Antibacterial and Antioxidant Activities of Biosynthesized Selenium Nanoparticles Using Marine Algae Turbinaria Conides

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Abstract: Biosynthesis of selenium nanoparticles (SeNPs) has gained great interest as a simple and eco-friendly alternative to conventional chemical methods. In this study, SeNPs were synthesized by using aqueous extract of marine Algae Turbinaria conoides. The formation of SeNPs using aqueous extract of Turbinaria conoides was confirmed visually by color change and their surface plasmon resonance peak at 270 nm, measured by UV-visible spectroscopy. Scanning electron microscopy was used to determine the size and form of the synthesised SeNPs. The synthesised SeNPs had an average size of about 76 nm and were almost spherical in form. The functional groups present in SeNPs was analysed by Fourier transform-infrared spectroscopy (FTIR). Antibacterial activity of SeNPs determined by an agar well diffusion assay demonstrated a significant activity against Escherichia coli,Staphylococcus aureus, Pseudomonas aeruginosa and BTurbinaria conoides. acillus subtilus.Antioxidant activity of synthesized SeNPs determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay and 2,20-azinobis-3-ethylbenzothiazoline-6-sufonic acid scavenging assayrevealed efficient results. Our findings demonstrate that aqueous extract of Turbinaria conoides is an effective reducing agent for synthesis of SeNPs with efficient antibacterial and antioxidant activities.

Keywords: Selenium Nanoparticles, Antibacterial, Antioxidant, Turbinaria conoides.

Introduction

Nanotechnology is a significant area of contemporary research that deals with the design, production, and manipulation of particles with structures between 1 to 100 nanometers in size (1). The cutting edge of the quickly expanding field of nanotechnology is represented by nanomaterials. They excel and are indispensable in many facets of human endeavour due to their exceptional size (2). The future of nanomaterials depends on the use of green chemistry. The creation of secure, environmentally acceptable NPs and widespread acceptance of nanotechnology are the ultimate goals of this field of nanoscience (3). Incredibly distinctive characteristics of nanoparticles (NPs) are their small size, high surface area, surface charge, surface chemistry, solubility, and multifunctionality (4). Nanotechnology research now focuses on the synthesis of nanoparticles with various chemical compositions, sizes, and regulated mono-dispersities (5). There are many ways to create nanoparticles, including physical, chemical, and biological processes. The most environmentally friendly alternative to physical and chemical processes is biological based synthesis, which uses enzymes, microorganisms, and plant extracts. The advantage of using plant-based synthesis techniques over other biological ones is that they do not require the labor-intensive process of maintaining cell cultures (6). Algae are also known as bionanofactories because they produce nanoparticles that are highly stable, manageable, and require no cell upkeep (7). Algae are the naturally available plant, which are important source of phytochemicals involved in the production of metallic nanoparticles (8). Selenium is well known for its semiconductor and photoelectric properties. Additionally, it has enormous potential in a variety of scientific disciplines, including biology, physics, chemistry, and medicine. Because of their interactions with several protein functional groups (C-O, C-N, NH, and COO-), selenium nanoparticles have strong adsorptive and biological activity (9). Although it can be challenging to create stable selenium nanoparticles, these nanoparticles also demonstrate antibacterial, anticancer, antioxidant, and enzyme inhibitory properties (10).

Materials and Methods

Collection of Alage sample

Marine Algae (Brown seaweed)Turbinaria conoides were collected from Mandapam, Rameshwaramand coastal area of south India. Seaweeds were washed with sea water to remove extraneous materials and it was brought to the laboratory. Seaweed sample was washed in running tap water to remove any associated debris and then with the distilled water. After washing, the samples were dried in a blotting paper for a week.

Extraction of Aqueous extract

Freshly collected marine Algae (seaweed)Turbinaria conoides were washed with distilled water and dried in shadow place for a week at room temperature to remove the moisture content. The dried seaweeds were cut into small pieces and grinded into seaweed powder. Aqueous extract was prepared by adding 2 grams of seaweed powder into 100 ml of distilled water and boiled in a boiling water bath at 60 °C for 30 mins. The boiled extract was filtered through Whatmann No.1 Filter paper. Then this aqueous extract was stored in a refrigerator for further studies (11).

Synthesis of Selenium Nanoparticles

To make up to 50 ml of selenium nanoparticles solution, 1.3 mg of sodium selenite was weighed and dissolved in 45 ml of distilled water. It was mixed on a magnetic stirrer for 1 hour. And then 5 ml of pure algal extract was added to it. The mixture was then stirred continuously for 3 hours to obtain a homogenous mixture. The pH was adjusted at 7 i.e., neutral. It was then subjected to incubation at room temperature in dark condition for 24 hours. After 24 hours a slight colour change was observed in the sample mixture. The sample mixture was then stirred for 72 hours for complete synthesis. The sample mixture was monitored for the formation of TSeNPs by monitoring the color reaction. Afer 3 days, color change was observed from brown to brownish red. Prepared Selenium nanoparticles (TSeNPs) was poured in a petri dish and kept in a hot air oven for drying. It was allowed to dry for 3 days. Then it was scraped by using a clean spatula and crushed into a fine powder. This fine powder was then collected in tubes for further studies and for characterization analysis (12).

UV-Visible spectroscopy

UV-Visible spectroscopy was used to confirm the synthesis of TSeNPs with aqueous extract 24 hrs, 48 hrs, 5 days, 7 days and 10 days to check the stability from the initiation of reaction using MORTAS SCIENTIFIC UV - Visible true double beam spectrometer, CFR compliance. 1ml of SeNPs was taken in a suitable glass cuvette where another cuvette filled with distilled water which serves as a blank. The scanning range of the samples was set as 200 to 800 nm at a scan speed of 480 nm/min. The UV-Visible absorption spectra of the SeNPs were recorded and the absorbance was read. The data in the spectrometer were recorded and analysed by “UV winlab” software (13).

Scanning Electron Microscopy (SEM)

The morphology and size of synthesized SeNPs was observed by scanning electron microscope(SEM). The images of TSeNPs were obtained in a scanning electron microscope

(TESCAN VEGA3). The details regarding applied voltage, magnification used and size of the contents of the images were implanted in the images itself (14).

Fourier Transform Infrared Spectroscopy (FTIR)

Functional groups of biosynthesized selenium nanoparticles were analyzedby using (SHIMADZU, INTRACER 100). Two milligrams of dry powdered TSeNPs was taken and encapsulated in 100 mg of Kbr pellet in order to prepare translucent sample discs. The pelleted samples specimens were subjected in the range of wavelength 400 to 4500 cm^{-1} with a resolution of 4 [cm^{-1}]. 25 scans were done to analyse the functional components. A Perkinelmer spectrometer was employed to record FTIR spectrum .

Antibacterial activity

The antibacterial activity of biosynthesized selenium nanoparticles from marine brown seaweed (Turbinaria conoides) were investigated by screening against gram positive bacteria (Staphylococcus aureus, bacillus subtilis) and gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa) by agar well diffusion assay. Fresh overnight cultures of innoculums of each culture were spread on to sterilized nutrient agar plates.Four wells of 5 mm were cut on the innoculum spreaded plates using a sterile cork borer and the wells were named as 1, 2, 3 and 4. In all the plates, using sterile micropipette tips 80 µL of aqueous extract of Turbinaria conoides, 40 µl of sodium selenite, 60 µL of SeNPs was added on well 1, 2 and 3 respectively. And then 20 µl of gentamycin was added well 4 which serves as a positive control. The plates were incubated and incubated at 37°C for 24 hours.The antibacterial activity was assayed by measuring the diameter of zone of inhibition formed around the wells (15).

Antioxidant activity

DPPH Radical scavenging activity

The DPPH scavenging activity of the TSeNPs was measured was measured by 1,1- diphenyl-2-picrlhydrazyl (DPPH) method. Briefly, 0.4mM solution of DPPH in methanol was prepared. 2 ml of this DPPH solution was added to different concentrations (20, 40, 60, 80, 100µl) of TSeNPs and was allowed to stand at room temperature for 20 mins, and then absorbance was read at 517 m against blank samples. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. The percentage of DPPH radical scavenging activity is calculated using the equation as given below (16).

DPPH (%) = (Absorbance control - Absorbance sample / Absorbance control) x 100.

ABTS Radical scavenging activity

ABTS radical scavenging activity of the TSeNPs was measured by 2,20-azinobis-3-ethylbenzothiazoline-6-sufonic acid. Briefly ABTS solution (ABTS stock solution (7mM) and potassium persulfate solution (100mM) in methanol was prepared. 2 ml of this ABTS solution was added to to different concentrations (20, 40, 60, 80, 100µl) of TSeNPs and was allowed to stand at room temperature for 20 mins, then absorbance was read at 734 nm against blank samples. Lower absorbance of the reaction mixture indicated higher radical scavenging activity.The percentage of ABTS radical scavenging activity is calculated using the equation as given below(17).

ABTS (%) = (Absorbance control - Absorbance sample / Absorbance control) x 100.

Results and Discussion:

Alage (Seaweed) are identified and authenticated according to morphological description as Turbinaria conoides. (Figure.1)

Figure 1 :Marine Algae Turbinaria conoides

Extraction of aqueous extract

The crude sample aqueous extract was obtained as light brown colour liquid after the filtration using whatmann no.1 filter paper(Figure.2). It was collected in a clean falcon tube and then stored in a refrigerator for further use.

Synthesis of Selenium nanoparticles

Selenium nanoparticles were synthesized by marine using brown seaweed Turbinaria conoides. This is a simple and high yielding method for the synthesis of selenium nanoparticles from the brown seaweed Turbinaria conoides byusing sodium selenite as a precursor. The formation of nanoparticles started after mixing the Turbinaria conoides aqueous extract with the sodium selenite solution. This reaction showed a time dependent color shift. At the primary stage, the color of the reaction mixture was light brown, which

progressively changed to brownish red over time. The visible color changes (light brown to brownish red) of the solution confirmed the formation of selenium nanoparticles from the aqueous extract of Turbinaria conoides. (Figure.2).Similarly, SeNPs synthesized from Cyanobacterium Spirulina plantenis was detected by the formation of red color. The reduction of Se (IV) to Se (0) results in the medium color change which could be used as a visual indicator for the formation of Se (0). Since the medium color change was used as a preliminary visual indicator for the formation of nanoparticles. The change of medium color continued for three more days and stopped at the sixth day following the start of the reaction. The results revealed that the reduction of selenite to SeNPs occurs at the logarithmic phase of growth. The results of this analysis were consistent with the observation of medium color change as the indicator for nanoparticle formation (18).

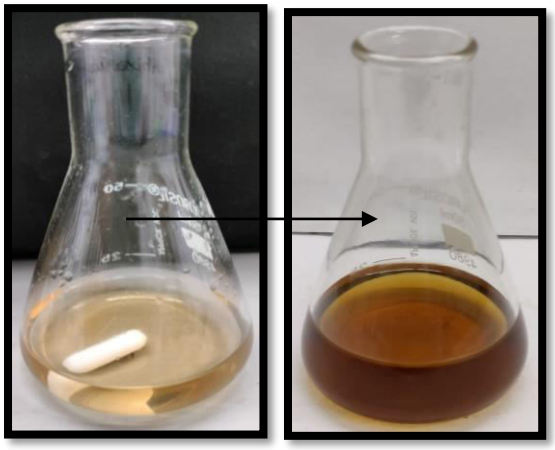


Figure 2 : synthesis of selenium nanoparticles

Ultra violet visible spectroscopy

UV-Visible spectroscopy was used to examine the formation of selenium nanoparticles. TSeNPs was visually identified by the color change of reaction mixture from brown to brownish red with time. After 72 hours of stirring and incubation, no more color change was observed. The dispersion of selenium nanoparticles showed intense color differences due to the plasmon resonance absorption. It was monitored by UV-Visible spectroscopy at a wavelength of 200–800 nm. The maximum absorption peak of SeNPs fabricated aqueous extract of Turbinaria conoides was shown at 270 nm. (Figure 3) shows a plodding increase in absorbance with respect to time of reaction, confirming the synthesis of TSeNPs. This observation revealed the completely formed selenium nanoparticles by biomolecules of marine brown seaweed Turbinaria conoides. The preliminary confirmation of selenium nanoparticles formation was concluded by plasmon resonance.Similar to this study, the maximum absorption peak of selenium nanoparticles fabricated by fruit aqueous extract of Emblica officinalis was shown at 270 nm. The data obtained show the efficacy of extracts

used as a biocatalyst for the reduction of SeO_3^{2-} to Se^0. The maximum peak noticed at 270 nm was responsible for the surface plasmon resonance of selenium nanoparticles.

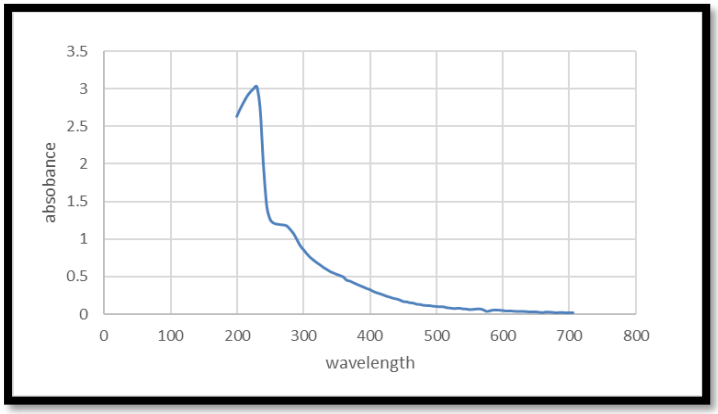


FIGURE 3 : UV–Visible spectrum of TSeNPs

Scanning Electron Microscopy (SEM)

Scanning electron microscope is employed to analyze the shape of the synthesized selenium nanoparticles. The scanning electron microscopic (SEM) images of selected selenium nanoparticles synthesized from Turbinaria conoides are presented in the (Figure. 4). According to the SEM images in Figure revealed that the shape of TSeNPs was spherical and variable in size. The particle size of TSeNPs was ranged from 55 to 76 nm that is around 100 nm. In a similar study of SeNPs synthesized from Leucas lavandulifolia confirmed that the majority of the SeNPs were spherical in shape with diameter range 56-75 nm. These particles were well distributed with good aggregation (18).

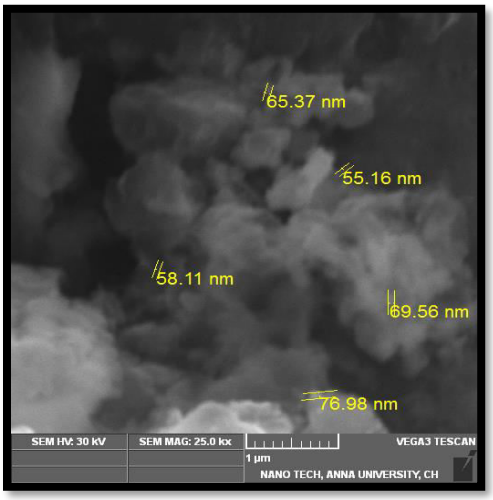


Figure 4 : SEM photographs of TSENPS

Fourier transform Infra Red Spectroscopy (FTIR)

Fourier Transform Infra-Red (FTIR) was performed using FTIR spectrometer for the detection and presence of functional groups involved in the synthesis of selenium nanoparticles. The main functional groups present in selenium nanoparticles synthesized from Turbinaria conoides are shown in (Figure.5). Wavelength absorbance reading of infra red radiations was checked for TSeNPs between the range of 400 – 4500 cm $^{-1}$. As can be seen in the FTIR spectra in the graph 2, there were six highlighted peaks centered at 3246.20 cm $^{-1}$, 2347.37 cm $^{-1}$, 1589.34 cm^{-1}, 1408.04 cm^{-1}, 1049.28 cm^{-1}, 700.16 cm^{-1}. The FTIR spectral analysis confirms the Se reduction. The absorption peak at 3246.20 cm^{-1} is due to O-H stretching of the alcohol group which denotesthe presence of alcohol and phenol groups. The peak at 2347.37 cm $^{-1}$ corresponds to strong O=C=O stretching of carbon dioxide. The small peak at 1589.34 cm $^{-1}$ is because of medium stretching N – H bending of amine group.The C–H bending form in alkanes is responsible for the shifted one at 1408.04 cm$^{-1.}$ The absorption peak at 1049.28 cm^{-1} is because of the presence of C – F stretch of alkyl and aryl halides. The strongest peak at 700.16 cm $^{-1}$ can be attributed to the strongC – Br stretch of alkyl and aryl halides. Similar to this study, the FTIR spectrum determined the active components functional groups according to the values of the infrared absorption bands. The absorption peaks at 3346.5464 cm^{-1} and 3435.0658 cm^{-1} resulted from -OH stretching of the aromatic rings, and the presence of alcohol and phenol groups. A peak at 2933.1506 cm^{-1} represented the stretching vibration of C-H of alkenes and ether-me (isothiocyanate). There were amide I bands at 1659.3297 cm^{-1} (C=O stretch of the ester group), amide II bands at 1536.7124 cm^{-1} (N-H bending), 1444.9020 cm^{-1} (C-H asymmetric bending in CH_2 and CH_3 groups), and bands at 1118.1212 cm^{-1} and 852.7263 cm^{-1} resulting from aliphatic amines and carboxylic acids, respectively, in the lower wavelength range. The absorption bands at 613.5960–789.6294 cm^{-1} (Phaeophyta and Chlorophyta) showed stretching of C=S, indicating the presence of sulfides in the tested algae (18).

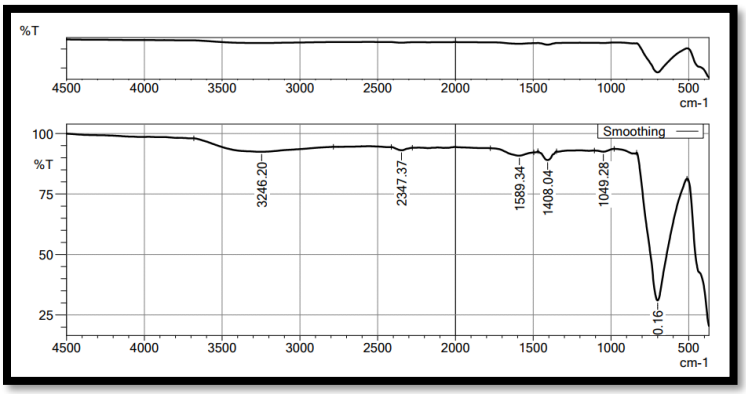
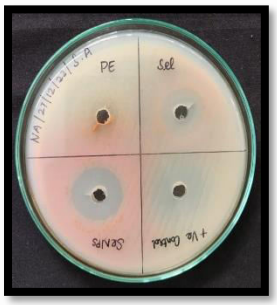


Figure 5 : FTIR spectra of turbinaria conoides mediated selenium nanoparticles

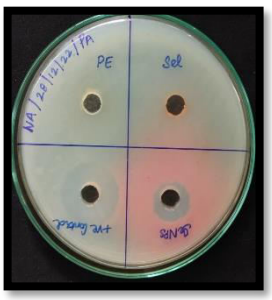
Antibacterial activity of Tsenps

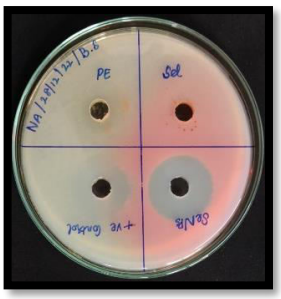
The nanoparticle stability is an important criterion for degrading bacterial cells, which is a critical prerequisite for many biological applications. SeNPs were well known to have strong antibacterial activities. The antibacterial activity of Turbinaria conoides mediated selenium nanoparticles were examined by agar well diffusion method against gram positive bacteria (Staphylococcus aureus, Bacillus subtilis) and gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa). In Antibacterial activity of biosynthesized TSeNPs, the zone of inhibition against the selected pathogens was shown in (Figure.6). Turbinaria conoides mediated selenium nanoparticles exhibited efficient zone of inhibition against the pathogens. The maximum zone of inhibition (25 mm) is observed specifically against Bacillus subtilis. The minimum zone of inhibition (17 mm) is observed against Pseudomonas aeruginosa, whereas the zone of inhibition against Staphylococcus aureus and Escherichia coli are 22 mm and 20 mm respectively. The results confirmed that SeNPs synthesized by Turbinaria conoides have potent antibacterial activity. Several studies revealed that the antibacterial activity of the Portulacaoleraceaaqueous extract mediated selenium nanoparticles tested against Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus)and gram-negative bacteria (Pseudomonas aeruginosa, Escherichia coli)exhibited 13.7 ± 0.6 mm, 14.3 ± 0.6 mm, 14.3 ± 0.6 mm and 14.4 ± 0.6 mm respectively. The activity increased with smaller sizes, and this is attributed to the increase in the surface-to-volume ratio, with small size leading to enhanced nanoparticles biological reactivity (19).






Escherichia coli **Staphylococcus aureus**






Pseudomonas aeruginosa **Bacillus subtilis**

Figure 6 :Antibacterial activity of Tsenps

Antioxidant activity of Tsenps

The DPPH radical-scavenging analysis could be utilized to assess the antioxidant potency. The antioxidant activity of selenium nanoparticles synthesized from Turbinaria conoides was analysed by using 1,1- diphenyl-2-picrlhydrazyl (DPPH) radical scavenging assay. The ability of free DPPH radical scavenging consists in measuring the change in absorption of purple DPPH radical solution. The disappearance of the violet color informs about the level of radical neutralization contained in the DPPH solution. In this assay, the color of DPPH solution slowly changed from purple to brown color. The antioxidant activity was observed at different concentrations (20, 40, 60, 80,100 µl).Turbinaria conoides mediated SeNPsshowed 34.04% of scavenging activity at the concentration of 100 (µg/ml) (Figure.8).

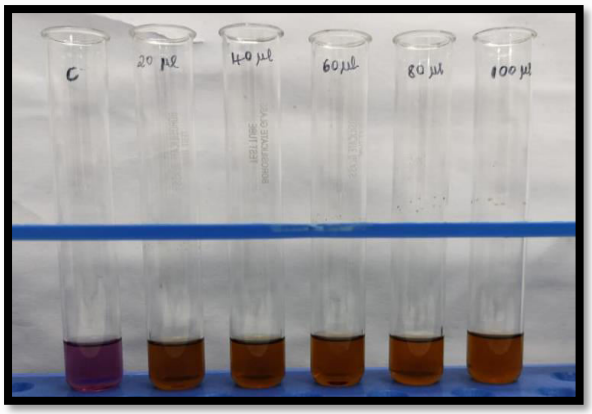


Figure 8 :DPPH radical scavenging activity of TSENPS

The ABTS radical-scavenging analysis could be utilized to assess the antioxidant potency. The antioxidant activity of selenium nanoparticles synthesized from Turbinaria conoides was analysed by using 2,20-azinobis-3-ethylbenzothiazoline-6-sufonic acid radical scavenging assay. The ability of free ABTS radical scavenging consists in measuring the change in absorption of green ABTS radical solution. The disappearance of the green color informs about the level of radical neutralization contained in the ABTS solution. In this assay, the green color of ABTS solution slowly. The antioxidant activity was observed at different concentrations (20, 40, 60, 80,100 µl).Turbinaria conoides mediated SeNPsshowed 22.70 % of scavenging activity at the concentration of 100 (µg/ml) (19) (Figure.9).

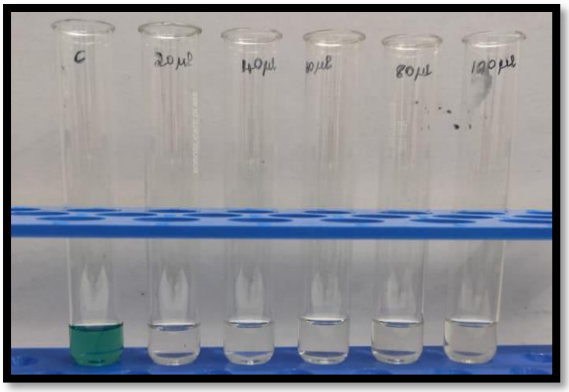


Figure 9 : ABTS radical scavenging activity of TSENPS

Conclusion

Selenium Nanoparticles (SeNPs) are gaining importance in the field of medicines due to their high surface area and unique properties than their other forms of selenium. Nowadays, green approaches are followed to synthesize the nanoparticles where the phytochemicals compounds present in act as a reducing agent for synthesizing nanoparticles. Biosynthesized SeNPs were characterized by using several characterization techniques such as UV - Visible spectroscopy, SEM, FTIR. The TSeNPs manifested potent antibacterial potentiality against gram positive and gram negative bacterial strains. They also showed an efficient antioxidant activity in DPPH and ABTS assay. The present study demonstrates that a simple, cost – effective, eco - friendly green synthesis of TSeNPs using brown seaweed Turbinaria conoides could be a competitive alternative to conventional chemicals methods. It is also suggested that the biosynthesized TSeNPs with efficient antibacterial and antioxidant hold huge potential for pharmaceutical applications. In future, this can be taken for further studies to bring selenium nanoparticles as a drug for cancer.

References

1. Colvin, V.L.S., M.C., Alivisatos, A. (2004), Light emitting diodes made from cadmium selenidenano crystals and a semiconducting polymer. Nature,370: 354-357.
2. Balashanmugam, Pannerselvam, and Pudupalayam Thangavelu Kalaichelvan. (2015), Biosynthesis characterization of silver nanoparticles using Cassia roxburghii DC. aqueous extract, and coated on cotton cloth for effective antibacterial activity.International journal of nanomedicine 10.sup2: 87-97.
3. Varma RS. (2012), Greener approach to nanomaterials and their sustainable applications. Curr Opin Chem Eng : 1:123–128.
4. S.M. Moghimi, A.C. Hunter, J.C. Murray, (2005), Nanomedicine: current status and future prospects, Faseb J.,19(3),pp.311-330.

5. Vyas, J. & Rana, S. (2017), Antioxidant activity and green synthesis of selenium nanoparticles using Allium sativum extract. Int. J. Phytomed. 9, 634 .
6. Jackson, T. C., Uwah, T. O., Ifekpolugo, N. L. & Emmanuel, N. A. (2018), Comparison of antimicrobial activities of silver nanoparticles biosynthesized from some Citrus species. Am. J. Nano Res. Appl. 6, 54–59.
7. Song JY, Kim BS. (2009), Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst. Eng., 32: 79–84.
8. Singaravelu G, Arockiyamari J, Ganesh Kumar V, Govindaraju K. (2007), A novel extracellular biosynthesis of monodisperse gold nanoparticles using marine algae, Sargassum wightii Greville. ColloidSurf B: Biointerf, 57: 97–101.
9. Husen, A. & Siddiqi, K. S. (2014), Plants and microbes assisted selenium nanoparticles: characterization and application. Journal of nanobiotechnology 12, 28
10. Jay Vyas and Shafkat Rana(2018), Synthesis of selenium nanopatocles using Allium sativum extract and analysis of their antimicrobial property against gram positive bacteria. The Pharma International journal 7(9), 262-266.
11. Dhanraj, Ganapathy, and Shanmugam Rajeshkumar. (2021), Anticariogenic effect of selenium nanoparticles synthesized using brassica oleracea. Journal of Nanomaterials: 1-9.
12. Alvi, G.B., Iqbal, M.S., Ghaith, M.M.S. (2021), Biogenic selenium nanoparticles (SeNPs) from citrus fruit have anti-bacterial activities. Sci Rep 11, 4811.
13. Alagesan, Venkatesan, and Sujatha Venugopal. (2019), Green synthesis of selenium nanoparticle using leaves extract of withania somnifera and its biological applications and photocatalytic activities, Bionanoscience 9: 105-116.
14. Zhang, Xi-Feng.(2016), Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches, International journal of molecular sciences 17.9: 1534.
15. Leela, K., Anchana Devi. (2017), Isolation, purification and application of secondary metabolites from lichen Parmelia Perlata, Biosciences Biotechnology Research Asia 14.4: 1413-1428.
16. Manzocco L, Anese M, Nicoli M.C(1998). Antioxidant properties of Tea extracts as affected by processing. Food science tech.31.pp-694-698.
17. Mossmann.T,(1983), Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods. 65: 55–63.
18. Kirupagaran, R., A. Saritha, and S. Bhuvaneswari. (2016), Green synthesis of selenium nanoparticles from leaf and stem extract of Leucas lavandulifolia Sm. and their application, Journal of Nanoscience and Technology, 224-226.
19. Gunti, Lokanadhan, Regina Sharmila Dass, and Naveen Kumar Kalagatur. (2019), Phytofabrication of selenium nanoparticles from Emblica officinalis fruit extract and exploring its biopotential applications: antioxidant, antimicrobial, and biocompatibility, Frontiers in microbiology 10 : 931.