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Original Article

ANTIOXIDANT ACTIVITY AND BIOGENIC SYNTHESIS OF SELENIUM NANOPARTICLES USING THE LEAF EXTRACT OF ALOE VERA

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ABSTRACT

Objective: The objective of this present study were to the biogenic synthesis of selenium nanoparticles using *Aloe vera* extract and check it's antioxidant potential by ABTS, DPPH and FRAP assays.

Methods: In this study we investigated the clove of *Aloe vera*, which is used for the synthesis of selenium nanoparticles were characterized by using UV-Visible (UV-VIS) spectrophotometer, Transmission electron microscopy (TEM), Fourier transform spectroscopy (FTIR) and Energy dispersive X-Ray spectroscopy (EDAX) and ABTS, DPPH and FRAP assays for checked it's antioxidant potential.

Results: The present study was carried out to synthesis of Selenium nanoparticles using extract of *Aloe vera*. UV-Vis Spectra at 350 nm with *Aloe vera* extract and observed as hollow and spherical particles in size ranging 7-48 nm 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. FT-IR analysis was carried out to identify the possible bio molecules and *Aloe vera* extract-metal ions interaction responsible for formation and stabilization of selenium nanoparticles. FRAP, ABTS and DPPH assay results sequester that Selenium nanoparticles prepared using *Aloe vera* extract possess more activity than extract alone.

Conclusion: The bio molecules of *Aloe vera* extract acted as stabilizing as well as capping agent leading to the formation of Selenium nanoparticles. 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.

Keywords: Biogenic synthesis, Selenium nanoparticles, *Aloe vera*, Antioxidant potential

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INTRODUCTION

Since the last decade, Nanotechnology is the active area of research. Nanotechnology is emerged as an interdisciplinary approach in biochemical application with focus on the cure of different disease like Alzimer, cancer etc [1].

Production of nanoparticles can be achieved through chemical and physical method [2, 3]. In bio nanotechnology the source of synthesis like microorganisms, enzyme, plants and plant extracts as possible methods to avoid the use of toxic chemical and expensive physical tools. The using of plant materials is an economical, eco-friendly and reliable technique [4].

In nanotechnology, nanoparticles defined as small object that behaves as a whole unit in terms of its transport and having one more dimensions of the order of 100 nm or less [5]. Nanoparticles shows size and shape dependent properties which are interest for application ranging from bio sensing and catalyst to optics, antimicrobial activity, antioxidant activity, computer transistors, electrometers, chemical sensors and wireless electronic logic and memory scheme [6].

Selenium(Se), belonging to group 16 of the periodic table having atomic no. 34 and atomic mass 78.96 amu. It is widely used for solar cells, rectifiers, photographic exposure meter and xerography due to its photoelectric and semiconductor properties. Selenium occurs in variety of oxidation state like selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), where in oxidation states+6 and+4 [4].

Selenium having great potential and properties in the field of medicine, physics, biology and chemistry. The selenium nanoparticles are also used as antioxidants, antimicrobial, anticancer agent but it's highly toxic so preparation of stable selenium nanoparticles as nontoxic biomedical application is still challenge [7].

Selenium nanoparticles has been synthesized by different biological source like Bacillus sp. MshI [8], *Klebsiella pneumonia* [9], *Bougainvillea spectabilis* [10], leaves of lemon [11], resin extract of grapes [12].

The fruits and vegetables are rich in antioxidants including polyphenolic compounds; vitamin E and vitamin C are believed to be the effective nutrients in the preventions of the oxidative stress related diseases [13].

The oxidative stress in occurs due to the reactive oxygen species(ROS) which is generated by redox process in metabolism these species are highly reactive and harmful to the cells the antioxidants enzyme and non enzymatic compounds for complete scavenging of ROS. A study also shows that prevention of free radicals induced disease by the antioxidant substances [14].

Aloe vera grows wild in tropical climates around the world and is cultivated for agriculture and medicinal uses. It is also used for decorative purposes and grows successfully indoors as a potted plant. *Aloe vera* leaf contains many vitamins mostly vitamin A, C and F. It also contains free anthraquinones and their derivatives Barbaloin, Anthron-c-glycosides and chromones. *Aloe vera* having antitumor, antioxidant, anti inflammatory, antimicrobial and anti fungal activities [15].

Therefore, the present study in aimed to evaluate the reducing potential of *Aloe vera* leaf in the synthesis and stabilization of selenium nanoparticles and compare the efficiency of ABTS, DPPH and FRAP assay to estimate antioxidant activity of *Aloe vera* extract and selenium nanoparticles synthesized by *Aloe vera* extract.

MATERIALS AND METHODS

Preparation of *Aloe vera* extract

A 30 gm portion of thoroughly washed *Aloe vera* leaves. Finely cut into 20 ml of sterile distilled water. The extract was then filtered through Whatman filter paper no.1 and stored at 4 °C for further work.

Synthesis of metal nanoparticles

Flask containing 25 ml 5 mmol Na_2SeO_3 solutions was kept on magnetic stirrer. Then drop wise addition of *Aloe vera* 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 h. After few days the color change of the solution was observed [11].

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 250 nm to 700 nm on UV-Vis spectrophotometer (Optizon Double beam 3220) [10].

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 *Aloe vera* 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) [11].

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 1 cm) and there discs were dried at $36^{\circ}c$ for 7 d [11].

Sample preparation for Fourier transform spectroscopy (FTIR)

Metal containing *Aloe vera* 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 h. After 1 h 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 [11].

Antioxidant assay

ABTS assay

For ABTS assay, the procedure followed the method of [16] 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 *Aloe vera* extract and the biogenic synthesized Se nanoparticles were separately mixed with 3 ml of 0.1 mmol 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 [17] 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 *Aloe vera* extract and the biogenic synthesized Se nanoparticles were separately mixed with 3 ml of 0.1 mmol 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 [18] 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 *Aloe vera* leaf extract at zero h. As the reduction proceed, the color of reaction medium is gradually changed to dark pink color after 24 h.

Fig. 1: Colour changed after reduction

UV–visible spectroscopy

In order to determine the formation of Selenium nanoparticles in the extract of *Aloe vera*, a spectral scanning procedure was carried out from 250 nm to 700 nm. Colloidal solution exhibited absorption maxima at 350 nm (fig. 2).

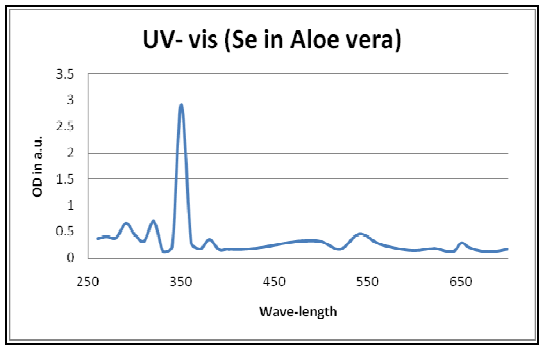

Fig. 2: UV-VIS spectra selenium nanoparticles

Initially the colloidal solution appeared white in color but after incubation of a period of 24 h, it turned to reddish brown in color. Building of absorbing maximum at 350 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. (fig. 3) shows that size of particles, generated using *Aloe vera* extract ranges from 7–48 nm. Formation of variable size of particles indicates that particles suggest that *Aloe vera* extract could form poly disperse nanoparticles. Fig. 3 shows Selected Area Electron Diffraction (SAED) of selenium nanoparticles.

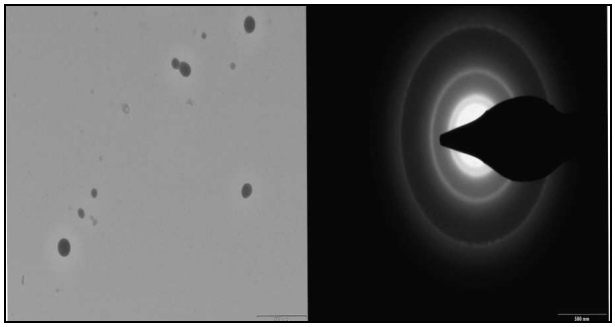

Fig. 3: TEM analysis of Selenium nanoparticles reveled size of particles 7-48 nm

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 silver nanoparticles. The result of FT-IR analysis of plant extract is presented in fig. 4. The fig. 4 shows the spectrum of both the sample control (A) and test (B). Fig. 4 (B) shows the spectrum of the sample that contains selenium metal in *Aloe vera* extract or fig. 4 (A) shows the spectrum of the *Aloe vera* extract that did not contain metal selenium. Spectra A show the peaks at 3439.34, 2926.57, 2856.55, 1630.80, 1412.22, 1384.92, 1315.09, 1112.49, 779.36, 618.64 and 522.63 cm-1. Similarly the transmission peaks of the sample (fig. 4 B) that did not contain metal selenium were obtained at 3421.21, 2925.59, 2856.55, 1740.26, 1610.60, 1415.00, 1319.54, 1250.14, 1077.04, 1033.46, 812.77, 775.97, 675.86, 599.98 and 540.99 cm-1.

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 CO_2. 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.

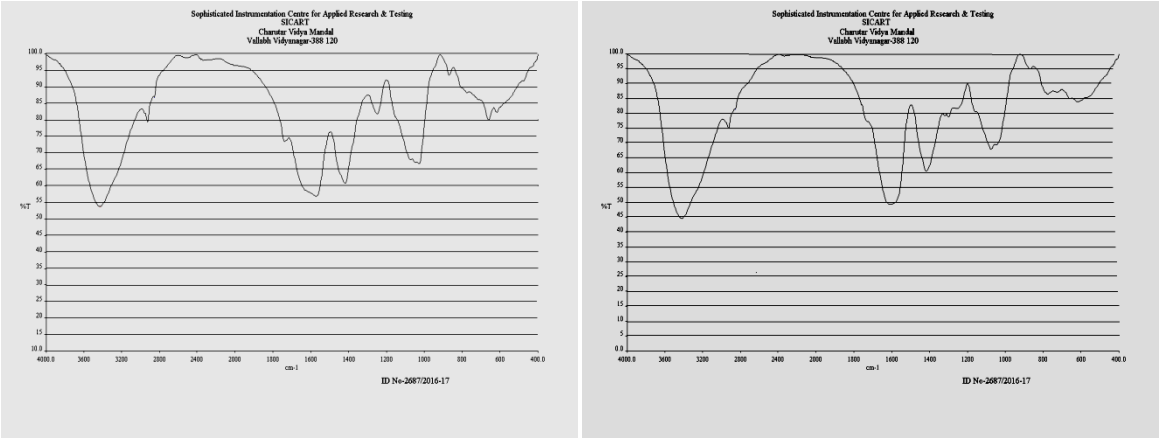

Fig. 4: FTIR spectrum of (A) *Aloe vera* extract and (B) Selenium nanoparticles synthesized by *Aloe vera* extract

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. Fig. shows the elemental profile of synthesized nanoparticles using *Aloe vera* extract. The analysis revealed the highest proportion of Selenium (25%) in nanoparticles followed by oxygen (20%), sodium (10%) P (10%) S (8%) etc.

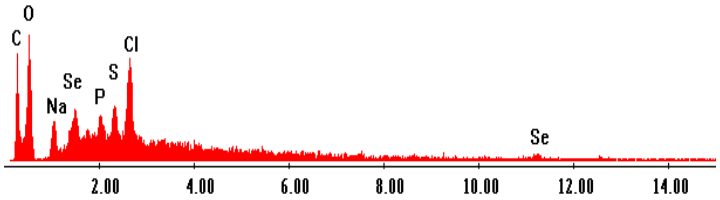

Fig. 5: EDAX spectrum of selenium nanoparticles

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 *Aloe vera* and synthesized Selenium nanoparticles. The results obtained in the ABTS assay showed effective free radical inhibition by both *Aloe vera* extract and synthesized Selenium

nanoparticles. The average percentage inhibition of synthesized Selenium nanoparticles was 73% as compared to *Aloe vera* extract 54% at different concentrations used in this study and the activity increased with increasing concentrations. Fig. 6 indicates that synthesized Selenium nanoparticles containing *Aloe vera* extract that relatively strong ABTS radical scavenging activity.

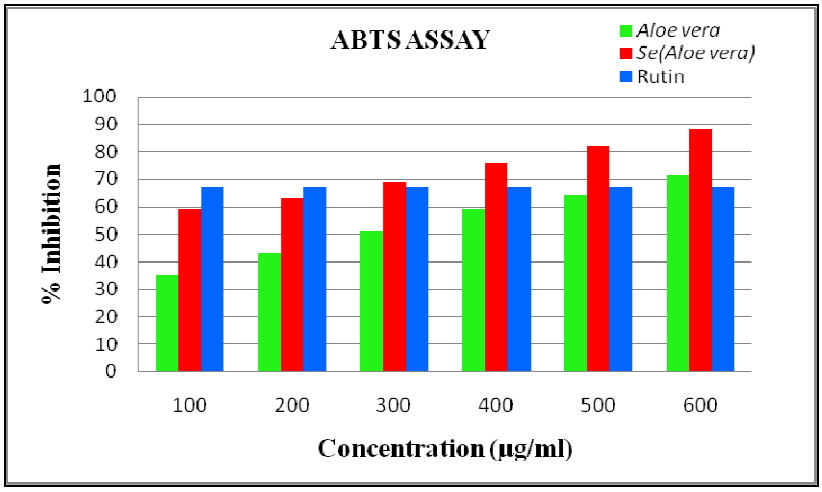

Fig. 6: ABTS assay showing enhanced antioxidant activity of synthesise selenium nanoaprticles

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 *Aloe vera* and synthesized Selenium nanoparticles. The results obtained in the DPPH assay showed effective free radical inhibition by both *Aloe vera* extract and synthesized Selenium nanoparticles. The average percentage inhibition of synthesized Selenium nanoparticles was 67% as compared to *Aloe vera* extract 57% at different concentrations used in this study and the activity increased with increasing concentrations.

Fig. 7 indicates that synthesized Selenium nanoparticles containing *Aloe vera* extract that relatively strong DPPH radical scavenging activity. The present study was aimed to assess the antioxidant activity of Selenium nanoparticles in *Aloe vera* 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.

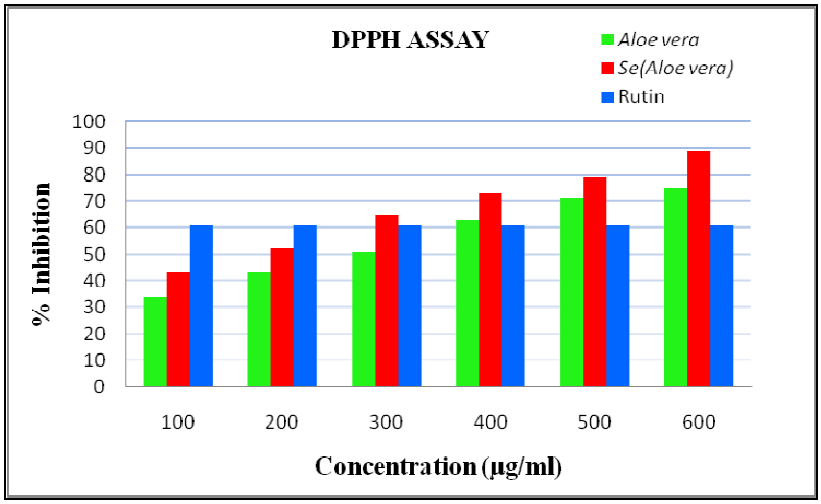

Fig. 7: ABTS assay showing enhanced antioxidant activity of synthesise selenium nanoaprticles

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 *Aloe vera* extract. According to FRAP assay Fig.8 shows reducing activity of biogenic synthesized Selenium nanoparticles and *Aloe vera* extract. Selenium nanoparticles showed more reducing activity than the *Aloe vera* extract and the reducing activity of Selenium nanoparticles was found with increasing concentrations.

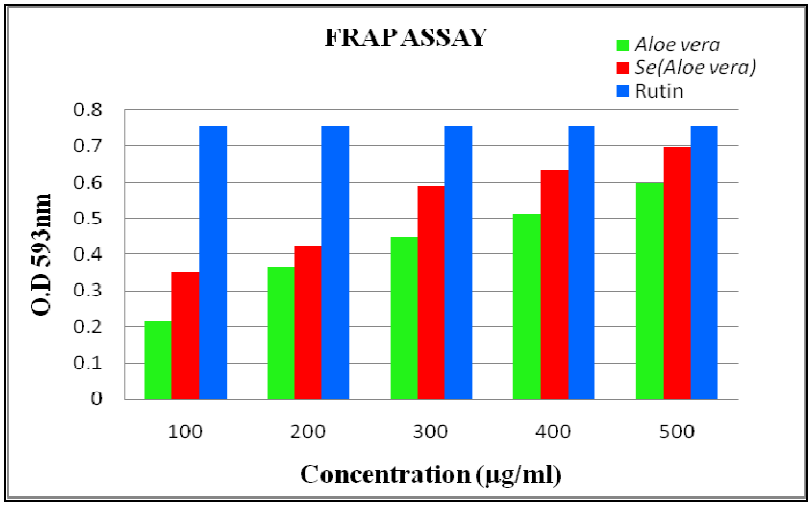

Fig. 8: FRAP assay showing enhanced antioxidant activity of synthesise Selenium nanoaprticles

CONCLUSION

The present study was carried out to synthesized Selenium nanoparticles using extract of *Aloe vera*. The bio molecules of *Aloe vera* extract acted as stabilizing as well as capping agent leading to the formation of Selenium nanoparticles. UV-Vis Spectra at 350 nm with *Aloe vera* extract and observed as hollow and spherical particles in size ranging 7-48 nm 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 *Aloe vera* extract possess more activity than extract alone.

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CONFLICT OF INTERESTS

Declare none

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