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Full Length Research Paper

Biosynthesis of silver nanoparticles using *Trigonella Foenum Graecum* and the determination of their antimicrobial activity

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Generally, nanoparticles are prepared by a variety of chemical methods which are not environmentally friendly. Use of plants in the synthesis of nanoparticles is quite novel leading to truly green chemistry which provided advancement over chemical and physical method as it is cost effective and environmentally-friendly. We have reported a fast, convenient and extracellular method for the synthesis of silver nanoparticles by reducing silver nitrate with the help of fenugreek seeds (*Trigonella Foenum Graecum*) extract. The characterization of nanoparticles was done by using UV- Vis Spectrophotometer. The morphology of silver nanoparticles was confirmed by Scanning Electron microscopy (SEM) and X Ray Diffraction Analysis (XRD). The antibacterial activity of these nanoparticles were studied against *E.coli, Streptococcus Pneumonia, Proteus Vulgaris, Pseudomonas Aeruginosa*and *Staphylococcus Aureus* comparing with the standard drug Ampicilin.

Keywords: Silver nanoparticles, biological synthesis, fenugreek seed extract, SEM, XRD, antibacterial activity, *E.coli, Streptococcus Pneumonia, Proteus Vulgaris, Pseudomonas Aeruginosa*, *Staphylococcus Aureus*, Ampicilin curve.

INTRODUCTION

Application of Nanoscale material and structures are usually ranging from 1-100nm and is an emerging area of nanoscience and nanotechnology. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms and have been studied extensively because of their unique physicochemical characteristics including catalytic activity, optical properties, electronic properties, antibacterial properties and magnetic properties (catauro et al., 2004; Crabetree et al., 2003; Krolikowska 2003; Zhao 1998). Synthesis of noble nanoparticles for applications such as catalysis, electronics, environmental and biotechnology is an area of constant interest (Hussain et al., 2003; Sharma Virendar, 2009). Generally, metal nanoparticles are synthesized and stabilized by using chemical methods such as chemical reduction (Balantrap Krishna et al., 2009; tripathi et al., 2010), electrochemical techniques (Rodriguez et al., 2000), photochemical reactions in reverse micelles (Taleb et al., 1997) and nowadays via green chemistry route (Begum Nazninara et al., 2009). Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective and environmentally-friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Nowadays we are using bacteria, fungi for the synthesis of nanoparticles (Lengkemaggy 2006; Binoj Nair, 2000; Shiying He et al., 2007; Holmes et al., 1995; Mukerjee et al., 2005; Ahamad et al., 2000; Kuber et al., 2006; et al., 2004; Gardea et al., 2002; to reduce the cost. We do not require any special culture preparation and isolation techniques.

A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route.

Here in, we report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell free aqueous extract of fenugreek seed. Further these biologically synthesized nanoparticles were found highly toxic against different multi-drug resistant and human pathogens.

Through elaborate screening process involving a number of plants, we observed that fenugreek (*Trigonella Foenum Graecum)* was a potential candidate for synthesis of silver nanoparticles. We also study the antibacterial property of silver nanoparticles against *E.coli, Streptococcus, Pneumonia, Proteus Vulgaris, Pseudomonas, Aeruginosa* and *Staphylococcus Aureus* comparing with the standard drug Ampicilin.

Fenugreek (*Trigonella Foenum-Graecum*) found in nature and is cultivated in India and Pakistan is a well known medicinal plant having properties of reducing blood sugar level, anthelmentic, antibacterial, anti-inflammatory, antipyretic, and antimicrobial. It contains lecithin and choline that helps to dissolve cholesterol and fatty substances, minerals, B. Complex, iron, Phosphates, PABA (Para-Amino Benzoic Acid), and vitamins A and D. It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system (Michael and Kumawat, 2003). The important chemical constituents are saponins, coumarin, fenugreekine, nicotinic acid, phytic acid, scopoletin and trigonelline.

MATERIALS AND METHODS

Plant materials and Preparation of seed extract

Seeds were collected from Choondal village, Thrissur district, Kerala. The seeds were initially washed thrice in distilled water and dried on paper toweling and sample (10g) ground into powder form. It was then boiled with 100ml of distilled water for 30 minutes. The extract was passed through Whatmann No.1 filter paper and the filtrates were kept 4^0C for further use.

Synthesis of Silver Nanoparticles

For synthesis of silver nanoparticles, the Erlenmeyer flask containing 1mM $AgNO_3$ was reacted with 10 ml of the aqueous extract of fenugreek seed. The set up was incubated for 72 hours in sunlight. A control set up was also maintained without fenugreek seed extract.

UV-VIS spectra Analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-VIS spectrum of each reaction mixtures at different time intervals within the range of 400-480nm in the UV-VIS spectrophotometer.

SEM analysis and XRD of silver Nanoparticles

The reaction mixtures prepared from the seeds were kept for 7 days at room temperature for stabilization and subsequently they were centrifuged at 2000 rpm for 1 hour and redispersed in distilled water. The procedure was repeated three times and remnant pellets were dried and powdered for SEM and XRD.

Antimicrobial Assay

Antimicrobial activities of the synthesized silver nanoparticles were determined using the agar well diffusion assay method. Approximately 20ml of molten and cooled media (Nutrient agar) was poured in sterilized Petri dishes. The plates were left overnight at room temperature to check for any contamination to appear. Agar wells of 5mm diameter were prepared with the help of sterilized stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labeled as A, B. ‘A’ well was loaded with Ag nanoparticles suspended ‘hydrosols’, ‘B’ well loaded with extract solution and a positive control drug (Ampicilin) was also added and marked ‘C’. The test organism should be added on the nutrient agar before the solutions added. The plates containing the bacterial and silver nanoparticles were incubated at 37^0C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells (Nascimento *et al.*, 2000). The diameter of such zones of inhibition was measured using a meter ruler and the value for each organism was recorded and expressed in millimeter. The test organisms used in the studies were *Streptococcus pneumoniae, Proteus vulgaris, Pseudomonas Aeruginosa, E.coli, Staphylococcus Aureus.*

RESULTS AND DISCUSSION

It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. As the fenugreek seed extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to light brown due to reduction of silver ion which indicated formation of silver nanoparticles. The

Table 1. Periodic colour change of the reaction mixture at different time intervals.

TIME/CONCENTRATION	1mM $AgNO_3$
0 Hr	+
1 Hr	++
2 Hrs	++
24 Hrs	+++
48 Hrs	++++
72 Hrs	++++

+ (light yellow), **++** (light brown), **+++** (brown), **++++** (dark brown)

Figure 1. Periodic colour change of the reaction mixture (fenugreek seed extract+ 1mM $AgNO_3$).

biosynthesized silver nanostructure by employing fenugreek seed extract was further demonstrated and confirmed by the characteristic peaks observed in the XRD image and the structural view under the scanning electron microscope.

Characterisation of Silver Nanoparticles

Characterization of nanoparticles is important to understand and control nanoparticles synthesis and applications. Characterization is performed using a variety of different techniques such as UV-VIS Spectrophotometry, scanning electron microscopy (SEM) and X-ray diffractometry (XRD). These techniques are used for determination of different parameters such as particle size, shape, crystallinity, and pore size. Fore instance, the morphology and particle size could be determined by SEM. Moreover X-ray diffraction is used for the determination of crystallinity.

UV-VIS Analysis

The figure 2 shows the UV-VIS absorption spectra recorded from the reaction from 0 hr to 5 hours. Absorption spectra of silver nanoparticles formed in the reaction media was increasing with time. This shows the reduction of silver ions to silver nanoparticles and the synthesis of nanoparticles.

SEM And XRD Analysis

Scanning Electron Microscopy (SEM) is one of the widely used technique for the characterization of the synthesized nanoparticles. The SEM image showing the

Figure 2. UV analysis of the reaction mixture (Trigonella Foenum Graecum seed extract with Silver nitrate solution) at different time intervals.

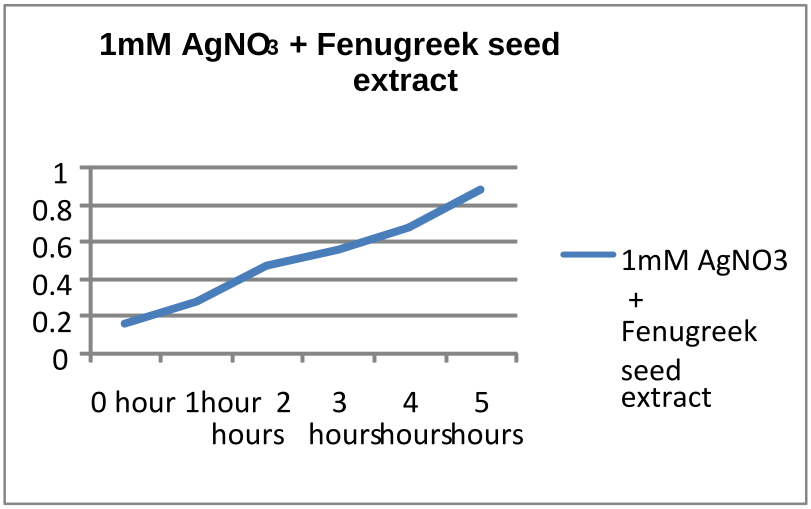
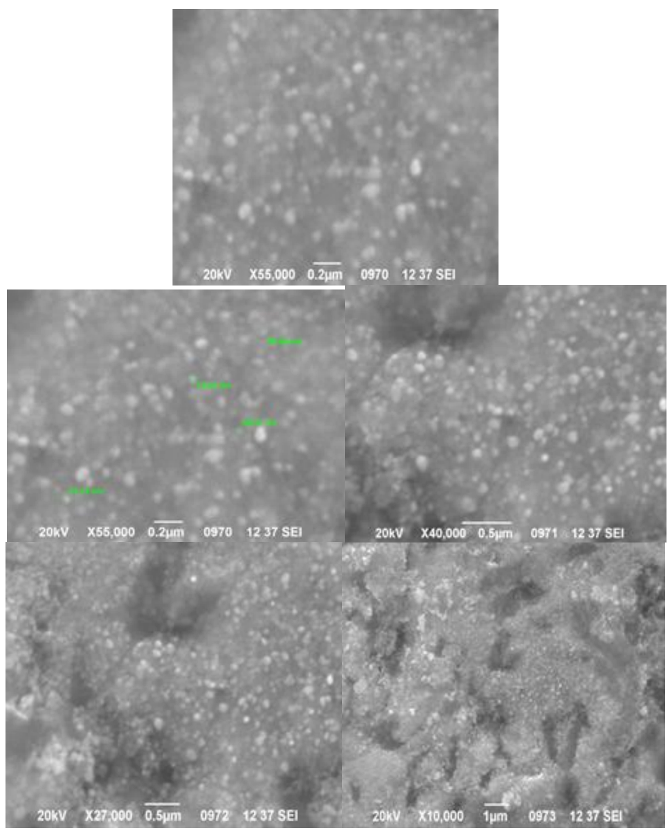


Figure 3. Photos of Scanning Electron Microscopic Analysis of the reaction mixture (Trigonella Foenum Graecum seed extract + 1mM $AgNO_3$).



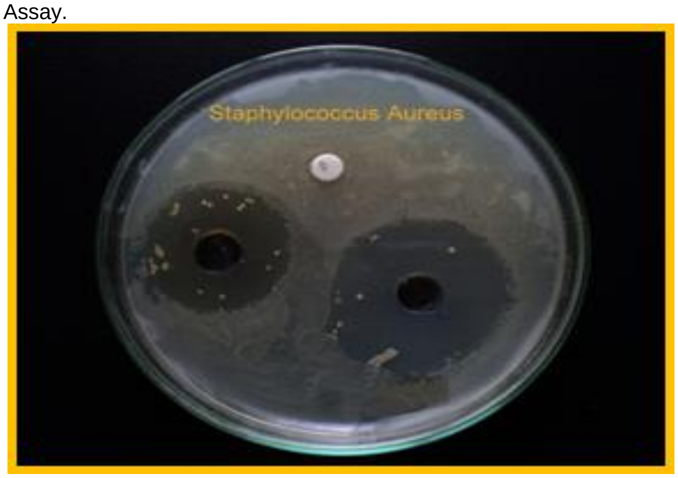
high density silver nanoparticles synthesized by Trigonella Foenum Graecum seed extract confirmed the development of silver nanostructure. Figure 3 shows the SEM analysis of the synthesized silver nanoparticles from

Table 2. Zone of inhibitory activity (in millimeter) of TrigonellaFoenumGraecum seed extract, silver nanoparticles and Standard drug (Ampicilin) against clinical and phytopathogenic bacteria.

Name of the test Organism	Zone of Inhibition(mm) of various Sample		
	STANDARD (Ampicilin)	EXTRACT (TrigonellaFoenum Graecum seed)	NANOPARTICLES (Silver) (1mM$AgNO_3$)
Streptococcus pneumoniae	-	15	17
Proteus vulgaris	-	10	12
Pseudomonas Aeruginosa	-	14	18
E.coli	-	-	-
Staphylococcus Aureus	4	9	11

'-' no activity

Figure 4. Photos of Inhibition zones obtained in the Anti Microbial Assay.



Trigonella Foenum Graecum seed extract. Average size of the particle synthesized were 48nm with size range 40 to 55nm

The biosynthesized silver nanostructure by employing Fenugreek seed extract was demonstrated and confirmed by the characteristic peaks observed in the XRD image. The XRD pattern showed three intense peaks in the whole spectrum of 2 value ranging from 25 to 50 theta

Antimicrobial assay

The silver nanoparticles synthesized via green route were highly toxic to multidrug resistant bacteria hence has a great potential in biomedical application. The antimicrobial activity of silver nanoparticles and pure extract against *E.coli, Staphylococcus Aureus, Streptococcus pneumoniae, Pseudomonas Aeruginosa, Proteus vulgaris* were investigated and compared with standard drug Ampicilin. The silver nanoparticles and extract of *Trigonella Foenum Graecum* has no activity against *E.coli*. the Standard Ampicilin has an activity only on *Staphylococcus*. The silver nanoparticles has a maximum activity against Pseudomonas where as extract has activity against *Streptococcus.*Table 2 shows the zone of inhibitory activities of *Trigonella Foenum Graecum* seed extract, standard (Ampicilin) and Silver nanoparticles. The figure 4 shows the photos of antimicrobial assay.

CONCLUSION

In conclusion, the bio reduction of aqueous Ag^+ ions by the seed extract of the Trigonella Foenum Graecum plant has been demonstrated. In the present study I found that seeds can be a good source for the synthesis of silver nanoparticles. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other inorganic materials (nanoparticles). Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents.

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