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# Green Synthesis of Silver nanoparticles from *Solanum torvum* and its Antibacterial Potential

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**Abstract:- A rising demand for nanoparticles have urged due to their various applications in Electronics, Chemistry, Energy and Medicine. Traditionally metallic nanoparticles are synthesized by wet chemical methods; however these methods have drawbacks such as use of toxic solvents and hazardous byproducts. Present investigation deals with a cost effective and eco-friendly method for green synthesis of Silver nanoparticles from 0.5 M $AgNO_3$ solution, using leaf extract of *Solanum torvum* as a reducing, as well as capping agent. Synthesized nanoparticles were characterized using UV-Visible spectroscopy, X-ray diffraction (XRD) and SEM. Further these biosynthesized nanoparticles were tested and proven to have remarkable antimicrobial activity against bacterial strains.**

***Keywords:-*** *Green synthesis, $AgNO_3$, XRD, SEM, Solanum torvum.*

## I. INTRODUCTION

Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic, as well as molecular level (Thakkar *et al.,* 2010). Nanotechnology is perhaps helping to probably improve, even revolutionize different technologies including Information Technology, Aerospace Engineering, energy, medicine, food industry, therapeutics and Environmental Science. A broad range of diversified nanoparticles comprising liposome (Hofheinz *et al*., 2005), stealth liposome (Moghimi and Szebeni, 2003), emulsions (Sarkar, 2005), polymers (Agnihotri *et al*., 2004), ceramic nanoparticles (Cherian *et al.,* 2000), metallic nanoparticles (Gupta and Gupta 2005), gold nanoparticles (Hirsch *et al*., 2006), carbon nanomaterials including fullerenes and nanotubes (Bosi *et al*., 2003; Pagona and Tagmatarchis, 2006) and quantum dots (Weng and Ren, 2006) are used for different purposes including biomedical imaging (Singh *et al.,* 2010), therapeutic drug delivery, treatment of burned patients, targeted drug delivery (Medina *et al*., 2007), endocytic capture (Lee, 2006), used in spectral selective coatings for solar energy absorption, biolabelling antimicrobial agents and electrical batteries (Zhang *et al*., 2008; Jeong *et al*., 2005; Savithramma *et al*., 2011; Saxena *et al*., 2010; Schultz *et al*., 2000; Vijayaraghavan *et al*., 2010; Crooks *et al*., 2001).

The much diversified research has been indulged to synthesize nanomaterials from a wide array of metals including sono-chemicals, electrochemical and microwave associated processes. They can be synthesized by different methods including chemical, physical and irradiation methods (Ram Prasad and Swamy, 2013). The chemical procedures involved in synthesis of nanomaterials generate a large amount of waste products and release environmental contaminants (Zhang *et al.,* 2013). In addition, chemically synthesized nanoparticles are toxic and potentially hazardous in concern with their biological application (Ankamwar *et al.,* 2005). They are reported to cause cardiovascular dysfunctions (Brook *et al*., 2004), induce platelet aggregation (Radomski *et al.*, 2005), induce the pro-inflammatory responses, inhibition of cell growth (Yamawaki and Iwai, 2006), also reported to show adverse effects and heavy injuries in kidney, liver and spleen (Chen *et al.*,2006).

To circumvent the difficulties encountered due to chemically synthesized nanoparticles, biological routes for synthesis have been broadly established. Biological synthesis of nanoparticles have been reported by using bacteria (Klimuthu *et al*., 2008; Nanda and Sarvanan, 2006), fungi (Bhainsa and D'Souza 2006; Vigneshwaran *et al.,* 2006; Siavash, 2011; Basavraja *et al*., 2008) and plants (Chandran *et al*., 2006; Huang *et al.* 2007 and Kavitha, 2013). The preparation and maintenance of fungal and bacterial cultures are time consuming and require aseptic conditions and also require large manual skill (Schultz *et al*., 2000). In this context, it is noteworthy to mention that green synthesis of nanoparticles provides advancement over other chemical methods as it is simple, cost effective and often results in more stable nanoparticles.

Synthesis using bio-organisms, especially plants that secrete the functional molecules for the reaction, is compatible with the green chemistry principles that pre-require the bio-organism to be eco-friendly, reducing agent and capping agent in the reaction. Different metals have been incorporated (Table 1) for the preparation of nanoparticles including Gold (Singaravelu *et al*., 2007; Ghodake *et al*., 2010), Paladium (Yang *et al.*, 2010), Lead (Jogalekar *et al.,* 2011), and Platinum (Song *et al.,* 2010). Variety of research has been carried out on biosynthesis of silver nanoparticles using plant leaves extract such as *Ficus benghalensis* (Saxena *et al.*, 2012), *Polyalthia longifolia (*Kaviya *et al.,* 2011), *Nicotiana tabacum* (Prasad *et al*., 2010), *Parthenium hysterophorus* (Sarkar *et al*., 2010) and *Pongamia pinnata* ( Panda *et al*., 2011).

*Solanum torvum* Sw. (Solanaceae), commonly known as Turkey berry is native and cultivated in Africa and West Indies (Adjanohoun *et al*., 1996). The fruits and leaves are widely used in Camerooninan folk medicine (Jaiswal, 2012). A decoction of fruits is given for cough ailments and is considered useful in cases of liver and spleen enlargement (Siemonsma, 1994). The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in

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the preparation of tonic and haemopoietic agents and also for the treatment of pain (Kala *et al.*, 2005).

The phytochemical screening of *S. torvum* shows active principles like isoquercetin (Lida *et al*., 2005), rutin (Lu *et al.*, 2009), kaempferol (Lu *et al.*,2009), quercetin (Gonzalez *et al*., 2004), neochlorogenin 6-O-β-D-quinovo-pyranoside (Zhu *et al*., 2003) and solagenin D-quinovopyranoside (Yahara *et al.*, 1996). Phytochemical screening of methanolic extract of sun dried *S. torvum* fruits gave positive tests for alkaloids, flavonoids, saponins, tannins, glycosides, fixed oil, vitamin B group, vitamin C and iron salts (Sivapriya *et al*., 2007 ; George *et al.*, 2011).

A potent scavenger of free radicals may serve as a possible preventive intervention for many diseases as the involvement of free radicals in the pathogenesis of a large number of diseases is well known (Waghulde *et al.*, 2011; Gyamfi *et al*., 1999). *S. torvum* was found to be a very potent antioxidant. It also exhibited outstanding reducing power, scavenging activity against DPPH and hydrogen peroxide (Jaiswal, 2012).

Methalonic and ethalonic extracts of *S. torvum* showed activity against *Xanthomonas campestris* pv *oryzae* (Lalitha *et al*., 2010), *Staphylococcus* and *Pseudomonas* (Govindaraju *et al*., 2010). Aqueous and solvent extracts of *S. torvum* revealed a significant inhibitory activity against *Fusarium oxysporum, F. solani, F. moniliformae, Pyricularia oryzae, Alternaria alternata* (Jaiswal, 2012), *Aspergillus niger* and *A. flavus* (Govindaraju *et al.*, 2010). Present investigation proposes a simple method for the extracellular biosynthesis of silver nanoparticles using *S. torvum* and demonstrate their capabilities as an alternative pathway to chemical and physical synthesis methods. In the present research work, preferably leaf extract of *S. torvum*, rather than decanted solution has been used as a reducing agent as well as capping agent.

## II. MATERIALS AND METHODS

➢ *Preparation of plant extract*

*S. torvum* leaves were collected from locality Kolhapur (India). Fresh leaves were brought to laboratory, washed thoroughly with running tap water to remove debris on it. It was followed by rinsing with distilled water and blotted to dry. Leaf mass weighing 40 grams was taken in a clean mortar pestle, to which 100 ml deionised water was added. The leaves were crushed and homogenate was filtered through non absorbent cotton. The filtrate was collected and used as plant extract for further experimentation, till which it was stored in refrigerator at $4^{\circ}$C.

➢ *Synthesis of Silver nanoparticles*

Silver nitrate ($AgNO_3$) analytical grade was purchased from Sigma-Aldrich Chemical Pvt. Ltd. and 100 ml of 0.05 M $AgNO_3$ solution was prepared. 40 ml of this solution was decanted in a clean grease free 500 ml beaker. The plant extract was added to $AgNO_3$ solution drop by drop with the help of burette at room temperature with constant steering on magnetic stirrer.

As soon as the plant extract was added to $AgNO_3$ solution, brown colored precipitation was observed in the solution, which was an indication of formation of Ag nanoparticles. After complete addition of 40 ml of plant extract, a sufficient amount of precipitate was observed and it was removed by high speed centrifugation (8000 rpm). The supernatant was discarded and the sediment was passed through Whatman filter paper No. 1 (pore size 25 µm). The residual precipitate was washed with alcohol for several times till the alcohol soluble impurities were removed. After complete washing, the solid mass was dried in microwave oven. The resultant solid mass was black in color, which was powdered in mortar and sampled for characterization.

➢ *Antibacterial Assay*

The bacterial cultures were sub cultured on liquid nutrient broth and they were incubated at 37°C for 24 hours in incubator with continuous stirring. The characterized nanoparticles were used for determination of antimicrobial potential by Microtitre Broth Dilution method on spectrophotometer against pathogenic bacteria *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* and *Bacillus subtilis*. The antibacterial activity of silver nanoparticles was measured by calculating percent inhibition and minimum inhibitory concentration (MIC).

## III. RESULT AND DISCUSSION

➢ *UV visible spectroscopy*

The black colored powder obtained was dissolved in deionised water and was sonicated for ten minutes. The absorbance was recorded by exposing the solution to UV visible radiation (400-450 nm) in a quartz cuvette. The absorption maxima were observed at 421 nm (Fig.1), which confirms the actual presence of silver nanoparticles in the solution.

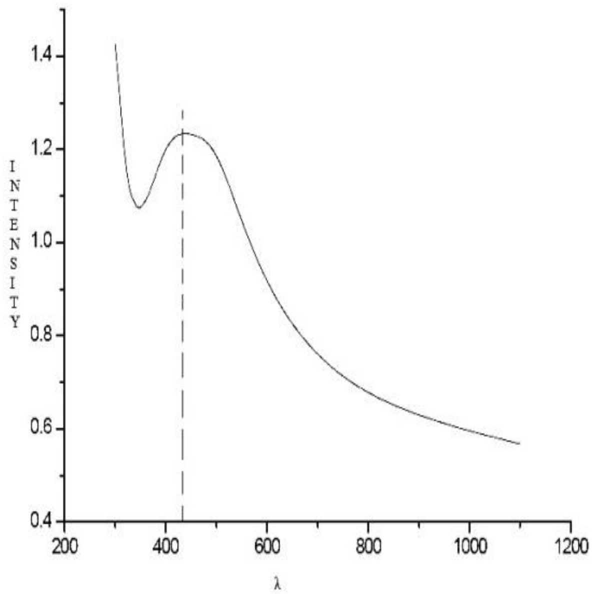


Fig 1. Absorption spectrum of synthesized nanoparticles.

➢ *XRD*

Synthesized Ag nanoparticles were characterized using Cu Kα radiation of wavelength 1.54056 A° for structural analysis and to identify crystalline structure. The XRD data was matched with JC PDS file for confirmation of formation of Silver nanoparticles.

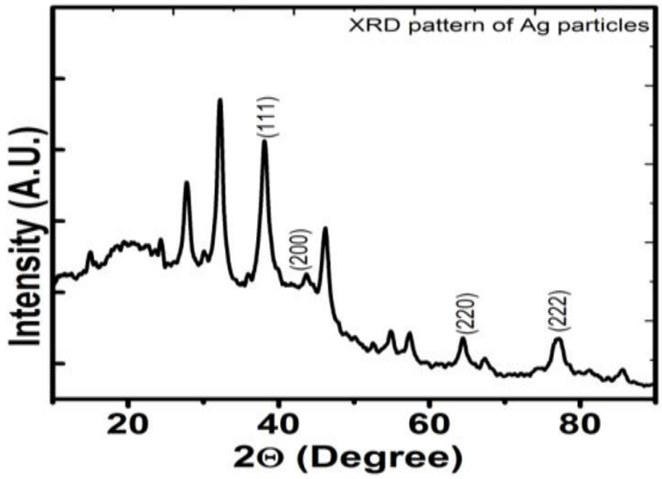


Fig.2 XRD pattern of Ag NP's synthesized from *S. torvum* leaf extract with 0.05 M aqueous $AgNO_3$ solution.

The X-ray diffraction pattern shows nanocrystalline nature. It shows that the synthesized particles are having cubic structure. Majority of the nanostructures were preferentially oriented along (111), (200), (220) planes with Bragg's reflection of 2Θ values 38.10° and 44.37°. Another less intense peaks were observed along plane (220) and (222) with Bragg's reflection having 2Θ values of 64.18° and 77.56° respectively. The observed d values of samples (from XRD pattern) correlate and are in well agreement with JC-PDS file no 00-00-11167. Thus the XRD pattern gives confirmation that silver nanoparticles were formed. Taking into account the angular position of the Bragg's peaks (Fig. 2), a face centered cubic structure was assigned to the silver nanoparticles.

➢ *SEM*

Morphological attributes of surface were studied using SEM. The SEM images were taken from VEGA3TESCAN (Department of Botany, Shivaji University, Kolhapur) having accelerating voltage 10 KV. The VEGA3TESCAN analysis gives the pixel depth of image 16 bits and image resolution of 512×572. Characterization for SEM was done by VEGA3TESCAN using accelerating voltage of 10 KV. The SEM image (Fig. 3) reveals a non-uniform pattern of polydisperse particles of oval shapes. Agglomeration of nanoparticles were seen in the SEM micrograph. The VEGA3TESCAN analysis gives the pixel depth of image 16 bits. The image magnification was 100X. From scanning electron microscope we obtained the average size of silver nanoparticles 40- 60 nm.

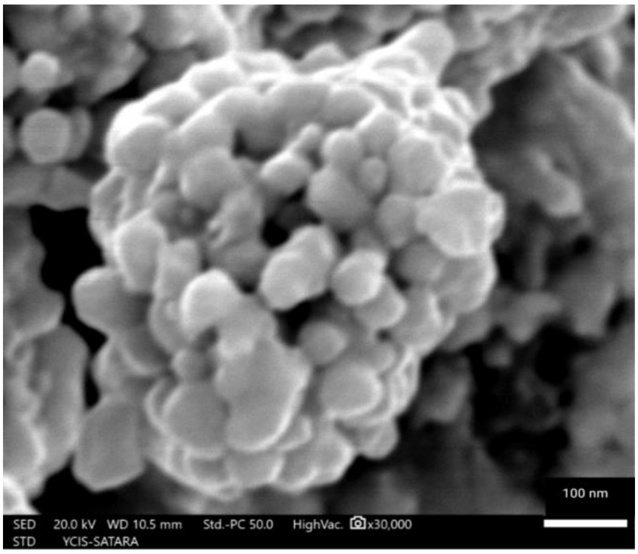


Fig. 3 SEM micrograph of synthesized Ag nanoparticles

➢ *Antimicrobial assay*

The antibacterial activity against *Pseudomonas aeruginosa* [ATCC 2021], *Klebsiella pneumonia* [ATCC 2075], *Escherichia coli* [ATCC 2065] and *Bacillus subtilis* [ATCC 2063] was tested. The MIC values were calculated by Microtitre Broth Dilution method. *Solanum torvum* based Ag NP's were used as samples for studying antimicrobial activity. To calculate the inhibition activity of synthesized nanoparticles on selected pathogenic bacterial cultures of different concentrations of Ag NPs were taken from mg/ml stock solution of Silver nanoparticles. Bacterial cultures were maintained in liquid nutrient broth. Antibacterial assay was carried out in 96-well microtitre plate. Nutrient broth was taken as positive control and antibiotic Streptomycin was taken as negative control. For the observation of antibacterial activity of Ag NP's on *Pseudomonas aeruginosa* 260 µl nutrient broth was taken along with 20 µl bacteria and 20 µl of Ag NP's solutions with different concentrations like 100 µl, 75 µl, 50 µl and 25 µl in each well for assay. The 96-well microtitre plate was incubated for 12 hours with time interval of 30 minutes in MultiscanSky_1530-00496C spectrophotometer and results were observed. Similar procedure was followed for streptomycin. By comparing the growth curve of control and other concentrations of silver nanoparticles the MIC was observed and reported for each bacteria. For *Klebsiella pneumonia*, *Escherichia coli* and *Bacillus subtilis* similar procedure was followed.

The lowest concentration which inhibits the growth of bacterial strain is taken as MIC of the Ag NP's for the pathogenic bacteria. When silver nanoparticles were tested for antimicrobial activity, *Pseudomonas aeruginosa* showed MIC value of (25 µg/ml), *Klebsiella pneumoniae* showed (25 µg/ ml), *Escherichia coli* showed (25 µg/ ml) and *Bacillus subtilis* (25 µg/ml). (Table 1).

In case of *Pseudomonas aeruginosa* using Streptomycin shows highest percentage inhibition 54% at 100 µl/ml and lowest inhibition percentage 40% at 25 µl/ml. In *Klebsiella pneumonia* Streptomycin shows highest inhibition percentage 58% at 100 µl/ml and lowest inhibition percentage 50% at 25 µl/ml. For *Bacillus subtilis* streptomycin shows the highest inhibition percentage 55% at 100 µl/ml and lowest inhibition percentage 42% at 25 µl/ml. While, in case of *Escherichia coli* antibiotic streptomycin shows highest inhibition percentage 56% at 100 µl/ml and lowest inhibition percentage 45% at 25 µl/ml. (Fig. 4).

The highest percentage inhibition observed for *Pseudomonas aeruginosa* using Ag NP's was 40% at 100 µl/ml and lowest was 30% at 25 µl/ml. In case of *Klebsiella pneumonia* highest percentage inhibition recorded was 45% at 100 µl/ml and lowest was 35% at 25 µl/ml concentration of Ag NP's. For *Bacillus subtilis* highest percent inhibition recorded was 48% at 100 µl/ml and lowest was recorded 35% at 25 µl/ml concentration of Ag NP's. While, highest percentage inhibition observed for *Escherichia coli* was 46% at 100 µl/ml and lowest was 38% at 25 µl/ml of Ag NP's. (Fig. 5).

**Table. 1 MIC Values of Silver nanoparticles with Streptomycin.**

| Sr. No. | Bateria | MIC values µg/ml | MIC values µg/ml |
|---|---|---|---|
| | | Ag NP's | Streptomycin |
| 1. | *Pseudomonas aeruginosa* | 25 | 100 |
| 2. | *Klebsiella pneumonia* | 25 | 50 |
| 3. | *Escherichia coli* | 25 | 100 |
| 4. | *Bacillus subtilis* | 25 | 100 |

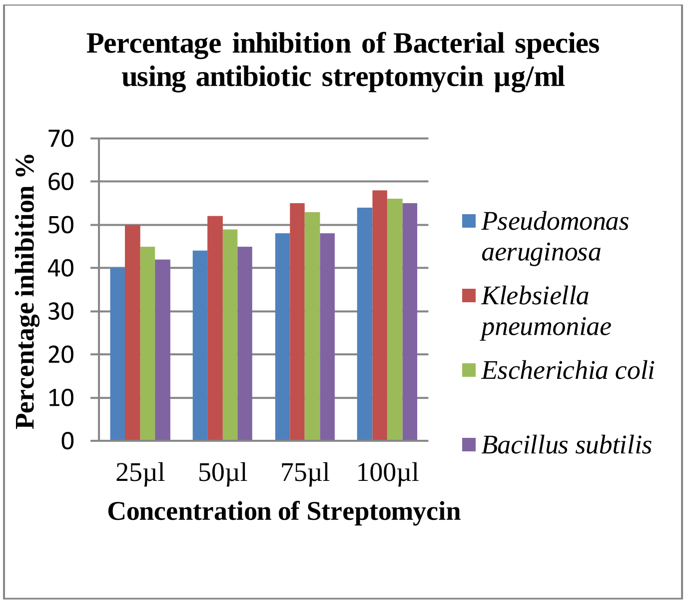


Fig 4. Percentage inhibition of Bacterial species using antibiotic streptomycin

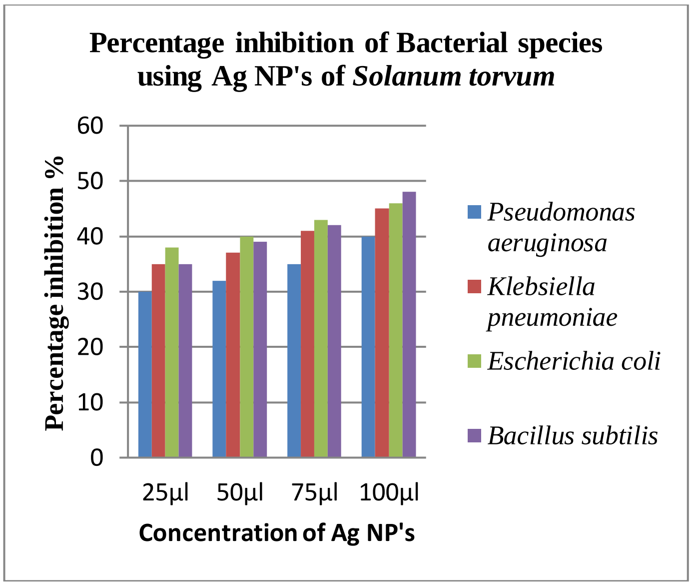


Fig 5. Percentage inhibition of Bacterial species using Ag NP's of *Solanum torvum*

- *Possible interaction between microbes and Silver nanoparticles*

Reactive metal oxide nanoparticles show excellent bactericidal effects (Stoimenov *et al.*, 2002). Thus investigating the use of other inorganic nanoparticles may help in knowing them as antibacterial materials. Very less information is known about the biocidal effect of noble metal particles (Sondi and Sondi, 2004).

Moreover, the antibacterial activity of Silver ions is less known (Slawson *et al.*, 1992; Zhao and Stevens, 1998; Spadaro *et al.*, 1974). Silver ion is highly toxic to most microorganisms (Jung *et al.*, 2008). While the antibacterial activity of elemental non toxic Silver in the form of nanoparticles have been reported by Sondi and Sondi (2004).

However, the actual mechanism of inhibitory action of Silver on bacteria is partially known. From the report of Feng *et al.* (2000), inactivation of cellular protein as well as inability of DNA to replicate are correlated with $Ag^+$ treatment. It was also found that $Ag^+$ binds directly to the functional group of protein resulting into denaturation of protein (Chaloupka *et al.*, 2010; Spadaro *et al.*, 1974). Nazeruddin *et al* (2014) states that as Silver is a soft acid and possess natural tendancy to react with the base, the resulting salt from the reaction leads to problems in DNA replication of bacteria leading to their death. Another report showed that the antimicrobial action of nanoparticles may be through a slow release of Silver ions via oxidation within or outside the cell (Mittal *et al.*, 2013). Additionally Silver nanoparticles affect the permeability of microbial cell membrane (Li *et al.*, 2010).

*E. coli* treated with highly reactive metal oxide nanoparticles exhibited a significant increase in permeability of cell membrane leaving the bacterial cell incapable of proper regulation and transport through plasma membrane, ultimately causing cell death (Stoimenov *et. al.*, 2002).

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Some reports in literature show electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles, which supports the fact that, nanoparticles serve as best bactericidal agents (Stoimenov *et al*., 2002; Hamounda and Baker, 2000). Significant changes were observed related to damage of bacterial membrane by forming pits on their surface. This was reported by Sondi and Sondi (2004) when they treated *E. coli* with Silver nanoparticles. The outer membrane of *E.coli* is predominantly composed of lipopolysaccharides which are tightly packed (Nikaido and Vaara, 1985). Metal depletion may cause irregular pits in outer membrane, thus altering the membrane permeability resulting into release of lipopolysaccharide and membrane proteins (Amro *et al*., 2000). It may thus be postulated that in *E. coli* same mechanism causes the degradation of cell membrane when treated with Ag nanoparticles.

## IV. CONCLUSIONS

Biological synthesis of nanoparticles using plant extracts for production of metallic nanoparticles is an economic and eco-friendly method. Additionally, it does not release any toxic byproducts and can be commercially scaled up. It can be further designed for treatment of various diseases in plants as well as animals. Thus biologically synthesized nanoparticles can serve as best substitutes for the over dose of antibiotics, chemical reducing and capping agents.

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