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Original article

Algal biomass as a source for novel oral nano-antimicrobial agent

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ABSTRACT

In the present study, sulphated polysaccharide Ulvan from *Ulva lactuca* was used for the synthesis of biogenic Selenium Nanoparticles (SeNPs) conjugate and Mouth rinse was prepared using this conjugate. The synthesis of nanoparticles was confirmed by UV–Visible spectrophotometry and characterized using Fourier transform infrared spectroscopy (FTIR), transmission electron microscope (TEM) and X-ray diffraction (XRD). TEM showed that the average size of the nanoparticle was 85 nm and spherical in shape. Furthermore, nanoparticle conjugates were evaluated for cell viability using MTT assay 3T3-L1 cell line and at 30 μl/ml showed 34% cell viability. The antimicrobial activity of SeNPs mouth rinse was tested against oral pathogens such as *Streptococcus mutans, Staphylococcus aureus, Lactobacillus,* and *Candida albicans* and it was effective against all tested microorganism at the concentration of 100 μl/ml. The present study has shown that Ulvan from algal biomass can be a safe and effective source for the development of oral nano-antimicrobial agents.

1. Introduction

Dental caries and periodontal disease are the most common infectious disease of the oral cavity. Plaque biofilm – which is a complex microbial community of bacteria and fungi forms a protective coating for the pathogens from host defense mechanisms and pharmaceutical agents (Fernandes et al., 2018).

There have been extensive efforts by the scientific community to develop anti-microbial agents to counter the plaque biofilm, these attempts have been futile due to low efficacy and other toxicity concerns (Song and Ge, 2019). The researchers discovered that they can synthesize nanoparticles using Nanotechnology, which has widespread application in the field of medicine. The increased surface areas of the nanoparticles provide better interactions with biological agents like bacteria compared to the traditional micron particles and they are having better chances to penetrate the bacterial cells (Webster and Tran, 2011). Thus the nanoparticle system seems to be a capable delivery vehicle for pharmaceutical agents, as bioactive materials.

Selenium is an essential micronutrient that has excellent antimicrobial, anticancerous, antidiabetic, and anti-inflammatory properties. However, in its traditional form, it has a low degree of absorption and high levels of toxicity. The nanoparticle system has addressed this problem; Nano-sized selenium possesses excellent biocompatibility with enhanced biological effects. The biological method of synthesis of Selenium nanoparticles has extensive application in the field of biomedicine due to low toxicity, targeted delivery of Nano drugs and stability (Vinković Vrček, 2018).

Seaweeds or marine algae are perennial source of chemical compounds which consists of a plethora of biologically active secondary metabolites. They are considered a potential source of antibiotic substances. *Ulva lactuca* is an edible green marine algae

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(Chlorophyta), which possess antibacterial activity against oral pathogens (Sujatha et al., 2012). Ulvan is the sulfated polysaccharide of the algae *Ulva* spp which is claimed to be responsible for its antibacterial activity and has no toxic effects (Tang et al., 2016).

Traditional oral anti-microbial agents in the form of mouth rinses or dental varnish are chemical-based containing alcohol, chlorhexidine gluconate, triclosan, etc. These chemicals causes taste disturbance, allergic stomatitis, and other side effects. This emphasizes the need for non-toxic natural products based mouth rinses which is also effective in reducing the bacterial load (Jeddy et al., 2018).

In the present study, it is hypothesized that biogenic synthesis of nano-selenium conjugate by Ulvan polysaccharide should have a synergistic effect and almost no toxic effects. Hence this study was done to assess the antimicrobial efficacy of a mouth rinse prepared from Selenium nanoparticle conjugate synthesized from Ulvan polysaccharide against potential oral pathogens such as *S. mutans, C. albicans, E. coli* and *S. aureus* in vitro.

2. Materials and methods

2.1. Ulvan extraction from Ulva lactuca

Ulva lactuca was collected from the Gulf of Mannar Biosphere in Mandapam, Rameswaram. The collected algae were washed in tap water, shade dried and stored in a dry dark place at room temperature. Ulvan extraction was done using the method described by Pengzhan et al. (2003). The mean yield of ulvan was 38.3 ± 1.2% (n = 6). One percent of the Ulvan extract was prepared by boiling 10gm of ulvan powder in 100 ml of double-distilled water in a water broth at 70 °C for ten minutes. Whatman number 1 filter paper was used to filter the solution following boiling and the obtained filtrate was used of nanoparticle synthesis.

2.2. Green synthesis of biogenic selenium nanoparticles

One ml of the filtrate was mixed with the solution containing 10 ml of 30 mM of selenous acid solution and 200 μl of 40 mM ascorbic acid. The solution was then placed in incubator cum shaker at 250 rpm until there was evidence of color change suggestive of nanoparticle synthesis. Confirmation of the SeNPs was performed using a UV–Visible spectrophotometer (Model UV-D3200) at 1, 12, 18, 24, 48 and 72 h, following which the solution was centrifuged at 10000 rpm for 30 min. The pellet obtained was washed with double distilled water, absolute ethanol and dried in a hot air oven at 80 °C for 2 h and stored in air-tight containers until further analysis.

2.3. Characterization of SeNPs

Visual observation in colour change of solution is one of the characteristic features of reduction of metal salts into nanoparticles. The solution was observed until color change was observed which was suggestive of NP synthesis. UV–vis spectrophotometric analysis was used to confirm SeNps synthesis by sampling 2 ml 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. The readings were recorded at 1, 12, 18, 24, 48, 72 h. The phase composition, crystal density, and size of the synthesized NPs was assessed with an X-ray diffractometer (PAN analytical X-Pert PRO) operating at 30 kV and 40 mA using CuKα radiation with about 1.54060 Å. Further, the surface morphology and size of the NPs were assessed using 200 kV transmission electron microscopy and FT-IR analysis of the NPs were carried out using the KBr pellet method at a resolution of 4 cm^{-1} (Shimadzu Model 400) to identify the biological compounds responsible for the synthesis of SeNPs and its stability.

2.4. Antioxidant activity - DPPH radical assay

The DPPH free radical scavenging activity of SeNPs was determined using the method described here (Qidwai et al., 2018) Typically, different concentration (10–50 μg/ml) of nanoparticles was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 μl of 50 mM Tris-HCl buffer(pH 7.4), and incubated for 30 min. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. Ascorbic acid was used as the standard controls. The percent inhibition was calculated from the following equation: % Inhibition = [Absorbance of control − Absorbance of test sample/Absorbance of control] × 100

2.5. Cell viability assay

The viability of the cells was assessed by MTT assay (Merloo 2011) using 3 T3 L1 cell line. The cells were plated separately in 96 well plates at a concentration of 1 × 105 cells /well. After 24 h, cells were washed twice with 100 μl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with the test material for 24 h. At the end of the treatment period, the medium was aspirated and serum-free medium containing MTT (5 mg/ml) was added and incubated for 4 h at 37 °C in a CO2 incubator. The MTT containing medium was then discarded and the cells were washed with PBS (200 μl). The crystals were then dissolved by adding 100 μl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometrical absorbance of the purple-blue formazen dye was measured in a microplate reader at 570 nm (Robonik ELISA analyzer). Cytotoxicity was determined using Graph pad prim5 software.

2.6. Preparation of mouth rinse

Ulva Selenium in an amount of about 98%; about 0.4% of essential oil (Thymol); and an emulsifier (Sodium stearoyl lactylate) in an amount of about 1.6%, was used to prepare the mouthwash.

2.7. Antimicrobial activity of Ulvan conjugate mouth rinse against oral pathogens

The agar well diffusion method was used to determine the antibacterial activity of different concentrations of SeNPs against oral pathogens such as *Streptococcus mutans*, *Lactobacillus*, *Candida albicans,* and *Staphylococcus aureus.* Secondary cultures of microbial suspension was dispersed evenly on the surface of Muller Hinton agar and rose Bengal agar plates using a sterile spreader. Different concentrations of nanoparticles (25, 50 & 100 μl) 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 (50 mg/ml) was used as positive control and the zone of inhibition (mm) was recorded for each plate and compared with control.

3. Results and discussion

Ulva lactuca, a green macroalgae showed the presence of many phytochemicals like alkaloids, flavonoids, phenols, xanthoproteins, and terpenoids which has anti-bacterial properties and antioxidant properties.

3.1. Characterization of SeNPs

3.1.1. UV–Vis spectra analysis

The synthesis of SeNPs was confirmed by visual observation with mild color change and UV–vis spectral findings. UV–Vis spectrophotometer is considered one of the best tools to assess the optical properties and synthesis of nanoparticles as it is highly sensitive towards the size of the nanoparticles synthesized. Fig. 1 showed the changes in the absorption band between 250 and 300 nm spectrum and the absorbance gradually increased from 2.05 to 2.25 indicating the reduction of nanoparticles, however, the maximum peak was found at 270 nm at 72 h observation. The color change occurred after 18 h, but it was evident only after 72 h and correlated with the peak found in the UV spectral reading at 270 nm suggestive of peak production of nanoparticle synthesis (Fig. 1). SeNPs synthesized using other plant extracts were found to have an absorbance spectrum in the range of 270–350 nm similar to ours (Rajeshkumar et al., 2018).

3.1.2. Transmission electron microscopy & X-ray diffraction analysis

The surface morphology assessment of the SeNPs performed using TEM revealed spherical and pseudo spherical shaped structures with a mean diameter of 85 nm with clear background (Fig. 2). Similar spherical shaped structures with a diameter of 50–100 nm was observed when synthesis of selenium nanoparticles using rhizobacterium (Kamnev et al., 2017).

The XRD analysis is shown in Fig. 3 confirmed the crystalline phase of the synthesized SeNPs with the intense peaks at 28.92, 44.12, and 66.23 corresponding to 111 of the face-centered cubic structure of selenium (00-001-0848). These results were similar to (Zhang et al., 2019). XRD also revealed some background noise that could be produced by the bioactive compounds conjugated with the selenium nanoparticles.

3.1.3. FT-IR assessment

FT-IR assessment is the major technique used to identify the chemical groups present in the nanoparticles powder and chemical group responsible for nanoparticle synthesis and its stability. Fig. 4 illustrates the peaks at 1096 cm^{-1} corresponding to C—O stretch ethers, 1387 $cm^{-1}$to N═O bend nitro groups, 1622 $cm^{-1}$to C═O stretch amides, and 3388 cm^{-1} represents N—H stretch secondary amines, confirming the presence of secondary metabolites and reducing groups present in the plant extract which are responsible for biogenic SeNPs synthesis. Further, the sharp band of brag peak confirms the stabilization of the synthesized Se NPs (Fig. 5).

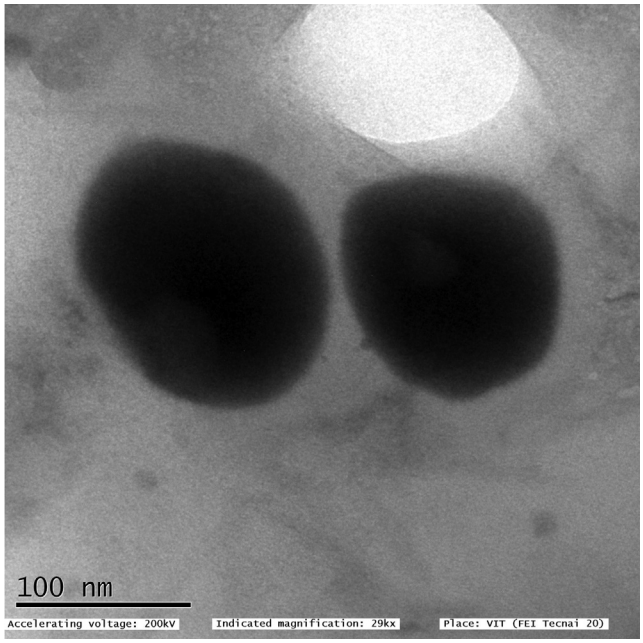

Fig. 2. Transmission electron microscope (TEM) micrograph of SeNPs produced by the Ulvan from *Ulva lactuca*.

3.2. Cell viability assay

MTT assay examined the cytotoxic effect of the SeNPs. As shown in Fig. 5, the SeNPs showed excellent cell viability on the 3T3 L1 cell line at all concentrations (5%, 10%, 20%, 30%). Previous studies have shown that SeNPs possess anti-cancer activity (Yu et al., 2012; Zhang et al., 2004). The cell viability assay shows that the SeNPs are non-toxic at different concentrations, proving its potential to be used as a safe agent.

3.3. Anti-oxidant activity

DDPH assay found biogenic SeNPs to possess effective antioxidant properties when compared to ascorbic acid at all the

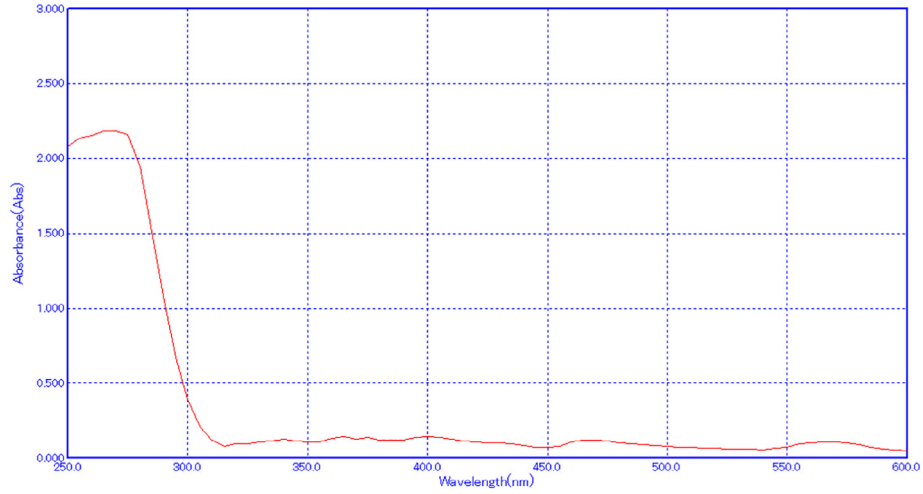

Fig. 1. UV–vis absorption spectrum of SeNPs produced by the Ulvan from *Ulva lactuca*.

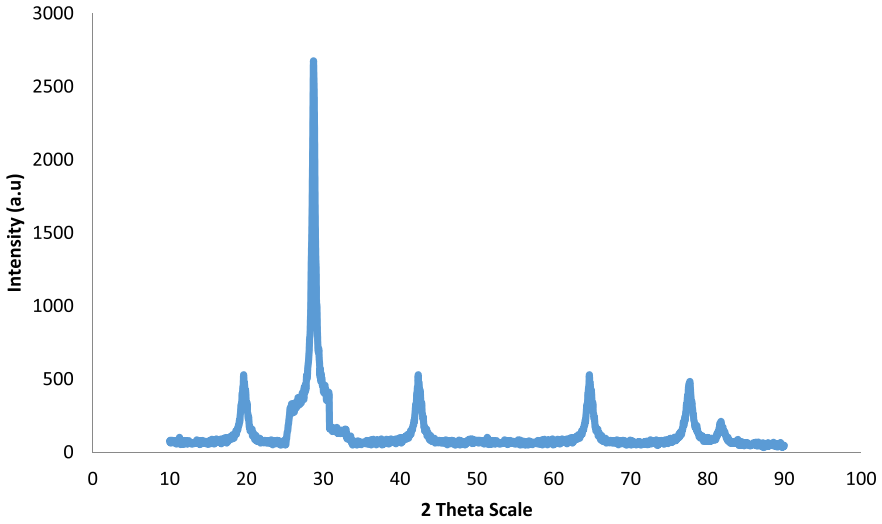

Fig. 3. X-ray diffraction (XRD) spectra of SeNPs produced by the Ulvan from *Ulva lactuca*.

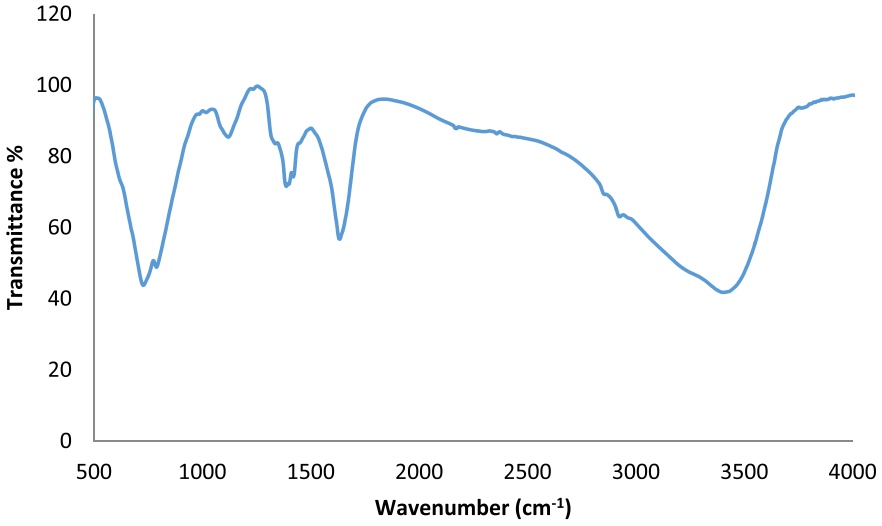

Fig. 4. Fourier transform infrared (FTIR) spectroscopy of SeNPs produced by the Ulvan from *Ulva lactuca*.

concentrations tested (Fig. 6). The efficacy was dose-dependent, and 50 µl/ml caused 93.15% inhibition. The synthesized biogenic SeNPs holds great potential as an antioxidant and could be used effectively in a myriad of medical applications.

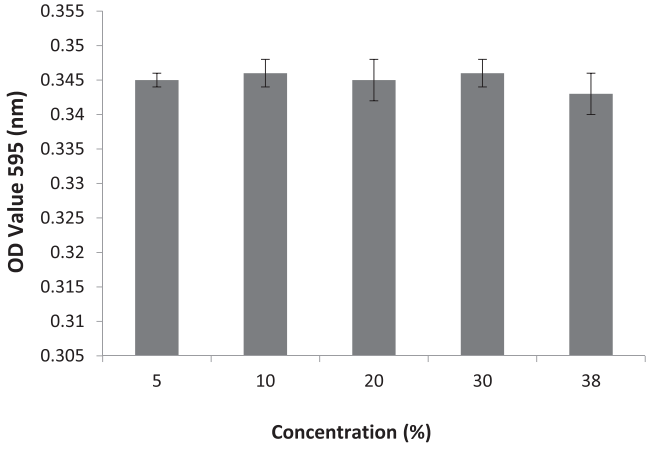

Fig. 5. Cell viability assay after treatment with SeNPs for 24 hrs compared to control.

3.4. Antimicrobial activity against oral pathogens

Antimicrobial efficacy of different concentrations of Ulvan-Selenium conjugate mouth rinse is presented in Figs. 7a and 7b. The mean zone of inhibition (ZOI) was found to increase as the concentration of NPs increased. 100 µl and 50 µl concentration of mouth rinse produced ZOI almost the same or superior to that of ampicillin/cycloheximide, but 25 µl 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 (Cremonini et al., 2016), However, Ulva Selenium conjugate mediated mouthwash was effective against all the organisms tested. The effectiveness of mouthwash could be considered superior to that of commercial ampicillin as the concentration of SeNPs was only 2.5 mg, 5 mg and 10 mg as compared to 50 mg of commercial antibiotics.

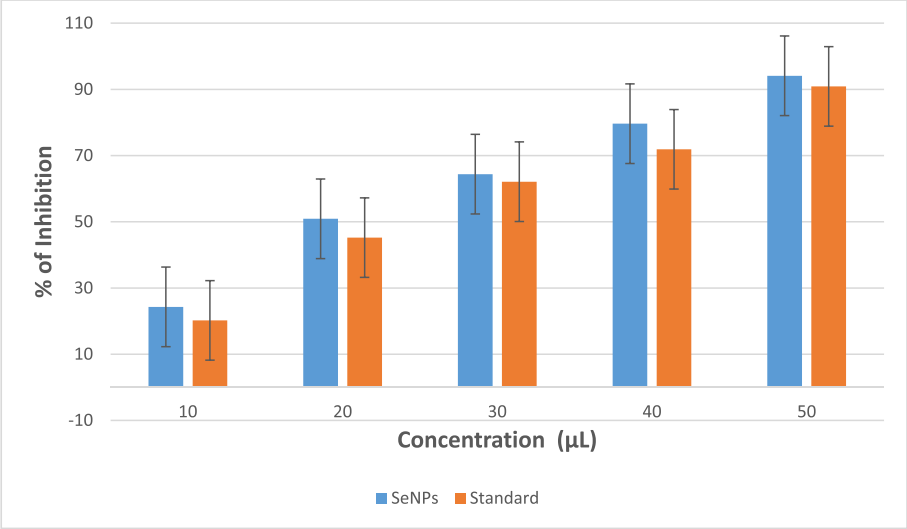

Fig. 6. DDPH anti-oxidant assay of SeNPs produced by the Ulvan from *Ulva lactuca*.

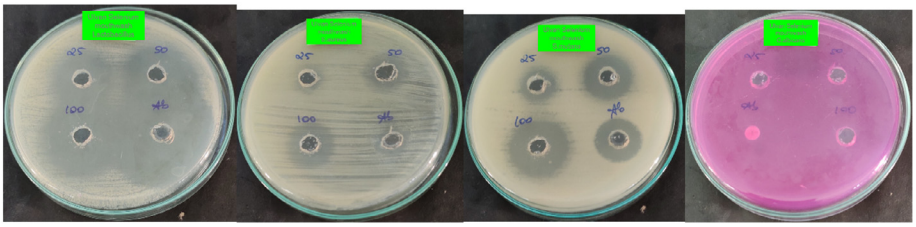

Fig. 7a. Antimicrobial activity of SeNPs produced by the Ulvan from Ulva *lactuca* (*Lactobacillus, S. aureus, S. mutans & C. albicans*).

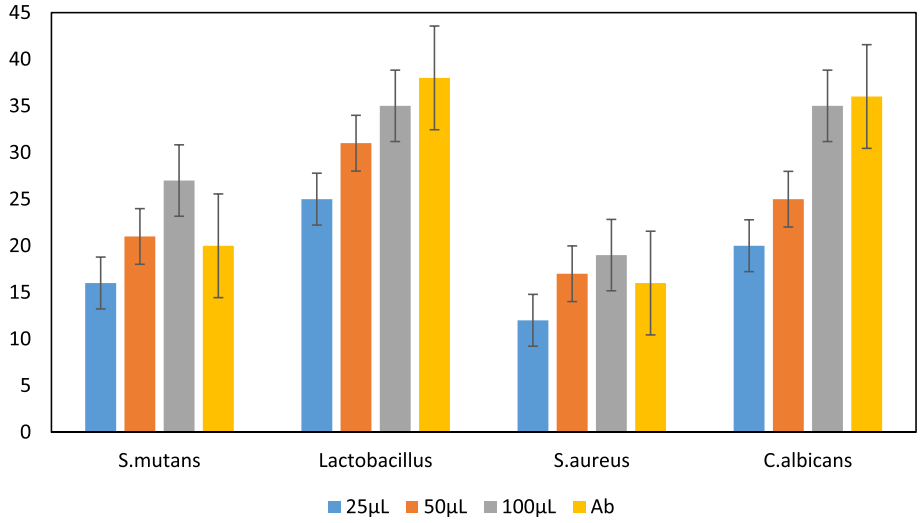

Fig. 7b. Anti-microbial activity of Ulvan-SeNPs mouth rinse against oral pathogens.

4. Conclusion

Ulvan polysaccharide extracted from *Ulva lactuca* was used for the synthesis of Selenium particle conjugate. The formation and stability of nanoparticles were confirmed by UV–Vis spectroscopy and TEM showed the average size of the nanoparticles to be 85 nm. The SeNPs possessed excellent anti-oxidant properties and also showed nil toxicity against cell lines. The anti-microbial activity of the mouth rinse showed they were as effective as antibiotics against *Lactobacillus, C.albicans* and superior effect on *S.mutans, S.aureus*. This study shows that mouth rinse from algal biomass as a source can be an excellent alternative to chemical-based oral anti-microbial products.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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