanoparticles possessing different desired physicochemical properties owing polydispersity, capping of biomolecules on the surface of silver nanoparticles and particle size was found to be nanoscale below 50nm. A green approach for development of silver nanoparticles using latex of *Syandenium grantii* appears an ease, economic, ecofriendly and expressive biosynthesis with aid of sunlight irradiation.

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