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Antioxidant activity and green synthesis of selenium nanoparticles using *Allium sativum* extract

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Abstract

The green synthesis of nanoparticles has provided cost effective, environment friendly procedure and raising safe strategies for synthesis of non-materials. In this study we investigated the clove of *Allium sataivum*, which is used for the synthesis of Selenium nanoparticles. The biosynthesized selenium nanoparticles were characterized by using UV-Visible (UV-VIS) spectrophotometer, Transmission electron microscopy (TEM), Fourier transform spectroscopy (FT-IR) and Energy dispersive X-Ray spectroscopy (EDAX). The selenium nanoparticles synthesized by garlic were observed as hollow and spherical particles in size ranging 7-45nm which is found more stable more than two months. The present study also reports the efficiency of ABTS, DPPH and FRAP assay to estimate the antioxidant potential of selenium nanoparticles which synthesized by garlic extract.
Keywords: Green synthesis, Selenium nanoparticles, *Allium sataivum*, antioxidant potential.

Introduction

Nanotechnology is the novel area for researchers because nanoparticles synthesis are bound with various limitations such as expensive approach, less stability and involving of hazardous toxic chemicals etc.[1] The field of nanotechnology has recently witnessed spectacular advance in the methods of nonmaterial fabrication and the utilization of their exotic physiochemical and optoelectronic properties[2]. The synthesis of nanoparticles of different chemical compositions, sizes and controlled monodispersity is an important area of research in nanotechnology. One of the fascinating aspects of nanotechnology is that on the nanometer scale all the natural sciences meet and intertwine. Physical meets life science as well as engineering, chemistry, material science and computational approaches, which altogether communicate and are closely linked. This inherent interdisciplinary nature of nanotechnology offers enormous potential for fruitful, cross fertilization in specialist areas [3]. Nanotechnology emerges from the physical, chemical, biological and engineering science where novel techniques are being developed to probe and manipulate single atoms and molecules.

In nanotechnology, a nanoparticle (10^{-9}) is defined as small object that behaves as a whole unit in terms of its transport and properties having one more dimensions of the order of 100 nm or less. [4] The development of reliable experimental protocols for the synthesis of non-materials over a range of chemical composition, sizes and high monodispersity is one of the challenging issues in current nanotechnology. In the context of the current drive to develop green technologies in material synthesis, nanotechnology is of considerable importance [5]. Nanoparticles have set up a wide range of applications in Bio engineering, Biosensors, cosmetics, nano-fabrics, catalysts, drug delivery, medicines etc [6].

Selenium has unique properties and great potential in the field of medicine, physics, biology and chemistry. The selenium nanoparticles are also used as antioxidant, enzyme inhibitors, anticancer agent but it is highly toxic, so preparation of stable selenium nanoparticles with biomedical application is still a challenge [7].

Selenium nanoparticles has been synthesized by different approaches like *Bacillus sp. MshI* [8], *Klebsiella pneumonia* [9], *Aspergillus terus* [10], *Saccharomyas cerevisiae* [11], *Bougainvillea spectabilis* [1], leaves of lemon [13], rasin extract of grapes [14].

The selenium is most important because of selenium deficiency can lead to heart disease, hypothyroidism and a weakened immune system [15]. Kashin-beck disease is also a result of selenium and iodine deficiency [16]. 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.

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The intake of vegetables and fruits are giving dietary antioxidants including polyphenolic compounds, vitamin E and vitamin C are believed to be the effective nutrients in the prevention of the oxidative stress related disease [17].

In metabolism, many of the redox process has generated reactive oxygen species (ROS), these species are highly reactive and harmful to the cells. If it is not eliminated then ROS can damage important molecules of cells like DNA, proteins and lipids. For the handling of these oxidative stress cells having antioxidants enzymes and non enzymatic compounds for complete scavenging of ROS. A study also shows that prevention of free radicals induced disease by the antioxidant substances [18].

Different types of assays for the estimation of antioxidants capacities in fresh fruits and vegetables are frequently used for the chemical studies, including 2,2-azinobis,3-ethyl benzothiazoline-6-sulfonic acid (ABTS) [19], 2,2-diphenyl-1-picrylhydrzyl (DPPH) [20], Ferric reducing antioxidant power (FRAP) [21,22] and the oxygen radical absorption capacity (ORAC) [23]. ABTS, DPPH, FRAP and ORAC assays gave comparable results for the antioxidant activity measured in methanol extract of guava fruit extract [24].

The aim of present study to report a simple green synthesis of selenium nanoparticles and compare the efficiency of ABTS, DPPH and FRAP assay to estimate antioxidant activity of Allium sativum extract and selenium nanoparticles synthesized by Allium sativum extract.

Material and Methods

Preparation of *Allium sativum* extract

Buds of garlic 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.

Synthesis of metal nanoparticles

Flask containing 25 ml 5 mM Na_2SeO_3 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.

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).

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).

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 $36\ c$ for 7 days.

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 plant extract. This metal containing plant 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.

Antioxidant assay

ABTS assay

For ABTS assay, the procedure followed the method of [19] with some modifications. The percent inhibition of ABTS radical by plant extracts were determined by the ability of plant extracts to scavenge the cationic free radical ABTS. Different concentrations (100 to 600 µg/ml) of *Allium sativum* extract and the biogenic synthesized Se nanoparticles were separately mixed with 3 ml of 0.1 mM ABTS and incubated in dark for 15 min. The extent of decolorization was measured at 745 nm. Rutin was used as standard and ABTS reagent without sample was used as control solution. The percent of scavenging inhibition capacity of ABTS+ of the extract was calculated from the following equation:

$$\% \text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100$$

DPPH assay

The DPPH assay was done according to the method of [20] with some modifications. The radical scavenging and antioxidant potential of the plant extracts were determined by the ability of plant extracts to scavenge the stable free radical DPPH and convert it into Diphenyl picryl hydrazine. The degree of decolourization from purple to yellow color was measured spectrophotometrically at 517 nm. Different concentrations (100 to 600 µg/ml) of *Allium sativum* extract and the biogenic synthesized Se nanoparticles were separately mixed with 3 ml of 0.1 mM DPPH and incubated in dark for 15 min. Rutin was used as standard and DPPH methanol reagent without sample was used as control. The reaction mixture was mixed well and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using this equation:

$$\text{DPPH Scavenging activity (\%)} = \frac{(\text{Abs control} - \text{Abs sample})}{(\text{Abs control})} \times 100$$

FRAP assay

The FRAP assay was done according to [21] with some modifications. This method is based on the reduction of ferric-tripyridyltriazine complex to its ferrous, coloured form in the presence of antioxidants. Readings of the coloured product (ferrous tripyridyltriazine complex) were then taken at 593 nm. Different concentrations (100–500 µg/ml) of Aloe vera extract and the biogenic synthesized Se nanoparticles of 0.5 ml was separately mixed with 2.5 ml of FRAP reagent allowed to react at room temperature in the dark. Rutin was used positive control in this test. An increase in the absorbance with increasing concentration is directly proportional to the reducing power. Results are expressed in µg Ascorbic acid equivalent (µg TE/mg de). BHT was used as reference.

Results and Discussion

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.

Figure. 1: Color change of reaction medium

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 (Figure. 2).

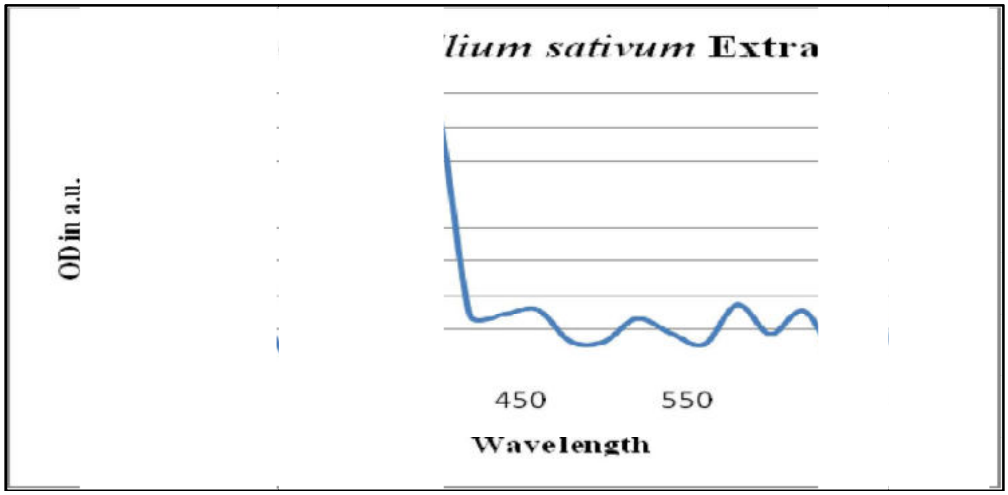

Figure. 2: UV- VIS spectra selenium nanoparticles

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.

Transmission Electron Microscopy (TEM)

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 7 – 45 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.

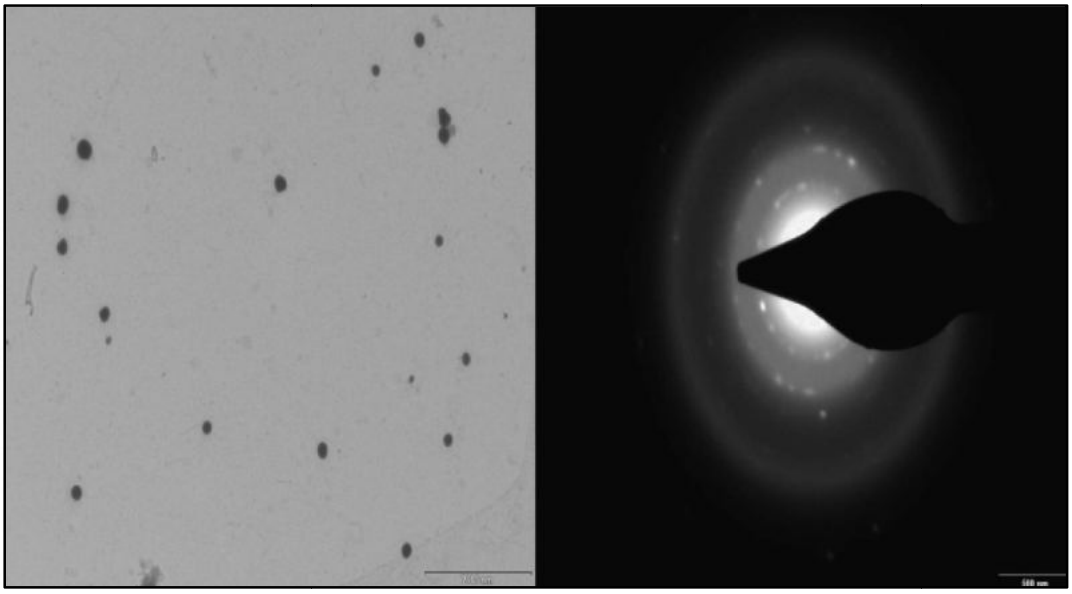

Figure. 3: TEM analysis of Selenium nanoparticles reveled size of particles 7-45 nm.

Results shows that particles are crystalline in nature as diffraction ring appeared which correspond to diffraction angle of (111, 121 and 311).

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 figure. 4 (B) shows the spectrum of the sample that contains selenium metal in *Allium sativum* extract or figure. 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 Figure. 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.

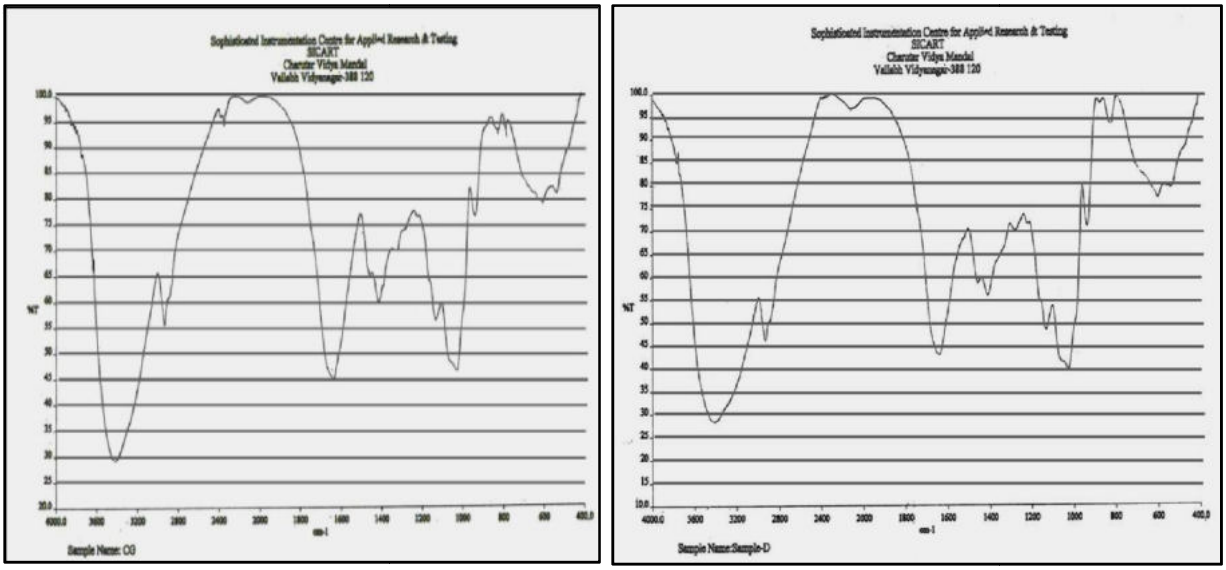

Figure. 4: FTIR spectrum of (A) *Allium sativum* extract and (B) Selenium nanoparticles synthesized by *Allium sativum* extract

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

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.

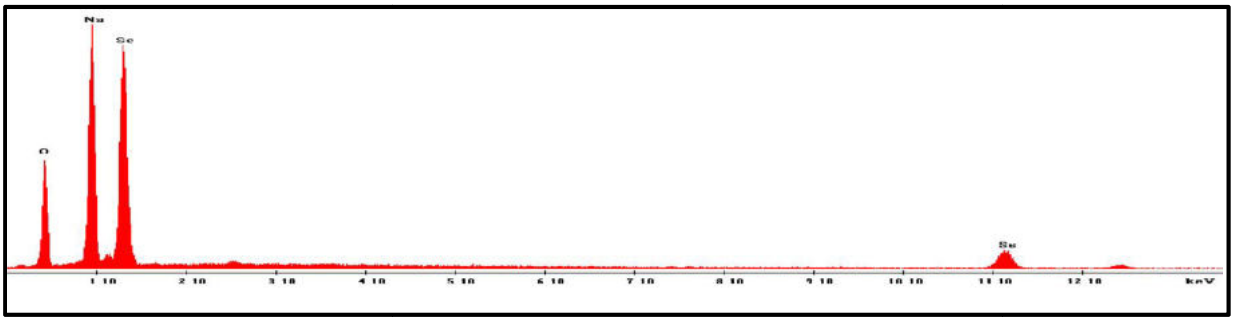

Figure.5 EDAX spectrum of selenium nanoparticles

Antioxidant assay

ABTS Assay

The reducing power of compounds is directly proportional to antioxidant activity of biogenic synthesized selenium nanoparticles was assessed by ABTS scavenging assay by using Rutin as a positive control. ABTS was a stable compound and accepts hydrogen or electrons from *Allium sativum* and synthesized Selenium

nanoparticles. The results obtained in the ABTS assay showed effective free radical inhibition by both *Allium sativum* extract and synthesized Selenium nanoparticles. The average percentage inhibition of synthesized Selenium nanoparticles was 75% as compared to *Allium sativum* extract 60% at different concentrations used in this study and the activity increased with increasing concentrations. Figure. 6 indicates that synthesized Selenium nanoparticles containing *Allium sativum* extract that relatively strong ABTS radical scavenging activity.

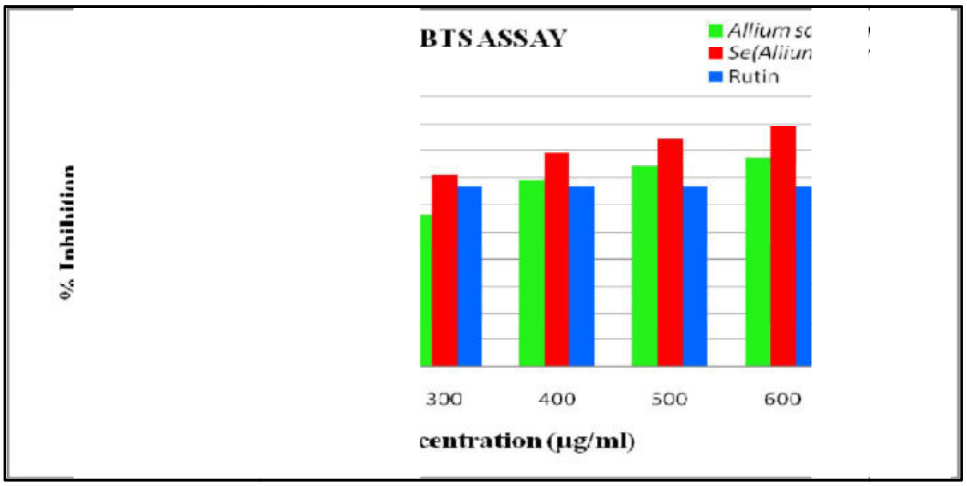

Figure. 6: ABTS Assay showing enhanced antioxidant activity of synthesise Selenium nanoaprticles.

DPPH Assay

Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. The reducing power of compounds is directly proportional to antioxidant activity of biogenic synthesized selenium nanoparticles was assessed by DPPH scavenging assay by using Rutin as a positive control. DPPH was a stable compound and accepts hydrogen or electrons from *Allium sativum* and synthesized Selenium nanoparticles. The results obtained in the DPPH assay showed effective free radical inhibition by both *Allium sativum* extract and synthesized Selenium nanoparticles. The average percentage inhibition of synthesized Selenium nanoparticles was 73% as compared to *Allium sativum* extract 65% at different concentrations used in this study and the activity increased with increasing concentrations. Figure. 7 indicates that synthesized Selenium nanoparticles containing *Allium sativum* extract that relatively strong DPPH radical scavenging activity. The present study was aimed to assess the antioxidant activity of Selenium nanoparticles in *Allium sativum* extract. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channeled their interest in preparing a new variety of natural antioxidants which are very effective to control the oxidative stress and hence prevent the initiation of disease propagation. The result of DPPH scavenging activity assay in this study indicates that the synthesize nanoparticles was potently active.

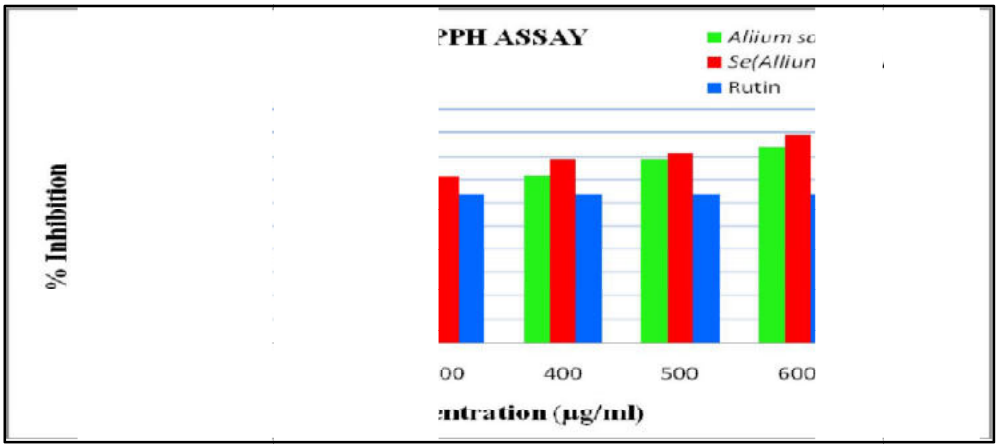

Figure. 7: DPPH Assay showing enhanced antioxidant activity of synthesise Selenium nanoaprticles.

FRAP Assay

In FRAP assay the change in absorbance is directly related to the combined or "total" reducing power of the electron donating antioxidants present in the reaction mixture of Selenium nanoparticles containing *Allium sativum* extract. According to FRAP assay Figure.8 shows reducing activity of biogenic synthesized Selenium nanoparticles and *Allium sativum* extract. Selenium nanoparticles showed more reducing activity than the *Allium sativum* extract and the reducing activity of Selenium nanoparticles was found with increasing concentrations.

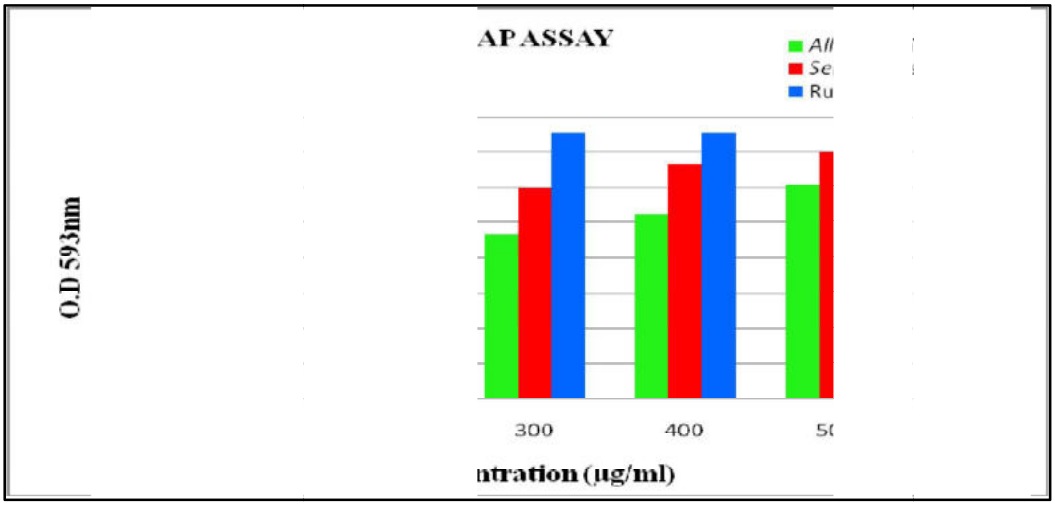

Figure.8: FRAP Assay showing enhanced antioxidant activity of synthesise Selenium nanoaprticles.

Conclusion

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. Selenite has been proven to have antioxidant activity and is being used as chemoprevention agent in cancer diagnosis but same time it is toxic also. Elemental Selenium i.e. Selenium nanoparticles are less toxic form of selenium. FRAP, ABTS and DPPH assay results sequester that Selenium nanoparticles prepared using *Allium sativum* extract possess more activity than extract alone.

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