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Synthesis of selenium nanoparticles using *Allium sativum* extract and analysis of their antimicrobial property against gram positive bacteria

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Abstract
Plant extract from *Allium Sativum* extract was used for the synthesis of selenium nanoparticles from sodium selenite solution. Selenium nanoparticles characterized by using UV-Visible (UV-VIS) spectrophotometer, Transmission electron microscopy (TEM), Fourier transform spectroscopy (FTIR) and Energy dispersive X-Ray spectroscopy (EDAX). The selenium nanoparticles synthesized by *Allium sativum* were observed as hollow and spherical particles in size ranging 8-52nm which is found more stable than two months. Antimicrobial activity of the selenium nanoparticles was performed by well diffusion method against *Staphylococcus aureus* and *Bacillus subtilis.* The selenium nanoparticles synthesized in this process has the efficient antimicrobial activity against pathogenic bacteria.

Keywords: Green synthesis, selenium nanoparticles, *Allium sativum*, *Staphylococcus aureus*, *Bacillus subtilis*

1. Introduction
Nanotechnology is novel as rapidly growing field with its vast application in science and technology for the purpose of manufacturing new materials at the nanoscale level (S.S. Shankar *et al*., 2004) [1]. The size of matter is important in nanoscience and nanotechnology is typically on the 0.2nm to 100nm scale. The properties of materials change as their size approaches the nanoscale (S. Eustis *et al*., 2006) [2]. Nanoparticles have wide range of application in bio-engineering, biosensor, cosmetics, nano-fabrics, catalyst, drug delivery, medicines etc (K.S. Kavita *et al*., 2013) [3].
Selenium has unique properties and great potential in the field of medicine, physics, biology and chemistry. The selenium nanoparticles are also used as antioxidant, antimicrobial, enzyme inhibition, anticancer agent but it is highly toxic so preparation of stable selenium nanoparticles with biomedical application is still a challenge (CH. Ramamurthy *et al*., 2013) [4].
Selenium nanoparticles has been synthesized by different approaches like *Bacillus sp. MshI* (M. Shakibaie *et al*., 2012) [5], *Klebsiella pneumonia* (P. J. Fesharaki et., 2010) [6], *Aspergillus terus* (B. Zare *et al*., 2013) [7], *Saccharomyas cerevisiae* (H. Hariharan *et al*., 2012) [8], *Bougainvillea spectabilis* (B. Deepa and V. Ganesan 2013) [9], leaves of lemon (K. S. Prasad *et al*., 2013) [10], rasin extract of grapes (G. Sharma *et al*., 2014) [11].
The selenium is most important because of selenium deficiency can lead to heart disease, hypothyroidism and a weakened immune system (D. M. Dick and A. Agrawal 2003)[12]. Kashin-beck disease is also a result of selenium and iodine deficiency (R. J. Coppinger and A. M. Diamond 2001) [13]. This disease affects bones and joints of growing children. One symptom is enlargement of cracking of small joints while a more serious symptom is distorted growth of long bones that leads to shorter structure. Recent supplementation of salt with selenium has reduced the occurrence of the disease.
Human beings are often infected by microorganisms such as bacteria, molds, yeast and viruses present in their surroundings. Gram positive *Staphylococcus aureus* and *Bacillus subtilis* were widely used to bacterial experiments. These bacteria mostly present on the body surface of mammals and also present in their surroundings but sometimes it occurs infection to them, so therefore *Staphylococcus aureus* and *Bacillus subtilis* strain were selected for the antibacterial activity by using selenium nanoparticles synthesized by *Allium sativum* extract in this study by well diffusion method. The selenium nanoparticles showing antimicrobial activity by zone of inhibition and it's increased with increasing concentrations.

2. Materials and Methods

2.1 Preparation of *Allium sativum* extract

Buds of *Allium sativum* 10gm were collected in a clean mortar. Buds were crushed using motor pestle and sufficiently diluted with water to make a thick paste. This paste was filtered through whaltman filter paper. The resulting pest was stored in refrigerator and used for further experiments.

2.2 Synthesis of metal nanoparticles

Flask containing 25 ml 5 mM Na2SeO3 solutions was kept on magnetic stirrer. Then drop wise addition of *Allium sativum* extract was made in flask containing Na2SeO3 solution until color of sodium selenite solution changed. From this solution 5 ml was taken which was used as a control. Remaining 20 ml solution was kept in shaker in dark for 72 hrs. After few days the color change of the solution was observed.

2.3 UV-Vis spectra analysis

The reduction of metallic selenium ions was observed by measuring the UV-Vis spectrum after 10 to 15 min of color change. A small aliquot was drawn from the solution and a wavelength from 250nm to 700nm on UV-Vis spectrophotometer (Optizon Double beam 3220).

2.4 TEM analysis

Transmission Electron Microscopic (TEM) analysis was performed with Techni 20 (Philips, Holland). A thin film of the sample was prepared on a carbon coated copper grid by dropping a very small amount of the sample on the grid. The *Allium sativum* extract containing Se nanoparticles were subjected to centrifugation at 13000 rpm for 10 min. The pellet thus recovered was subjected to washing by its re-suspension in de-ionized water followed by centrifugation at 13000 rpm for 10 min, to remove possible organic contamination present in nanoparticles. Finally, pellet was freeze dried using a lyophilizer (Labconco, Kanas, USA).

2.5 EDAX analysis

EDAX analysis was carried out on EDAX XL-30 operating at 15-25KeV.Incorporation of selenium nanoparticles in gauze cloth. Nanoparticles suspension was poured on the gauze cloth discs (diameter 1cm) and there discs were dried at $36^{\circ}c$ for 7 days.

2.6 Sample preparation for Fourier Transform Spectroscopy (FTIR)

Metal containing *Allium sativum* extract for Fourier Transform Infrared (FT-IR) analysis was prepared by mixing 5 mg metal salt in 10 ml *Allium sativum* extract. This metal containing *Allium sativum* extract was incubated at room temperature for 1 hour. After 1 hour incubation, this metal containing leaf extract was dried in Petri plate. After drying, particles were scraped using blade. So, powder of synthesized nanoparticles was obtained. Then spectral scan analysis was carried out at wave number ranging from 400-4000 cm^{-1} by using a FT-IR spectrometer (Perkin Elmer, Spectrum GX) with resolution of 0.15 cm^{-1} to evaluate functional groups that might be involved in sorption process.

2.7 Antibacterial studies

The selenium nanoparticles synthesized from *Allium sativum* extract was tested for their antimicrobial activity by well diffusion method against pathogenic organisms like *Staphylococcus aureus* and *Bacillus subtilis*. Each strain was spread uniformaly on the individual plates using glass spreader. Well of size 6mm have been made on Muller Hilton agar plates using gel puncture. Using micropipette 25µl, 50µl, 75µl and 100µl of the sample of nanoparticles solution and also Ampicillin as control were poured into wells. After incubation at 37ºc for 24 hrs the different level of zone of inhibition measure.

Results and Discussion

3.1 Visual observation

Reduction of metal salts into metal nanoparticles by the bio-molecules is always accompanied by the color change of reaction medium. In the present study the colorless solution of sodium selenite is changed in light pink color after drop wise addition of *Allium sativum* extract at zero hour. As the reduction proceed, the color of reaction medium is gradually changed to dark pink color after 24 hours.

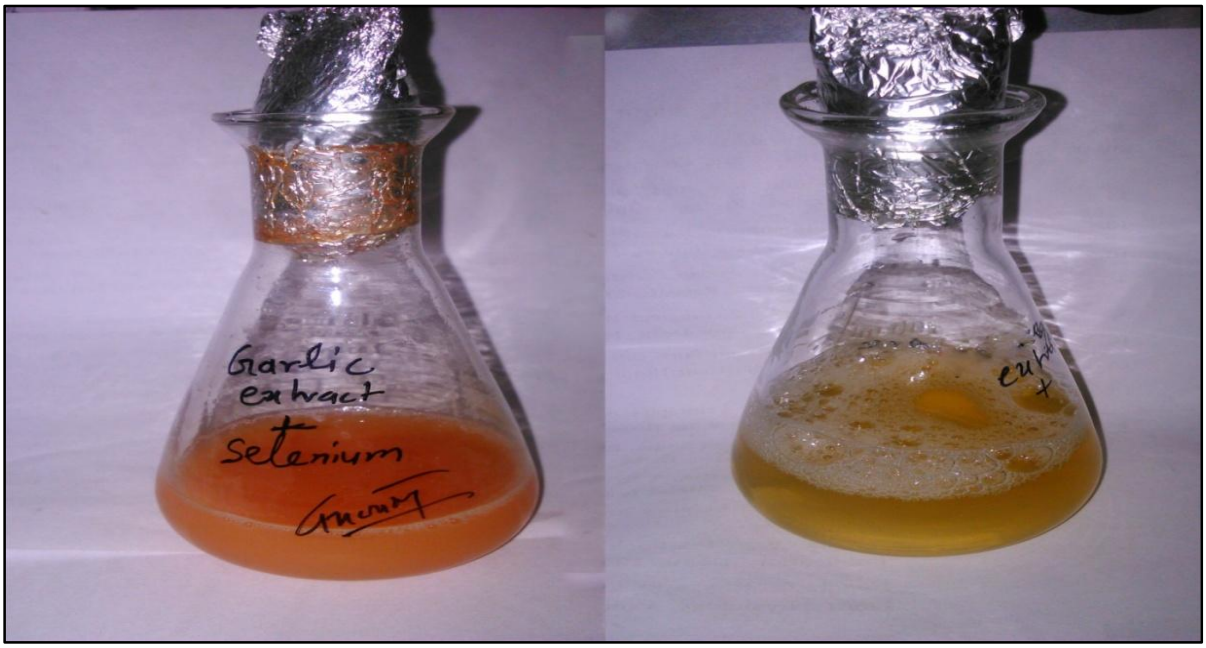


Fig 1: Color change of reaction medium, the color of reaction medium is gradually changed to dark pink color after 24 hours.

3.2 UV - Visible Spectroscopy

In order to determine the formation of Selenium nanoparticles in the extract of *Allium sativum*, a spectral scanning procedure was carried out from 250 nm to 700 nm. Colloidal solution exhibited absorption maxima at 400 nm (Fig. 2).

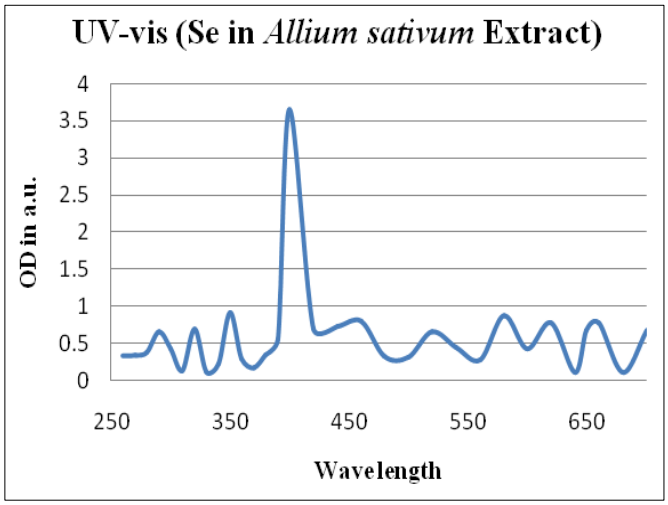


Fig 2: UV- VIS spectra selenium nanoparticles synthesize by *Allium sativum* extract and gets peak at 400nm

Initially the colloidal solution appeared white in color but after incubation of a period of 24 hours, it turned to reddish brown in color. Building of absorbing maximum at 400 nm clearly indicates the gradual formation of particles during the incubation period.

3.3 Transmission Electron Microscopy (TEM)

The results obtained from the transmission electron microscopy (TEM) and selected area electron diffraction (SEAD) study gives clear indication about the shape, size and size distribution of the nanoparticles.

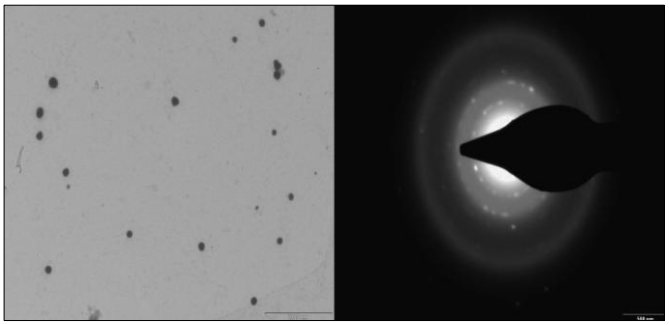


Fig 3: TEM image (left) and SEAD pattern (right) of Selenium nanoparticles synthesized using extract of *Allium sativum*.

TEM analysis of colloidal solution indicated the formation of selenium nanoparticles. (Figure 3) shows that size of particles, generated using *Allium sativum* extract ranges from 8 – 52 nm. Formation of variable size of particles indicates that particles suggest that *Allium sativum* extract could form polydisperse nanoparticles. Figure 3 shows Selected Area Electron Diffraction (SAED) of selenium nanoparticles results shows that particles are crystalline in nature as diffraction ring appeared which correspond to diffraction angle of (111, 121 and 311).

3.4 Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR analysis was carried out to identify the possible bio molecules and plant extract-metal ions interaction responsible for formation and stabilization of selenium nanoparticles. The result of FT-IR analysis of *Allium sativum* extract is presented in figure 4. The figure 4 shows the spectrum of both the sample control (A) and test (B). The fig. 4 (B) shows the spectrum of the sample that contains selenium metal in *Allium sativum* extract or fig. 4 (A) shows the spectrum of the *Allium sativum* extract that did not contain metal selenium. Spectra B show the peaks of both control and test, similarly the Fig. 4 (A) is showing transmission peaks of the control sample. Around 600 and 500 may be due to the partial duitriation of amine or carboxyl group.

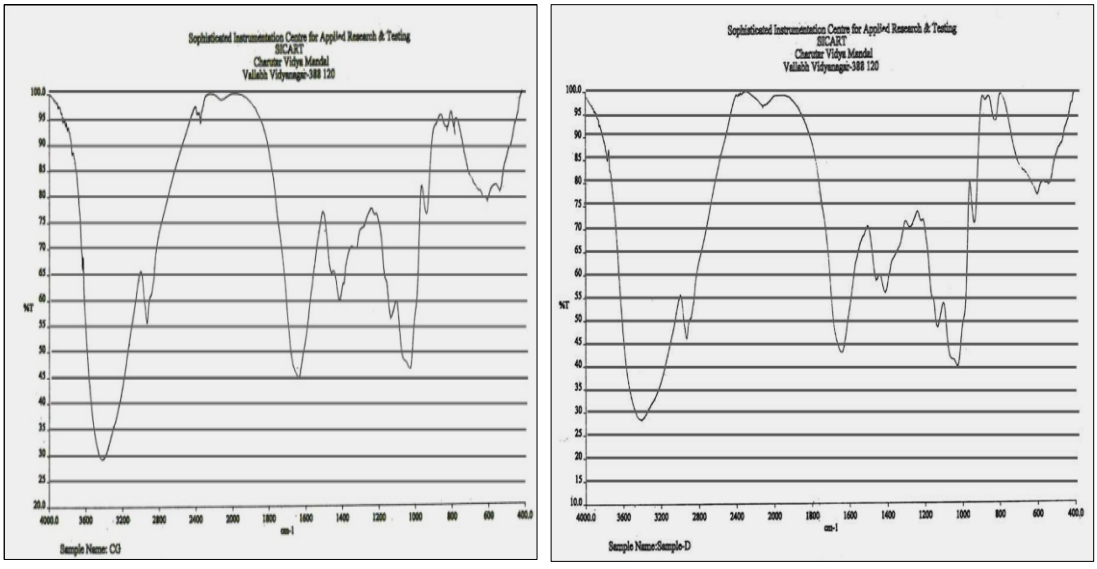


Fig 4: FTIR spectrum of (a) *Allium sativum* extract and (b) Selenium nanoparticles synthesized by *Allium sativum* extract

Two absorption peaks located around 3400 and 4000 can be assigned as the absorption peak of N-H. The peaks located around 3000 and 3200 may be due to the presence of C-H group. The absorption peaks around 2300 and 2000 can be assigned as the peaks of CO2. The absorption peaks around 1500 and 1800 can be assigned as the absorption peaks of C=O / C=N / C=C. The peaks around 1200 and 1100 were attributed to the stretching vibration of carboxyl group (C=O).

The peaks around 1100 and 1000 may be due to the presence of C-O group. Two absorption peaks around 600 and 500 may be due to the partial deuteration of amine or carboxyl group.

3.5 Energy Dispersive X-Ray Spectroscopy (EDAX)

EDAX analysis gives qualitative as well as quantitative status of elements that may be involved in formation of nanoparticles. Figure shows the elemental profile of synthesized nanoparticles using *Allium sativum* extract. The analysis revealed the highest proportion of Selenium (55%) in nanoparticles followed by oxygen (15%), sodium (28%) etc.

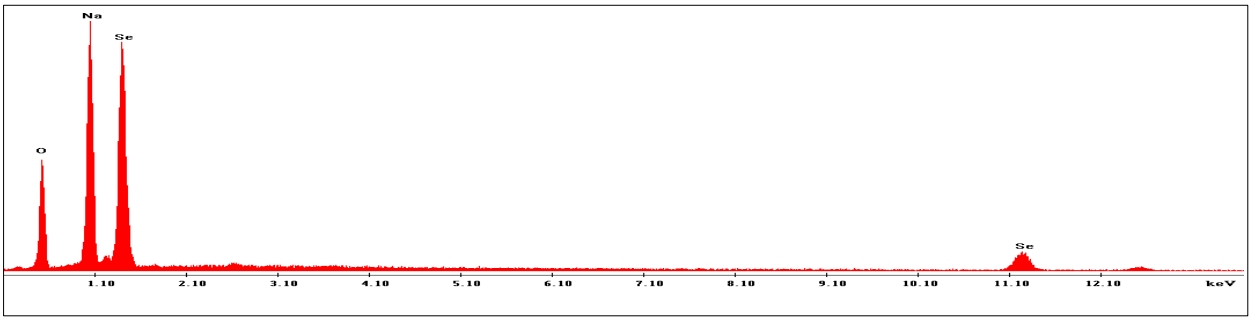
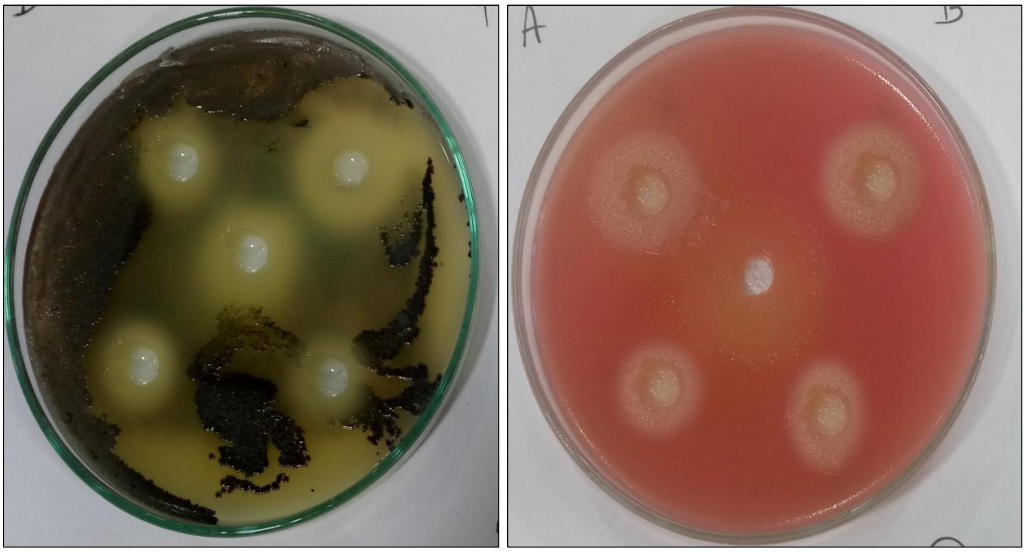


Fig 5: EDAX spectrum of selenium nanoparticles synthesize using extract of *Allium sativum*

3.6 Antimicrobial activity by well diffusion method

It is well known that selenium ions and nanoparticles are highly toxic to microorganisms. Selenium nanoparticles have been known to have inhibitory and bacterial effect and thus we extend its application as an antibacterial agent. The antimicrobial activity is estimated by the zone of inhibition. Several studies propose that selenium may attach to the surface of the cell membrane disturbing permeability and respiratory function of the cell. It is also possible that selenium nanoparticles not only interact with the surface of membrane but can also penetrate inside the bacteria.

The antimicrobial activity of selenium nanoparticles synthesized by *Allium sativum* extract was investigated against pathogenic organisms such as *Staphylococcus aureus* and *Bacillus subtilis* using well diffusion method.



a) b)

Fig 6: Antimicrobial activity by well diffusion method. Zone of inhibition shows in plates (a) *Staphylococcus aureus (b) Bacillus subtilis*

It may be observed that increasing quantity of selenium nanoparticles have comparatively higher antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis* probably due to thinner peptidoglycan layer and presence of porins.

Table 1: Zone of inhibition of Selenium nanoparticles synthesized by *Allium sativum* extracts against pathogenic bacteria

Sample	*Staphylococcus aureus*	*Bacillus subtilis*
Zone of inhibition (mm)		
25µl	13	11
50µl	19	17
75µl	25	23
100µl	32	28

The susceptibility of pathogenic bacteria to selenium nanoparticles shown in Table 1. The diameter of inhibition zone (mm) around each well with selenium nanoparticles synthesized by *Allium sativum* were found to have highest antimicrobial activity against *Staphylococcus aureus*(32mm) and *Bacillus subtilis* (28mm) at 100µl and the lesser antimicrobial activity of selenium nanoparticles synthesized by *Allium sativum* extract was found at 25µl *Staphylococcus aureus* (13mm) and *Bacillus subtilis* (11mm).

4. Conclusions

The present study was carried out to synthesis of Selenium nanoparticles using extract of *Allium sativum*. The bio molecules of *Allium sativum* extract acted as stabilizing as well as capping agent leading to the formation of Selenium

nanoparticles. UV-Vis Spectra at 400nm with *Allium sativum* extract and observed as hollow and spherical particles in size ranging 7-45nm which is found more stable more than two months. EDAX analysis was carried out to check the presoak of Selenium in nanoparticles. Results of EDAX, confirmed its present. TEM and SEAD represented addition evidence of formation of nanoparticles whereas SEAD indicates the particles were crystalline in nature. The zones of inhibition were formed in the antimicrobial screening test indicated, that the selenium nanoparticles synthesized by *Allium sativum* extract in this process has the efficient antimicrobial activity against pathogenic bacteria. The biologically synthesized selenium nanoparticles could be of immense use in medical field for their efficient antimicrobial function.

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