ANTIMICROBIAL ACTIVITY OF SELENIUM NANOPARTICLES SYNTHESIZED USING MARINE GREEN ALGAE ULVA LACTUCA

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INTRODUCTION : Nanomedicine is an interdisciplinary field, where nanoscience, nanoengineering, and nanotechnology interact with the life sciences (1). Nanoparticles (NPs) are used as delivery vehicles for pharmaceutical agents, as bioactive materials. Selenium nanoparticles (SeNPs) are gaining importance owing to their excellent antibacterial properties. Ulva lactuca (Linnaeus), a green seaweed is reported to have potential anti-microbial properties (2). In this study the green seaweed Ulva lactuca (Linnaeus) is used for the green synthesis of selenium nanoparticles and studied its antibacterial activity against Oral pathogens.

Materials and methods

- Biogenic synthesis of selenium nanoparticles- Ulva lactuca was collected from the Gulf of Mannar Biosphere in Mandapam, Rameswaram. 1% aqueous extract of Ulva was prepared and filtered using Whatman number 1 filter paper, and the obtained filtrate was used for nanoparticle synthesis.(Fig 1)
- 1ml of the filtrate was mixed with the solution containing 10ml of 30mM selenious acid solution and 200 µl of 40mM ascorbic acid (Fig 2). The solution was then placed in an incubator cum shaker at 250 rpm until there was evidence of colour change suggestive of nanoparticle synthesis.
- Confirmation of the SeNPs was performed using UV-Visible spectrophotometer (Model UV- D3200) at 1, 12, 18, 24, 48, and 72 hours, following which the solution was centrifuged at 10,000 rpm for 30 minutes.
- The pellet obtained was washed with double distilled water, followed by absolute ethanol and dried in a hot air oven at 80 °C for 2 hours and stored in air-tight containers until further analysis.

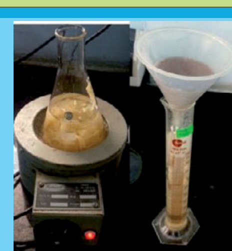


Fig 1. Preparation of Ulva filtrate

Fig 2a. Selenous acid solution 2b. Mixture of Ulva filtrate with Selenium Preparation

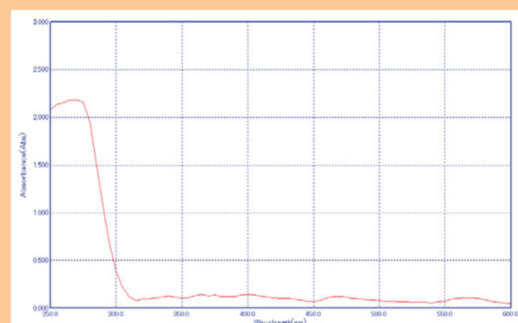


Fig 3.UV-Vis Spectroscopy

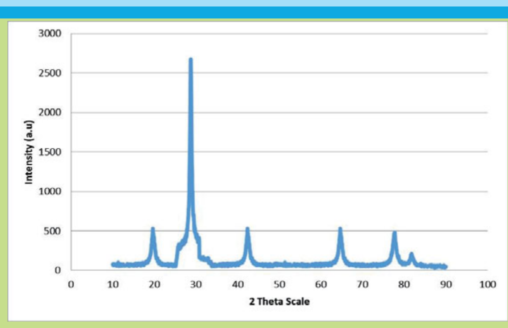


Fig 4. XRD Analysis

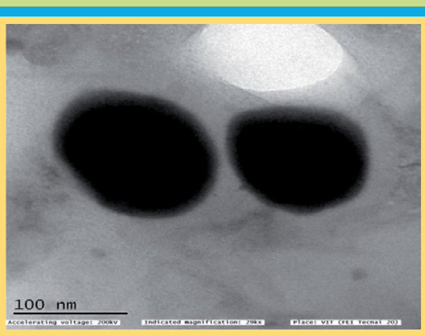


Fig 5 Transmission Electron Microscopy image

Characterisation of SeNPs- Visual observation of colour change in solution is one of the characteristic features suggesting the reduction of metal salts into nanoparticles. The solution was observed until a change in colour was evident suggestive of NP synthesis (Fig 2b).

UV-vis spectrophotometric analysis was used to confirm SeNPs synthesis by sampling 2ml aliquots of the prepared solution at periodic intervals using Shimadzu 1,700 UV–Vis spectrophotometer at a wavelength ranging between 200 and 650 nm with a scanning speed of 1,856 nm/min (Fig 3). The readings were recorded at 1, 12, 18, 24, 48, and 72 hours.

The phase composition, crystal density, and size of the synthesised NPs was assessed with an X-ray diffractometer (PAN analytical X-Pert PRO) operating at 30kV and 40mA using CuK radiation with about 1.54060Å (Fig 4).

Further, the surface morphology and size of the NPs were assessed using 200 kV high resolution transmission electron microscopy (Fig 5).

Antimicrobial activity of SeNPs against oral pathogens - Agar well diffusion method was used to determine the antibacterial activity of different concentrations of SeNPs against oral pathogens such as S. mutans, E. faecalis, C. albicans and S. aureus.

Secondary cultures of microbial suspension were dispersed evenly on the surface of MHA and rose Bengal agar plates using a sterile spreader. Different concentration of nanoparticles (25, 50 & 100 l) were incorporated through a sterile micropipette into the wells created on the agar plate using a sterile cork borer.

The plates were then incubated at 37°C for 24 h to 48 h. Commercial antibiotic ampicillin (50mg/ml) was used as positive controls for S. mutans, E. faecalis, and S. aureus, but for C. albicans, cycloheximide was used. The zone of inhibition (mm) was recorded for each plate and compared with the control. All the tests were replicated in triplicate for analysis(Fig 6).

Fig 6a

Fig 6b

Fig 6c

Fig 6d

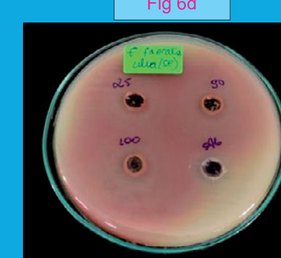
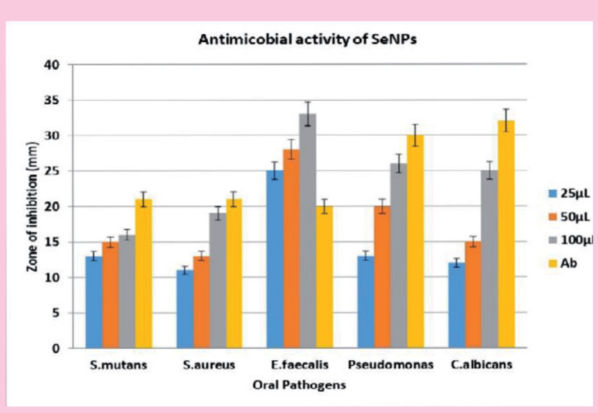


Fig 6e

Results and discussion

- Ulva lactuca, a green macro algae showed the presence of many phytochemicals like alkaloids, flavonoids, phenols, xanthoproteins and terpenoids which have anti-bacterial properties and anti-oxidant properties. These phytochemicals could have aided in reducing selenious acid to SeNPs. Huang et al. have reported plant derived flavanols and phenolic to be effective reducing agents towards nanoparticle synthesis and also function as its stabilizer (3).
- The synthesis of SeNPs were confirmed with colour change and UV-Vis spectral findings. (4) Fig 2. Shows changes in absorption band between 250-300 nm spectrum. The absorbance gradually increased from 2.05 to 2.25, indicating the reduction of nanoparticles; however ,the maximum peak was found at 270nm at 72 hr observation.
- The surface morphology assessment of the SeNPs performed using TEM revealed smooth spherical/ball shaped structures with diameter ranging from 16-132nm is, depicted in Fig 3.
- The XRD analysis (Fig 4) confirmed the desired crystalline phase of the synthesised SeNPs with the peak corresponding to 101 of the face centred cubic structure of Selenium (00-001-0848) ,similar to the results of Sharma et al.(6)



Graph 1 Mean Zone of Inhibition

The average size of the SeNPs when calculated using the Scherrer's formula was found to be 85nm. XRD also reveals background noise which could be produced by the bioactive compounds conjugated with the SeNPs.

Antimicrobial efficacy of different concentrations of SeNPs are presented in Fig 6 (A-D) and Graph1. The mean zone of inhibition (ZOI) was found to increase as the concentration of NPs increased; however, the maximum was found for ampicillin/cycloheximide except for E. fecalis (Graph 1). 100µl concentration of SeNPs produced ZOI almost that of ampicillin/cycloheximide, but 25µl and 50µl concentrations were not as effective as ampicillin/cycloheximide. Only limited evidence exists about the antimicrobial efficacy of biogenic SeNPs. Studies have reported better antimicrobial efficacy of SeNPs against gram positive bacteria as compared to gram negative and yeasts (7). However, Ulva mediated SeNPs were effective against all the organisms tested.

The effectiveness of Ulva mediated SeNPs could be considered superior to that of commercial ampicillin as the concentration of SeNPs were only 2.5 mg, 5 mg and 10 mg as compared to 50 mg of commercial antibiotics.

Conclusion :

In conclusion, the synthesized biogenic SeNPs synthesized using Ulva lactuca exhibits potential to be used as an oral antimicrobial agent. Further animal studies should be conducted to validate the above results and their application in improving the oral health of the community.

References

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