

## BIOGENIC SYNTHESIS AND CHARACTERIZATION OF SELENIUM NANOPARTICLES USING CYANOBACTERIAL SPECIES AND ITS ANTI-BACTERIAL ACTIVITY

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The biogenic potential of two fresh water cyanobacterial sp. (Anabaena UU2493, Calothrix UU24112) and two

marine water cyanobacterial sp. (Lyngbya BDU72401 and Phormidium BDU7060) are used to synthesize

selenium nanoparticles. The characterization of synthesized selenium nanoparticles was done using UV-VIS spectroscopy, TEM, FTIR, and X-RD. The X-RD data revealed that freshwater cyanobacterial species synthesize

amorphous SeNPs, whereas, marine water cyanobacterial species synthesize SeNPs in crystalline form. The

functional groups involved in the bio-reduction of selenium nanoparticles were monitored by FTIR spectroscopy. The synthesized SeNPs in combination with chloramphenicol (100ug/ml, 200ug/ml, 300ug/ml and 400ug/ml)

against E. coli MG1655 were evaluated and the SeNPs synthesized by Calothrix UU24112 showed higher antibacterial activity. The synthesized SeNPs by Calothrix UU24112 were assessed for photo catalytic dye

degradation, safranin and crystal violet activity through UV-Vis irradiation techniques and play a pivotal role in

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ABSTRACT

photocatalytic activity.

## **KEYWORDS**

Cyanobacteria Selenium Nanoparticles Antibacterial properties

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## **INTRODUCTION**

Nanotechnology is gaining the attention of researchers because of the wide application of nanomaterials in various fields like biomedical, agriculture, catalysis, optical, and electronic. (Kannan and Subbalaxmi, 2011; Kirtane *et al.*, 2021). Nanoparticles are widely used in the medical field due to their advantages over conventional drugs. (Vivek *et al.*, 2018; Afzal *et al.*, 2022). Both metallic and non-metallic nanoparticles are widely used, but compared to non-metallic nanoparticles, metallic nanoparticles gain a great interest in science because of their physico-chemical and optoelectronic properties (Krolikowska *et al.*, 2003; Krishnani *et al.*, 2022).

Selenium is an essential trace element and cofactor of many enzymes like glutathione peroxidase, thioredoxin reductase anddeiodinase(deOliveira Maia et al., 2020; Bevinakoppamath et al., 2021). The diverse roles of selenium as seleno-proteins are: antioxidant activities (Qamar et al., 2021), reproduction (Liang et al., 2016; Salam et al., 2021), muscle function (Otieno, 2017) and tumor prevention (Hatfeild, 2011). Se also modulates the photosynthesis antenna complex, protecting the chlorophyll pigment and increasing the yield of crops (Lanza and Dos Reis, 2021), like other micronutrients (Si, Zn, B, and Ca), that had a substantial influence on vegetative development of plants, floral blooming, spike longevity, and enhanced nutrient status of fruits (Lalithya et al., 2014; Sharma et al., 2013). Selenium in the nano form has gained importance as a possible supplement in the treatment of diseases due to changes in the properties and efficacy of the material (Ferro et al., 2021; Khurana et al., 2019; Zhang et al., 2004). Nano selenium has been shown to have higher biocompatibility, bioefficacy, and lower toxicity than other organic and inorganic forms of selenium (Skalickova et al., 2017; Kumar and Prasad, 2021). Biological synthesis of nano-particles mediated by plants (Husen and Siddiqi, 2014), fungi (Zare et al., 2013), algae (Ramamurthy et al., 2013), cyanobacteria (Hnain, 2013) and bacteria (Fayaz, 2010) is more acceptable in nano-engineering because of its cost-effective and ecofriendly nature (Shang et al., 2019). Selenium nanoparticles have anticancer (Kong, et al., 2011), anti-fungal (Kheradmand et al., 2014), anti-bacterial (Tran and Webster, 2011; Menon et al., 2020), anti-inflammatory (Malhotra et al., 2016), and anti-oxidant (Ramya et al., 2015) activities. It is also known to suppress the production of biofilms (Abu-Elghait et al., 2021). Presently nanoparticles of various metals using different plants are synthesized with different goals (Pawar et al., 2023; Dandapat et al., 2023., Sheikh et al., 2023).

Studies reveal that cyanobacteria are rich in bio-active molecules and polysaccharides (Celis-Plaet *al.*, 2021) protein (Kerfeld, 2004), vitamins (Raja, 2016), pigments (Mourelle et *al.*, 2017) and secrete many secondary metabolites (Engene, 2011) that help in reduction, synthesis, and capping of nanoparticles (Rezaei *et al.*, 2019). Both fresh water, and marine water cyanobacteria, has the potential to synthesize nanoparticles (Anyaogu, 2008). Nanoparticles show tremendous activity in industrial dye stuff degradation for water treatment. Many CNTs and oxide nanoparticles were used to degrade the dyes like methylene blue, crystal violet, safranin and textile dye (Sankar *et al.*, 2014, Raman *et al.*, 2016). In this study, the biosynthesis of selenium nanoparticles (SeNPs) *via* a single-step reduction of selenium oxyanion at room

temperature by using cyanobacteria isolated from both fresh and marine water has been done and their antimicrobial and photocatalytic activity has been analysed.

### MATERIALS AND METHODS

## Culture of cyanobacteria

Purified cyanobacteria samples (Phormidium sp. BDU7060, Lyngbya sp. BDU72401, Calothrix sp. UU24112 and Anabaena sp. UU2493), maintained in Laboratory, and were used in this study. The samples were washed 2 times in double distilled water and centrifuged. 2ml of samples were inoculated in 200ml of suitable culture media for both fresh water (Anabaena sp. and Calothrix sp.) and marine water (Lyngbya sp. and Phormidium sp.) in BG-11 (Allen and Stainer, 1968) and ASN-III medium (Castenholz., 1988) respectively. The cultures were grown in a controlled laboratory setting at 26  $\pm$ 2°C and 16 hours of light/8 hours of darkness. After one month of inoculation, the mass of cyanobacteria was harvested. After centrifugation and pellets were washed with double distilled water twice and dried at room temperature. After drying, the sample was crushed in a mortar and pestle for powder preparation.

### **Biosynthesis of Selenium nanoparticles**

For the synthesis of selenium nanoparticles, 1ml of cyanobacterial biomass, 8.8ml of (0.5mM/1mM) sodium selenite solution and 0.2ml of 5mM ascorbic acid were taken. Ascorbic acid was used as an initiator of the reduction reaction (Pyrzynska and Sentkowska., 2021). Sodium selenite and ascorbic acid solutions were transparent in color, which turned greenish after the addition of algal extract. The pH was maintained at neutral by adding 0.1N NaOH and 0.1N HCl solution. 0.5mM/1mM of sodium selenite along with ascorbic acid without cyanobacterial biomass was taken as control. Beakers containing solutions were covered with aluminium foil and kept in a dark room. All the steps were carried out at room temperature for 7 days to 15 days. The formations of SeNps were monitored by observing color changes. For separation and purification of SeNPs from crude matrix, samples were allowed to centrifuge at 10,000 rpm for 20 min. The pellets were washed two times with double distilled water, followed by three washes with absolute ethanol, and allowed to air dry overnight. The powder form of the extract was used for further analysis.

All the chemicals and cyanobacteria culture media (ASN-IIIand BG11) were purchased from Sigma Aldrich (https://www.sigmaaldrich.com/).

#### **Characterization of SeNPs**

#### **UV-VIS Absorbance Spectroscopy Analysis**

Nanoparticle formation was monitored by observing the changes in colour of the extract after addition of sodium selenite and ascorbic acid (0.5 mM) and incubation for scheduled time period. The bio-reduction of Sodium selenite in the extract solution was analyzed by taking the absorbance with the Shimadzu- 1900 UV-Vis spectrophotometer at 250-600 nm in room temp (Premasudha *et al.*, 2015). Double distilled water was used as a control.

#### Transmission electron microscopy(TEM) analysis

The size and shape of the green synthesized SeNPs was

determined using transmission electron microscopy (JOEL JEM-1230) at 80 kV. The SeNPs were sonicated for uniform distribution. After that, a drop of aqueous sample was placed on a carbon coated copper grid. It was then dried under an infrared lamp prior to photography (Fesharaki *et al.*, 2010).

### X-ray diffractometer (X-RD) analysis:

The physical nature of SeNPs was analyzed by X-Ray diffractometer (Model-Xpert Pro P analytical). Briefly, a dried thin film of SeNPs solution was made on a glass slide and observed under an X-ray diffractometer. The X-ray diffractometer machine operates at a voltage of 40 kV and a current of 40 mA with CuK( $\alpha$ ) radiation of 1.54 A° wavelength.

The scanning was carried out with a 2 angle from  $2^{\circ}$  to  $90^{\circ}$  with a constant time. (Srinivasan et *al.*, 2017).

## Fourier-transform infrared spectroscopy observations (FT-IR) analysis

To determine the functional group of the bio-molecule capping on the surface of SeNPs, the centrifuged and dried samples of SeNPs were subjected to FTIR analysis (Perkin Elmer, Model Spectrum-GX Range/resolution MID IR range). For FTIR, a small amount of dried biomass was grounded with KBr pellets at room temperature with a resolution of 4 cm<sup>-1</sup> and a range of 450-4000 cm<sup>-1</sup> (Ramamurthy et *al.*, 2013)

#### Antibacterial susceptibility test

The antibacterial activity of SeNPs was determined by an agar disc diffusion assay (Ho et al., 1998). The LB agar medium was prepared according to the procedure, and was sterilized by autoclaving at 121°C. After sterilization, the media was poured onto sterile Petri-plates and allowed to cool until the agar solidified. Then 200ul of bacterial culture were inoculated on to the Petri-plates. Filter paper discs (6mm) were placed on LB agar. Different concentrations of antibiotics (100ug/ml, 200ug/ml, 300ug/ml, and 400ug/ml) were given to disc with SeNPs, while 400ug/ml of chloramphenicol and 5mM Sodium selenite were given as a control. The plates incubated at 37°C for 24 hours and the zone of inhibition around the disc was calculated.

#### Photo catalytic activity of biogenic SeNPs

To examine the efficiency of SeNPs for dye reduction, the stock solution of crystal violet and Safranin 10mg/L were prepared. 1ml of dye stock solution and nanoparticles synthesized from Calothrix UU24112 were added and stirred for 30 minutes until absorption-desorption equilibrium was reached. The photocatalytic degradation was calculated by the given equation:

### $D\% = (C1-C2/C1) \times 100$

Here, C1 is the initial prepared concentration of solution, and C2 is the end point concentration of dye. The degradation rate is annotated as D%.

#### **RESULTS AND DISCUSSION**

#### Synthesis of selenium nanoparticles

The synthesis of selenium nanoparticles was primarily observed by the change in the color of the solution from greenish to a brick red color. This color change is may be due to surface Plasmon resonance, which indicates the synthesis

	Zone of Inhibition (mm)								
Dose of SeNPs (mg/mL)	Control (Na <sub>2</sub> SeO <sub>3</sub> )	Calothrix	Anabaena	Lyngbya	Phormidium				
0.1		$21 \pm 1.4$	$21 \pm 2.3$	$21 \pm 2.06$	$20 \pm 1.4$				
0.2	$18.7 \pm 1.2$	$25.6 \pm 2.01$	$22 \pm 1.05$	$23 \pm 1.4$	$20.5 \pm 2.5$				
0.3		$29.4~\pm~1.8$	$22.8~\pm~1.6$	$29 \pm 3.11$	$21.8 \pm 1.17$				
0.4		$34.1 \pm 1$	$23.1 \pm 2.1$	$31 \pm 2.06$	$22 \pm 2.4$				

Tabl	e 1:	Calcu	lated	inhibitior	zone	by se	lenium	nanop	particle	es on l	E Co	li usin	g disc	: diffu	ision	assay
													0			



Figure 1. Formation of SeNPs afollowing incubation of cyanobacterial species with different molab concentration (0.5mM and 1 mM) of Sodium Selenite at room temperature for 7 days and 15 days

of selenium nanoparticles. The color change is faster in *Calothrix sp. UU24112,Lyngbya sp.BDU 72401,* and *Anabaena sp. UU2493,* but a very slow color change was seen in *Phormidium sp. BDU7060.* 

# Characterization of SeNPs by UV-Visible (UV-Vis) spectral analysis

Nanoparticle synthesis was further confirmed by taking UV-Vis spectra at 250-600nm. The absorption peak was recorded on the 7<sup>th</sup> and 15<sup>th</sup> days (Fig. 1a and b). The absorption maxima differ with the time interval. This indicates the synthesis of selenium nanoparticles. The SeNPs synthesized from 0.5 mM sodium selenite showed better absorbance than 1 mM. The SeNPs synthesized from 0.5 mM sodium selenite were taken for further characterization. The absorption maxima were seen in the range of 420-460nm.

#### Determination of size of SeNPs using TEM analysis

The size and shape of selenium nanoparticles were determined using TEM. Nanoparticle shape and size varies from species to species (Fig. 2 a-f). The size of SeNPs obtained from *Anabaena* sp. *UU2493* was found to be 100 nm, while the size of SeNPs from *calothrix sp.* (*UU24112*), *Phormidium sp.*  (BDU7060), and Lyngbya sp. (BDU72401), were observed to be 50 nm, 100 nm and 80 nm respectively. All are spherical in shape. The smallest size SeNPs were observed in *calothrix* sp. UU24112 and the largest size SeNPs were observed in the case of *Phormidium* sp. BDU7060.

#### Determination of crystal structure of SeNPs using X-RD

The crystal structure of SeNPs was determined by X-RD.The number of Bragg reflections with 2  $\theta$  values in the X-RD patterns was found to be 31.64° of SeNps synthesized from *Lyngbya sp. BDU 72401*, The SeNps synthesized by *Phormidiumsp.* BDU7060 should three peculiar 2  $\theta$  values *i.e.*, 31.64° 31.74° and 45.40° (Fig. 3 c, d). The SeNPs synthesized by both the fresh water species (*Calothrix UU24112* and *Anabaena UU2493*) does not show any Bragg reflection 2 $\theta$  values reflecting with synthesize amorphous nature of SeNPs (Fig. 3 a, b). This clearly illustrates that the SeNPs formed from the sodium selenite by cyanobacteria were crystalline as well as amorphous in nature.

## Determination of bio-reducing agent through functional group analysis using FTIR

The FTIR analysis was carried out to find out possible bio-



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Figure 2: TEM Micrographs displaying the size and morphology of SeNPs formed following incubation of cyanobacterial extract with Sodium Selenite for 15 days (a-c) Phormidium and Lyngbya (d-f) Calothrix and Anabaena

reducing agents present in the cyanobacteria extract, which helps in reducing and capping of SeNPs. The FTIR spectral

peaks of SeNPs of Anabaena sp.UU2493 were found at 3424.18 cm<sup>-1</sup>, 2921.41 cm<sup>-1</sup>, 1650.82 cm<sup>-1</sup>, 1036.00 cm<sup>-1</sup>, calothrix sp. UU24112, at 3430.28 cm<sup>-1</sup>, 2923.19 cm<sup>-1</sup>, 1638.14 cm<sup>-1</sup>, 1403.45 cm<sup>-1</sup>, 1032.80 cm<sup>-1</sup>, 673.65 cm<sup>-1</sup>, Lyngbya sp.BDU 72401 at 3422.87cm<sup>-1</sup>, 2923.70 cm<sup>-1</sup>, 2853.05 cm<sup>-1</sup>,1638.11 cm<sup>-1</sup>, 1403.43 cm<sup>-1</sup>,1044.59 cm<sup>-1</sup>, 859.99 cm<sup>-1</sup>, 583.85 cm<sup>-1</sup> and in Phormidium sp. BDU7060at 3489.40 cm<sup>-1</sup>,3289.87 cm<sup>-1</sup>, 1647.48 cm<sup>-1</sup>, 1451.77 cm<sup>-1</sup>, 858.26 cm<sup>-1</sup>and 522.65 cm<sup>-1</sup> as shown in figure 4. The strong absorption band of 3424.18 cm<sup>-1</sup>, 3430.28 cm<sup>-1</sup>, 3422.87 cm<sup>-1</sup>, 3289.87 cm<sup>-1</sup>, and 3489.40 cm<sup>-1</sup> represent the O–H stretching vibration modes of hydroxyl functional group in alcohols, and N-H stretching vibrations in amines. The absorption peak at 2921.41cm"1, 2923.19 cm-1, 2923.70 cm-<sup>1</sup> with medium intensity and 1451.77 cm<sup>-1</sup>, 859.99 cm<sup>-1</sup>, 858.06 cm<sup>-1</sup> having strong intensity corresponds to C-H stretching vibration modes of the alkanes and alkenes. The absorption 1036 cm<sup>-1</sup>, 1032.80 cm<sup>-1</sup>, 1044.59 cm<sup>-1</sup> and 1046.78 cm<sup>-1</sup> represent C-N stretching of amine. The absorption bands 1650.82 cm-1, 1638.14 cm-1, 1647.48  $cm^{-1}$  and 1451.77  $cm^{-1}$  represent C = O and C = C stretching of amide and alkene. The absorption bands 858.26 cm<sup>-1</sup>, 673.65 cm<sup>-1</sup> and 859.99 cm<sup>-1</sup> represent C-X stretching of alkyl halides and peaks at 583.85 cm<sup>-1</sup>, and 522.65 cm<sup>-1</sup> shows C-N-C bending in amines during characterization.

The broad absorption bands of OH functional group in Calothrix indicate the presence of OH pool which may be a reason that *Calothrix* synthesizes good nano-material.

## Assessment of Antimicrobial activity of SeNPs using disc diffusion method

The antibacterial studies of SeNPs against *E. coli* (MG1655) synthesized from all the cyanobacterial species showed a significant zone of inhibition at concentrations ranging from 0.1mg/ml to 0.4mg/ml when treated in combination of chloramphenicol. In this study, the antibacterial activity of selenium nanoparticles (Table 1 and Fig. 5) was evaluated using a disc diffusion test at concentrations of 0.1, 0.2, 0.3, and 0.4 mg/l with 0.4g/ml chloramphenicol, and the zone of inhibition was measured. 0.4ig/ml sodium selenite and chloramphenicol were taken as standard control for this measurement. The zone of inhibition for *Calothrix sp., UU24112* with chloramphenicol was 34.1±1mm at 0.4mg/ml, while the other three species, *Anabaena UU2493, Lyngbya* 

*BDU72401*, and *phormidium BDU 70601* had zones of inhibition of  $23.1 \pm 1$ ,  $31 \pm 1$ , and  $22 \pm 1$  respectively against *E. coli* at 0.4mg/ml.

## Assessment of photo catalytic degradation of Crystal violet and Safranin dye

Pollution from industrial dyestuffs is a major concern at the moment because they settle in bodies of water and enter the food chains of both vertebrate and invertebrate animals. For this purpose, we intend to explore the photocatalytic degradation of industrial dye by SeNps from *Calothrix UU24112*. The dye degradation study by UV-Vis absorbance of crystal violet (Fig. 6a) and Safranin (Fig. 6b) compared with control were represented. The absorbance peak comes at 500-600 nm. After each reading at a 5-minute time interval, the SeNps of *Calothrix UU24112* show a decline in absorbance compared to control in both crystal violet and Safranin solution.



Figure 3: X-ray diffraction pattern of SeNPs synthesized by Anabaena UU2493, Calothrix UU24112, Lyngbya BDU72401, Phormidium BDU70601



Figure 4:FTIR spectrum of SeNPs left panel Anabaena UU2493, Calothrix UU24112 and right panel Lyngbya BDU 72401, Phormidium BDU 70601



Figure 5: Determination of Zone of inhibition of SeNPs produced by (a) Anabaena UU2493, (b) CalothrixUU24112, (c) Lyngbya BDU72401 and (d) Phormidium BDU70601 against E. coli MT1655.

This study reveals that all chosen cyanobacterial species have the potential to synthesize SeNPs. The synthesis of SeNPs was initially determined by the color change, which clearly suggested the possible role of cyanobacteria in reducing the Na<sub>2</sub>SeO<sub>3</sub> solution into SeNPs. The exact mechanism of reducing the Na<sub>2</sub>SeO<sub>3</sub> solution into SeNPs is not very well known. However, it is supposed that the enzyme, protein, amino acids, and several other groups might be involved in the reduction of the Na<sub>2</sub>SeO<sub>3</sub> solution into SeNPs (Menon et *al.*, 2019).*Lyngbya sp.* BDU 72401, *Anabaena sp.* UU2493, and Calothrix sp. UU24112 showed very fast synthesis of SeNPs because they contain more bio-molecules or secondary metabolites that are responsible for the synthesis of selenium nanoparticles.

Color changes in cyanobacteria species took 7 days after which distinct color changes were observed due to a gradual increase in SeNPs until 15 days. Tugarova (2017) reported the synthesis of selenium nanoparticles using enzymes and thiols. Cyanobacteria contain enzymes known as nitrate reductase and sulphite reductase, which are involved in the reduction of Na<sub>2</sub>SeO<sub>3</sub> into SeNPs. It is also proposed that a number of cyanobacterial photosynthetic pigments and secondary metabolites such as phycobiliproteins, mycosporine-like amino acids (MAAs) in cyanobacterium *Anabaena variabilis* PCC (7939) (Singh, *et al.,2010)*, and carotenoids are well-known photoprotective compounds present in the cell extract, which might be involved in the synthesis of SeNPs (Maksimov, *et al.*, 2015; Zinicovscaia, and Cepoi, 2016)

UV-Vis spectroscopy is a significant and sensitive technique to give primary information about SeNPs synthesis (Boroumand et al., 2019). The synthesis of SeNPs was monitored by a change in colour from greenish to brick red due to the reduction reaction. The colour change has been reported by various authors during the synthesis of SeNPs in fungi like *Gliocladium roseum* sp. (Srivastava and Mukhopadhyay, 2013); bacteria like *Enterococus faecalis* (Shoeibi, and Mashreghi,2017; Narayanan and Sakthivel 2010) in the angiosperm *Allium sativum* (Anu et al., 2017).

Other researchers proposed that the  $\theta_{max}$  for SeNPs is 245 nm in Aspergillus terrus (Zare et al., 2013), 260 nm in garlic (Anu et al., 2017), 261 nm in Bacillus, and 271 nm in Emblica officinalis (Gunti et al., 2019). However, the higher  $\theta_{max}$  was observed at330nm in Zooglea ramera (Srivastava and Mukhopadhyay, 2013), 390 nm in Terminila arjuna (Prasad and Selvaraj, 2014) and in Citrus limon (Prasad et al., 2013), the  $\theta_{max}$  is 395.In this study, the absorbance peak ( $\theta_{max}$ ) was found at 442 nm and the continuous increase in the absorbance with increasing time of incubation clearly indicates



Figure 6. Photodegradation activity of SeNPs against (a) Crystal violet dye (b) Safranin dye following UV irradiation for 5 mins of time interval.

a gradual increase in the production of SeNPs. These data were in accordance with the previously reported spectroscopic data for SeNPs (Srivastava and Mukhopadhyay, 2016), suggesting the potential role of cyanobacteria in the synthesis of SeNPs. The UV-Vis absorption spectra of synthesized nanoparticles from cyanobacteria were exhibited in the region of 442 nm.

Evaluation of the size of selenium nanoparticles by TEM analysis of two freshwater and two marine water species suggested that *Phormidium* sp. BDU70601 synthesized large 100nm-sized nanoparticles as compared to the other three species, and Calothrix sp. showed smaller size nanoparticles. The size of nanoparticles is in the range of 1-100 nm. X-RD offers unparalleled accuracy in the measurement of atomic spacing and is the primary tool for detecting the presence of nanomaterials (Sharma et al., 2012). From X-RD data, it was confirmed that both fresh water cyanobacteria sp. (Anabaena and Calothrix) synthesizes amorphous SeNPs, which supports the findings of Yu et al. (2016), but both marine water cyanobacteria species (Lyngbya and Phormidium) synthesize crystalline as well as amorphous SeNPs, which supports the findings of Naveena and Prakash (2013). The crystalline nature of selenium nanoparticles is because of the bio-system synthesis that forms a coating that makes them thicker and larger in size. FTIR analysis was done to detect the presence of different functional groups. From this data, it was found that the amine group, carbonyl group, and hydroxyl group were involved in the reduction of sodium selenite to SeNPs, the same has been validated by Kumar and Prasad (2021). The reduction of sodium selenite to selenium nanoparticles is by stretching vibration modes of OH hydroxyl functional group in alcohols, N-H stretching vibrations in amines and having strong intensity corresponds to C-H stretching vibration modes of the alkanes and alkenes, also C-X stretching of alkyl halides and C-N-C bending in amines during characterization.

The antibacterial study showed the highest zone of inhibition in the case of SeNPs isolated from *Calothrix* UU24112, *i.e.*,  $34.1 \pm 1$  mm, while SeNPs obtained from all the other three cyanobacteria under study showed an average zone of inhibition of 22  $\pm$  1 mm. Here it is strongly mentioned that SeNPs synthesized from biogenic sources show minor inhibition, almost negligible when treated against the E. coli (MG1655) strain, but in combination with chloramphenicol they show fair antibacterial activity. In this study, small-sized nanoparticles showed the maximum zone of inhibition may be due to their small surface area to volume ratio (Singh et al., 2014). When the antibacterial activity of sodium selenite and SeNPs was compared, it was discovered that SeNPs synthesized from calothrix sp., UU24112, had the highest antibacterial activity against E. coli MG1655. Earlier reports revealed that selenium nanoparticles showed anti-fungal activities against A. fumigatus and C. albicans (Shakibaie et al., 2015), S. aureus (Tran and Webster, 2011), Rhizoctonia solani in faba beans (Hashem et al., 2021). The biogenic SeNPs synthesized are safe and can be used in other applications such as dye degradation and addition to paints for durability and shine (Shnoudeh et al., 2019). Photocatalytic degradation of Crystal Violet and Safranin was done with synthesized selenium nanoparticles of Calothrix UU24112. As selenium nanoparticles are synthesized from biogenic sources, they may be nontoxic in nature, and SeNPs of Calothrix UU24112 showed excellent dye degradation activity (Cittrarasu et al., 2021). This suggests the SeNPs synthesized from cyanobacteria will also eradicate dye stuff from industrial sites.

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