

# Biogenic Synthesis and Characterization of Selenium Nanoparticles Using the Flower of *Bougainvillea spectabilis* Willd

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**Abstract:** The present study reports that the flower of *Bougainvillea spectabilis* Willd. has the potentiality to reduce sodium selenate and synthesize selenium nanoparticles. The exposure of aqueous sodium selenate to the flower broth of *B. spectabilis* leads to the synthesis of stable selenium nanoparticles within five days. The colour change of the reaction medium from purple to brown colour indicates the process of reduction. The mechanism of reduction was analyzed through UV-Visible (UV-Vis) Spectrophotometer and the  $\lambda_{max}$  in the Surface Plasmon Resonance (SPR) gradually shifted from 348nm to 326nm over the period of incubation and the absorbance was gradually raised from 0.21 to 1.276 a.u. during the period of reduction. The emission and excitation spectrum showed major peaks at 337.5 and 365.5 nm respectively. Fourier Transform Infra-Red Spectroscopic (FT-IR) analysis substantiated the role of alcohols, amines and ketones in the formation and stabilization of selenium nanoparticles. The X-ray Diffraction (XRD) study confirmed the presence of crystalline selenium nanoparticles with an average size of 53nm. The Energy Dispersive X-ray pattern (EDAX) shows that the synthesized nanoparticles are of selenium. Further the structural view of the synthesized selenium nanoparticles has been documented with the help of Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The synthesized selenium nanoparticles were hollow and spherical with an average size of  $24.24 \pm 2.95$  nm and found to be stable for more than three months. This biogenic synthesis using the flower of *Bougainvillea spectabilis* is a cost effective, simple and eco-friendly method.

**Keywords:** Biogenic synthesis, selenium nanoparticles, *Bougainvillea spectabilis*, eco-friendly approach.

## 1. Introduction

The unique features of nanoparticles made it as a core element in the field of Nanotechnology. The conventional methods employed in the synthesis of nanoparticles are bound with various limitations such as expensive, generation of hazardous toxic chemicals etc., [1]. Use of natural resources like biological systems becomes essential to overcome these limitations [2]. Varieties of living organisms are employed as a "bio-factories" for the synthesis of different metal nanoparticles. Among the biological systems, plants tender a superior option for the synthesis of nanoparticles, as the protocols involving plant sources are free from toxicants and the availability of natural capping agents [3]. Apart from the synthesis of gold, silver and platinum nanoparticles, the synthesis of selenium nanoparticles also gained much interest due to its excellent antioxidant [4, 5] and antifungal [6] properties.

Selenium is widely synthesized by chemical reduction method [7]. Elevated temperature and high pressure involved in the synthesis of selenium nanoparticles are hazardous to the environment [8, 5]. Biomimetic approaches paved the way for the synthesis of selenium nanoparticles using biological systems in an eco-friendly manner. There are few reports in the literature regarding the synthesis of selenium nanoparticles using biological systems. They are *Bacillus cereus* [7], *Klebsiella pneumonia* [9], *Shewanella* sp.HN-41 [10], *Pantoea agglomerans* [5], *Aspergillus terreus* [11], *Bifidobacterium* [12], leaves of lemon [13], *Terminalia arjuna* [14], raisin extract of grapes [15] and seed extract of fenugreek [16]. Therefore, the present study is aimed to evaluate the reducing potential of flower of

*Bougainvillea spectabilis* Willd. (Family : Nyctaginaceae) in the synthesis and stabilization of selenium nanoparticles.

## 2. Materials and Methods

The flowers of *Bougainvillea spectabilis* Willd. (Fig. 1) are true, perfect, small, tubular and white and a cluster of three flowers is surrounded by three showy



Figure 1: Flower of *Bougainvillea spectabilis* Willd

petaloid bracts. This plant is referred to as "paper flower" because the bracts are thin and papery. Fresh and healthy flowers of *B. spectabilis* along with its purple colour bract were collected from Sivakasi, Tamilnadu and air dried for two days at room temperature. 10 grams of the dried flowers were cut into fine pieces and added with 100ml of sterile double distilled water. It was kept in a water bath at 70°C for five to ten minutes. Then the solution was filtered through the cheese cloth and the filtrate was used as flower broth and it was purple in colour. 10ml of the freshly prepared flower broth was added to 90ml of 10mM aqueous solution of

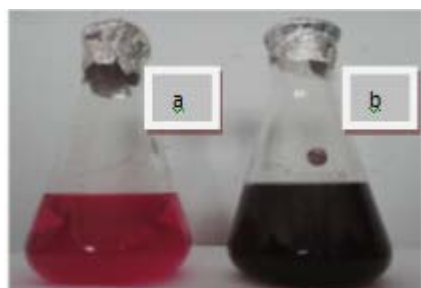
sodium selenate. This reaction medium was kept in an incubator cum shaker (Orbitek) with 250rpm at 36°C for five days.

Spectral analysis of the reaction medium was done with a small amount of the diluted reaction medium using Labomed (Model UV- D3200) UV-Visible spectrophotometer at an interval of 24hr for five days. After five days, the emission and excitation spectrum of the reaction medium were obtained through the analysis with Luminescence Spectrophotometer (Perkin Elmer – Model LS45). The reaction medium was centrifuged at 10,000 rpm for 10 minutes using Remi centrifuge – Model R-24. The pellet was completely dried in hot air oven to carry out further analyses using FT-IR spectrophotometer (Shimadzu), XRD (X'Pert PRO X-ray diffractometer), SEM (Quanta FE G250) coupled with EDAX (Ametek) and TEM (Philips-Techno 10).

### 3. Results and Discussion

#### 3.1 Visual Observation

Reduction of metal salts into metal nanoparticles by the biomolecules is always accompanied by the colour change of the reaction medium. In the present study, the colourless solution of sodium selenate acquired purple colour due to the addition of purple coloured flower broth of *B.spectabilis* at zero hour. As the reduction proceeds, the colour of the reaction medium gradually changed from purple colour to dark brown colour after five days (Fig.2).

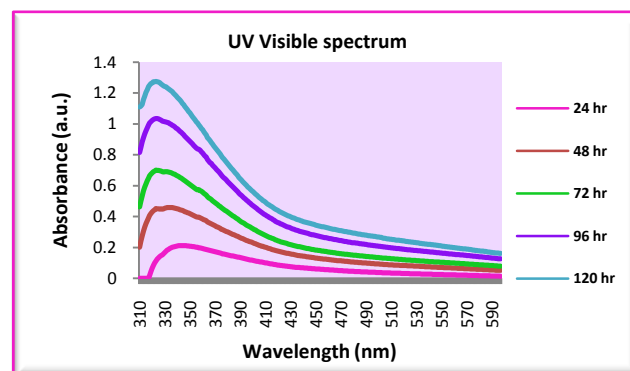


**Figure 2:** (a) Initial colour of the reaction medium containing flower broth of *B.spectabilis* and 10mM Sodium selenate in the ratio of 1:9; (b) Colour change of the reaction medium after 120 hr.

The flower broth of *B.spectabilis* took 120 hours for the complete reduction of sodium selenate into selenium nanoparticles, while the supernatant of *Aspergillus terreus* [11] and *Pantoea agglomerans* [5] took 60 minutes and two hours respectively to complete the formation of selenium nanoparticle. It was reported that *Stenotrophomonas maltophilia* [17] took 28 hours for the formation of selenium nanoparticles and in this microbe mediated synthesis they used sodium selenite (Se IV) as metal salt for the synthesis of selenium nanoparticles. But, we used sodium selenate (Se VI) for the synthesis procedure in this present study. It implies that the nature of selenium salts used and their concentrations play a vital role in the time taken for the completion of synthesis of selenium nanoparticles.

#### 3.2 UV-visible spectroscopic analysis

The UV-visible spectra (Fig.3) of the reaction medium which obtained at different time intervals showed

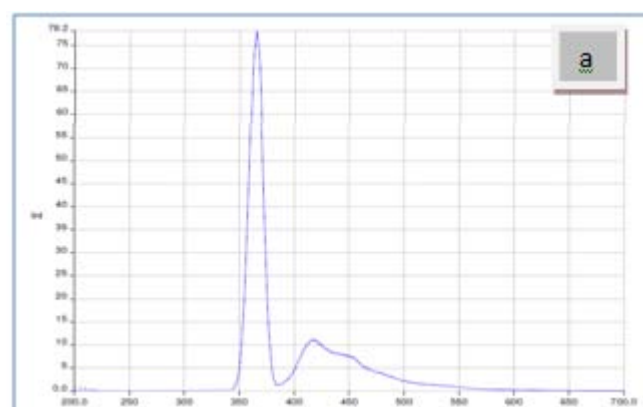


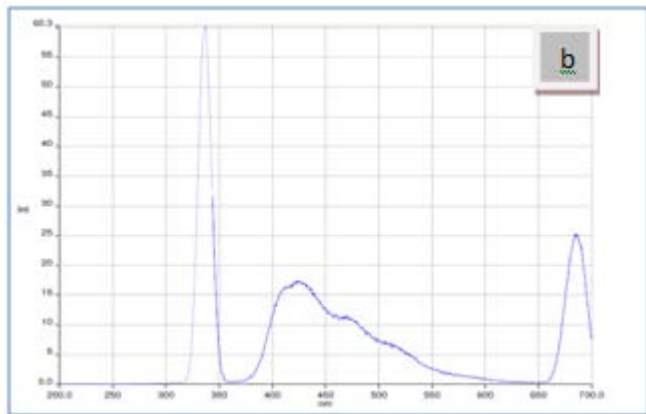
**Figure 3:** UV-visible absorption spectra recorded as a function of time for selenium nanoparticles synthesized using flower broth of *B.spectabilis* Willd.

the increase in Surface Plasmon Resonance (SPR) bands with increasing reaction time. The appearance of strong bands in the spectral patterns is due to the excitation of the localized surface plasmons which causes strong light scattering by an electric field at a wavelength where resonance occurs [18]. The reaction medium showed the maximum absorbance at 348, 336, 334, 326 and 326nm for 24, 48, 72, 96 and 120 hr respectively. It clearly indicates that the absorption maximum is blue shifted with decrease in the size of the particles [19, 20]. The absorbance was gradually raised from 0.21 to 1.276 a.u. during the period of reduction indicating the increase in the synthesis of nanoparticles. Interestingly, the UV-visible absorbance spectra of selenium nanospheres recovered from the culture broth of *Bacillus cereus* gave characteristic peak at 590nm [7] while, selenium nanoparticles synthesized by *Aspergillus terreus* showed the peak at 245nm [11]. The large variations in the spectral peaks were attributed to the diversity of enzymes that catalyze the reduction of selenium oxyanions into selenium nanoparticles [21, 7].

#### 3.3 Luminescence spectroscopic analysis

The excitation spectrum (Fig.4a) shows a peak at 365.5 nm and the emission spectrum (Fig.4b) shows peaks at 337.5, 425 and 686.5 nm. The excitation spectrum was found to coincide almost with  $\lambda_{max}$  of UV-visible spectra of the reaction medium. Earlier reports



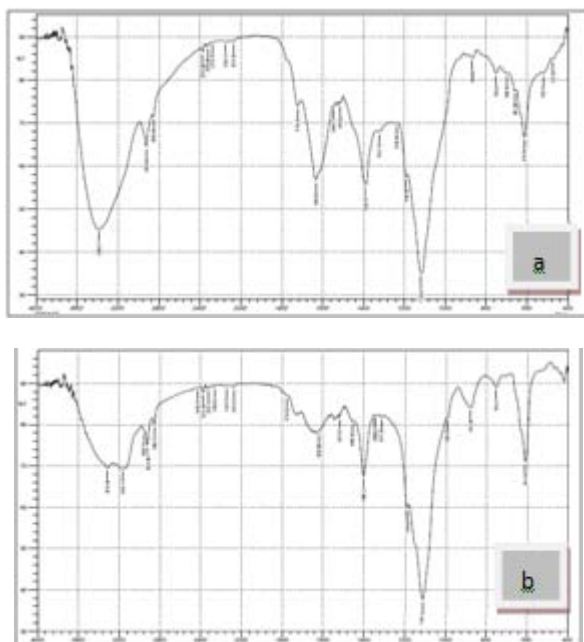


**Figure 4:** Excitation (a) and emission (b) spectra of reaction medium

elucidated the formation of selenium nanoparticles with a peak at 682 nm in the emission spectrum [22, 4]. The peak at 686.5 nm in the emission spectrum of the present study may be ascribed to the formation of selenium nanoparticles.

### 3.4 FT-IR Spectroscopic Analysis

The functional groups involved in the synthesis of selenium nanoparticles were detected with the help of FT-IR analysis. A comparative study on the FT-IR spectrum of *Bougainvillea spectabilis* flower (Fig.5a) and synthesized selenium nanoparticles (Fig.5b) predicts



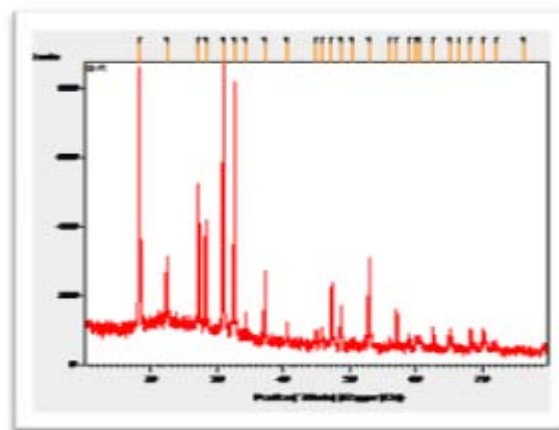
**Figure 5:** FT-IR Spectrum of flower broth (a) and selenium nanoparticles (b).

that the disappearance of the absorbance band at  $1236\text{ cm}^{-1}$  which corresponds to the stretching vibration of  $\text{-NH-}$  of amines and a shift in the band from  $1724$  to  $1776\text{ cm}^{-1}$  which corresponds to the stretching vibration of  $\text{-C=O-}$  of ketones and from  $1321$  to  $1317\text{ cm}^{-1}$  corresponds to the stretching vibration of alcohol. These biomolecules may be the functional groups that involved in the reduction and stabilization of nanoparticles. These biomolecules may be derived from the phytochemicals such as alkaloids and

flavonoids present in the aqueous flower extract of *B.spectabilis* [23]. However, lignin derived from raisins [15], carboxyl ( $\text{-C=O}$ ), hydroxyl ( $\text{-OH}$ ) and amine ( $\text{-NH}$ ) derived from the lemon plant extract [13] were the reported functional groups involved in the reduction and stabilization of the selenium nanoballs.

### 3.5 X-Ray Diffraction (XRD) Analysis

X-ray powder diffraction is an efficient analytical technique used to identify and characterize unknown crystalline material using monochromatic X-rays. The XRD pattern of selenium nanoparticles synthesized using the flower broth of *B.spectabilis* (Fig.6) showed number of Bragg's reflections. The clear peaks of cubic phases at  $22.37^\circ$  (100),  $30.89^\circ$  (101),  $32.50^\circ$  (101),  $40.44^\circ$  (110),  $44.94^\circ$  (102),  $45.80^\circ$  (111),  $52.86^\circ$  (201),  $56.08^\circ$  (112),  $62.56^\circ$  (103),  $65.12^\circ$  (210),  $72.02^\circ$  (113) and  $76^\circ$  (203) confirmed the crystalline nature of selenium nanoparticles.

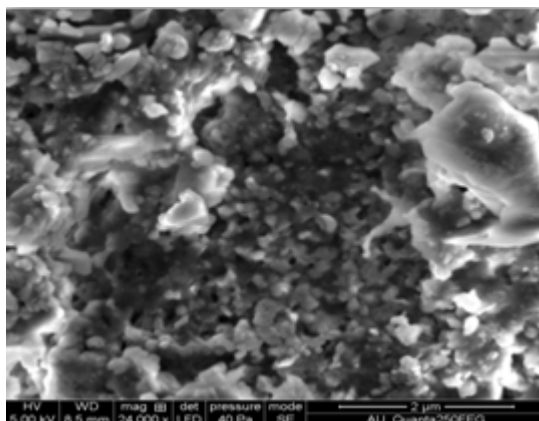


**Figure 6:** XRD Pattern of synthesized selenium nanoparticles

These sets of lattice planes had been indexed on the basis of the face centered cubic structures (fcc) of standard selenium PDF card -00-001-0848 of International Centre for Diffraction Data, 2013. The calculated average size of the selenium nanoparticle was found to be 53nm. Selenium nanoparticles of 60 nm were synthesized by simple wet chemical technique using sodium selenosulphate as a precursor and glucose as a stabilizer [24]. But the present study proved that the flower broth of *B.spectabilis* played a dual role as reducing and stabilizing agent.

### Scanning Electron Microscopy (SEM) and Energy Dispersive Atomic X-ray (EDAX) spectroscopic analyses

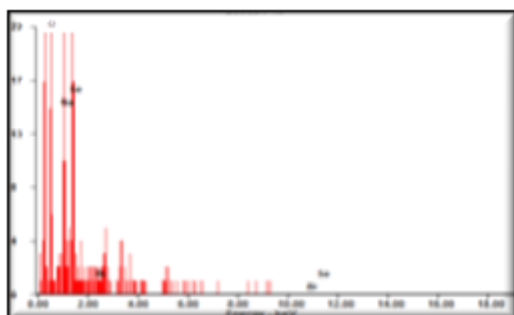
The topographic structure of the nanoparticle surface and the distinction of different phases were analyzed through the SEM analysis. It showed the presence of spherical selenium nanoparticles (Fig.7).



**Figure 7:** SEM images of selenium nanoparticles synthesized using flower broth of *B.spectabilis* Willd. (at 24,000X Magnification)

There is a strong relationship between the size of the nanoparticles and their biological activity [25, 5, 26]. The culture supernatant of *Aspergillus terreus* produced spherical selenium nanoparticles of 47 nm [11], *Klebsiella pneumonia* produced elemental selenium nanoparticles of 245 nm [9] and the *Bacillus cereus* strain CM100B isolated from coalmine soil produced the nanoparticles of 150-200 nm [7].

In EDAX analysis, the vertical axis shows the amount of X-ray counts and horizontal axis shows energy emitted in keV. The EDAX spectrograph (Fig.8) and



**Figure 8:** EDX image of selenium nanoparticles synthesized using flower broth of *B.spectabilis* Willd.

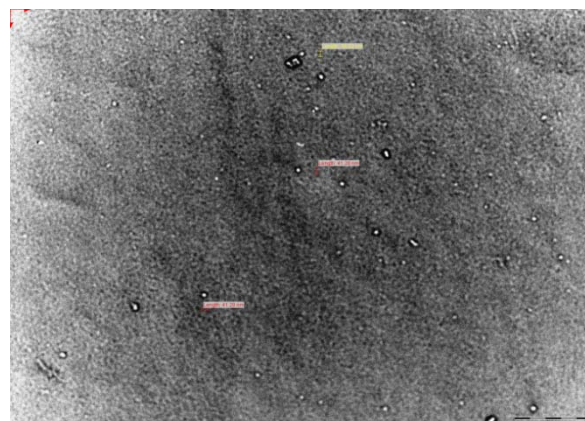
Elemental microanalysis (Table.1) derived from the synthesized selenium nanoparticles indicate the presence of 43.46% of selenium nanoparticles. The electron dense Selenium nanoparticles showed typical absorption for SeL $\alpha$  and SeK $\alpha$  peaks approximately at 1.37 keV and 11.22 keV respectively [6, 7, 11]. There are also reports for the presence of SeK $\beta$  nanoparticles which had a peak at 12.49 keV [7]. This sort of elemental selenium provided in the form of nanoparticles was widely used in the preparation of antifungal formulations [6]. Other peaks detected for Na and O may be from the mixed components present in the plant extract [27].

**Table 1:** EDAX elemental microanalysis

Element	Weight %	Atomic %
SeL	43.46	14.78
OK	37.63	63.14
NaK	18.91	22.08
<b>Total</b>	<b>100</b>	<b>100</b>

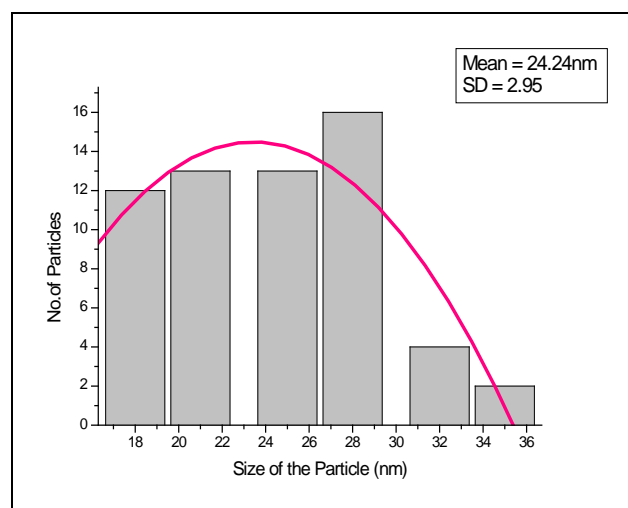
### 3.6 Transmission Electron Microscopic analysis (TEM)

Transmission electron microscopic image elucidates that the synthesized selenium nanoparticles are spherical and hollow in nature with an average size of 24.24 $\pm$ 2.95 nm (Fig.9).



**Figure 9:** TEM image of synthesized Se nanoparticles

The histogram of selenium nanoparticles (Fig.10) indicates that they range from 18 to 35 nm. The extract of dried raisins synthesized selenium nanoballs with a size range of 3-18 nm [15] while, the culture of *Klebsiella pneumoniae* produced nanoparticles of 245 nm [9].



**Figure 10:** Histogram showing the distribution of synthesized Se nanoparticles

### 3.7 Stability of selenium nanoparticles

The synthesized selenium nanoparticles are found to be stable for more than three months without forming any aggregates. Generally the stability of nanoparticles correlates with the zeta potential. Either large positive or negative zeta potential tends the nanoparticles to repel each other. Thus it prevents the aggregation and thereby makes the nanoparticle a stable one [7].

## 4. Conclusion

To conclude, we have reported the biogenic synthesis of selenium nanoparticles using the flower broth of *B.spectabilis*. Gradual blue shift in the Surface Plasmon Resonance (SPR) peak of UV-visible spectrum indicates the

formation of smaller sized selenium nanoparticles. FT-IR analysis detected the role of characteristic functional groups such as amide I band and ketones derived from the alkaloids or flavonoids in the reduction and stabilization. XRD data confirmed the formation of crystalline selenium nanoparticles. The electron dense selenium nanoparticles with typical absorption peaks noted in the EDAX spectrograph proved its elemental nature. Further TEM analysis confirmed that the synthesized selenium nanoparticles were hollow with an average size of  $24.24 \pm 2.95$  nm. This sort of biogenic synthesis of highly stable selenium nanoparticles is a simple, low-cost and eco-friendly method.

## 5. Acknowledgement

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