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Morinda Citrifolia Mediated Biogenic Synthesis of Selenium Nanoparticles and Evaluation of its Antimicrobial and Antioxidant Activity

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***Abstract*---**Purpose: The aim of the study is to assess its potential to synthesize selenium nanoparticles (SeNPs) with Noni fruit extract and augment its antimicrobial and antioxidant activity. Materials and methods: The synthesized SeNPs were confirmed using UV-vis spectroscopy and transmission electron microscopy (TEM). Antimicrobial activity against *S. mutans, S. aureus, E. faecalis, C. albicans* was tested using agar diffusion; antioxidant potential assessed on 2,2-diphenyl-2-picrylhydrazyl hydrate oxidation. Results: UV-vis, and TEM confirmed the synthesis of SeNPs and revealed smooth spherical nanoparticles (25-100nm). SeNPs exhibited significant antimicrobial activity for the provided concentration against all organisms and excellent antioxidant potential in a dose dependent manner (84% inhibition/50 µl concentration). Conclusion : *Morinda citrifolia* augmented biogenic SeNPs is a cost effective yet efficient method and they show great potential as oral antimicrobial agent with superior antioxidant properties and is eco-friendly.

***Keywords*---**noni fruit extract (NFE), selenium nanoparticles, green synthesis, antimicrobial activity, antioxidant, local drug delivery

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Introduction

The quest for ideal oral antibacterial agent has galvanized oral health scientists over the centuries with limited success. The use of nano particles as anti-microbial agents has been promising owing to their physical and chemical properties (1) . The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at a therapeutically optical dose (2). Selenium (Se) is an essential element in human and animal body in less concentration. It is a necessary dietary constituent of at least 25 human proteins and enzymes(3). Selenium nanoparticles (SeNPs) is usually synthesized through physical methods such as laser ablation, UV radiation, and hydrothermal techniques(4). Chemical synthesis is mediated by precipitation, acid decomposition, and catalytic reduction using ascorbic acid, glucose, sulfur dioxide, and sodium dodecyl sulfate which is extremely toxic and unsafe for biological applications(5) whereas green synthesis or biological synthesis of SeNPs is safe, eco-friendly, inexpensive and nontoxic . Moreover, biologically made SeNPs are more stable due to the natural coating of organic materials over the surface, which do not allow nanoparticles to be aggregated with the time period(6). Biosynthesis of nanomaterials using plant extracts has more advantages than other biological methods because it is inexpensive and does not require any special conditions(7)

Noni is the common name for Morinda Citrifolia in India, a small to medium sized tree (3–10 m high) with a pantropical distribution(8). Noni fruit and leaves have been widely used throughout history among Pacific Islanders as well as in Southern and Southeast Asia. Recent evidences has suggested noni fruit extract has a high anti oxidant potential(9). The antioxidant properties of noni juice, likely, contribute to its immune modulating activity. This attribute can be least partially responsible for noni's anti-inflammatory activity. The relationship among inflammation, the immune system, and reactive oxygen species (ROS) is well established in the scientific literature(10). There are evidences of biogenic synthesis of SeNP using leaf extract of Capsicum annuum, seed extract of fenugreek , leaf extract of lemon, flower extracts of Bougainvillea spectabilis, aqueous extract of Allium sativum. (11)There is no research on green synthesis of SeNP with noni extract. Thus it makes a ideal component for synthesis for Selenium Nanoparticles

Thus the aim of the present study is to assess the efficacy of fruit extract of Morinda Citrifolia in synthesizing biogenic SeNPs with bioactive compounds, and to evaluate its potential to augment the antimicrobial efficacy of SeNPs against potential oral pathogens such as *S. mutans*, *C. albicans*, *E. coli*and *S. aureus*in vitro. Furthermore, we also assessed the antioxidant potential of the biogenic SeNPs and its toxicity using brine shrimp lethality assay(BSLA).

Materials and Methods

Biogenic synthesis of Se Nano particles

Noni was obtained from dedicated ayurvedic pharmacy and inspected for purity and phytochemical composition prior to use. Aqueous extract of Noni was

prepared by boiling 1 gm of Noni powder in 100 mL of double distilled water in a water broth at 70^{o}C for ten minutes to obtain 1% of the Noni extract. Whatman number 1 filter paper was used to filter the solution following boiling, and the obtained filtrate was used for nanoparticle synthesis.0.519 gm of 20MM of sodium selenite dissolved in 70 mL of distilled water. 70 mL of sodium selenite solution + 30 mL of noni filtered extract is mixed to obtain the SeNP. 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 10000 rpm for 30 minutes. The pellet obtained was washed with double distilled water, followed by absolute ethanol and dried in hot air oven at 80^{o}C for 2 hours and stored in air tight containers until further analysis.

Characterization of SeNPs

Visual observation of colour change in solution is one of the characteristic features suggesting a reduction of metal salts into nanoparticles. The solution was observed until a change in colour was evident , suggestive of NP synthesis 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 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 hours. The phase composition, crystal density and size of the synthesized NPs were assessed with a X--ray diffractometer (P(PAN analytical XAN analytical X--Pert PRO) operating at 30 Pert PRO) operating at 30 kV and kV and 4040 mA using CuKα radiation with about 1.54060mA using CuKα radiation with about 1.54060 Å. Further, the surface morphology and size of the NPs were assessed using 200 kV high resolution TEM (Fig size of the NPs were assessed using 200 kV high resolution TEM). Analysis of the NPs were carried out using the KBr pellet method at a resolution of 4 cm pellet method at a resolution of 4 cm--1 1 (Shimadzu Model 400) to identify the biological (Shimadzu Model 400) to identify the biological compounds responsible for the synthesis and stability of SeNPs.

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 Muller Hinton agar and rose Bengal agar plates using a sterile spreader. Different concentration of nanoparticles (25, 50 & 100 µL) were incorporated through a sterile micropipette into the wells created on the agar plate using sterile cork-borer and 100 µL of the solution had a SeNPs concentration of 10 mg. The plates were then incubated at 37°C for 24 h to 48 h. Commercial antibiotic ampicillin (50mg/mL) was used as positive control for *S. mutans*,*E. faecalis, S. aureus* but for *C. albicans,* cycloheximide was used and the zone of inhibition (mm) was recorded for each plate and compared with SeNPs. All the tests were replicated in triplicate for analysis .

Antioxidant activity -DPPH assay

The 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH) free radical scavenging activity of SeNPs was determined to assess its antioxidant potential. Various concentrations (10-50 µg/mL) of nanoparticles were mixed with 1 mL of 0.1 mM DPPH in methanolsolution 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 controland the percent (%) inhibition was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of test sample}] \times 100}{\text{Absorbance of control}}$$

Results

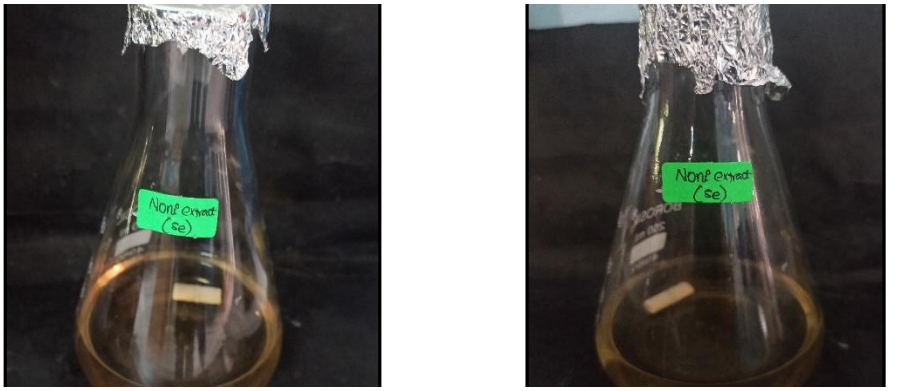
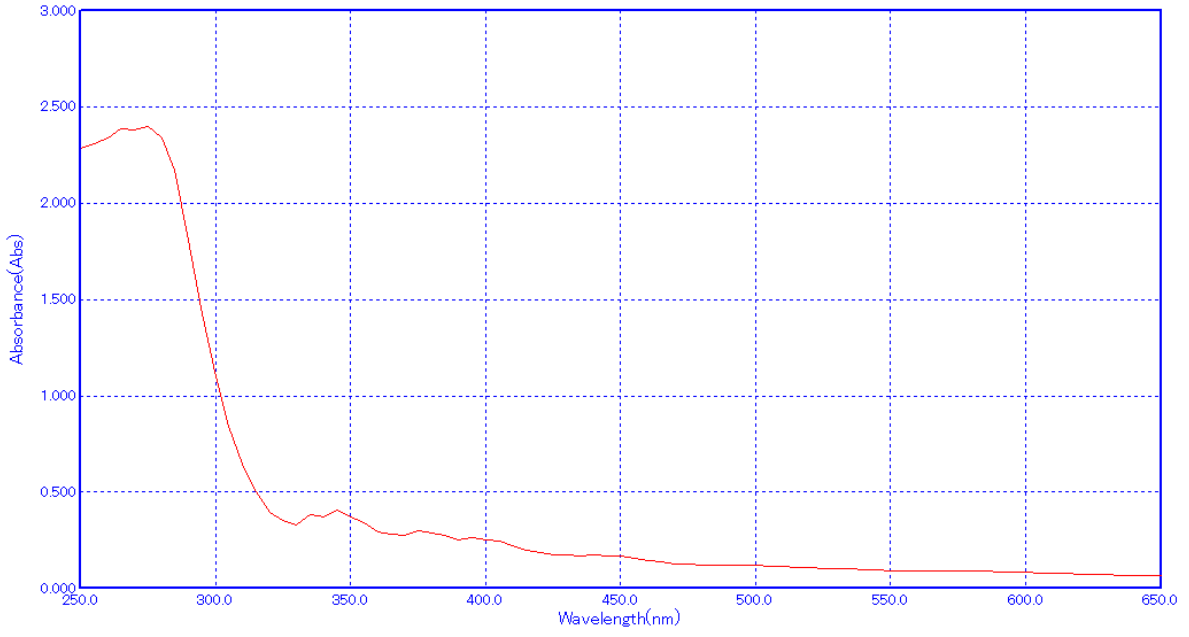


Figure 1 : Visual observation of colour change after 24 hours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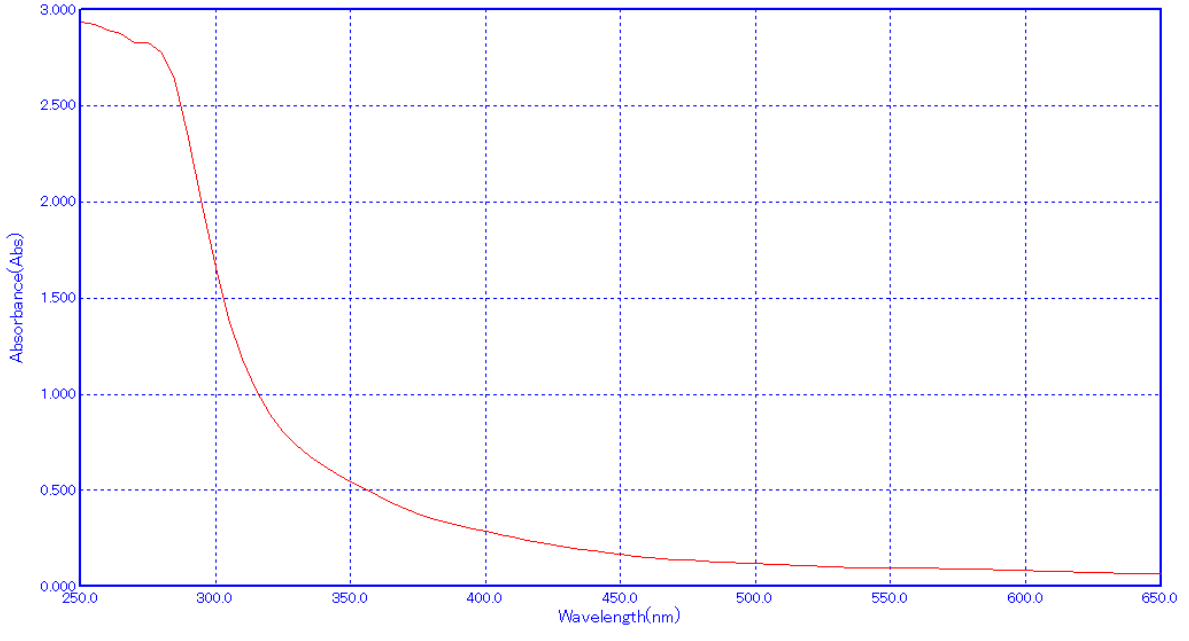
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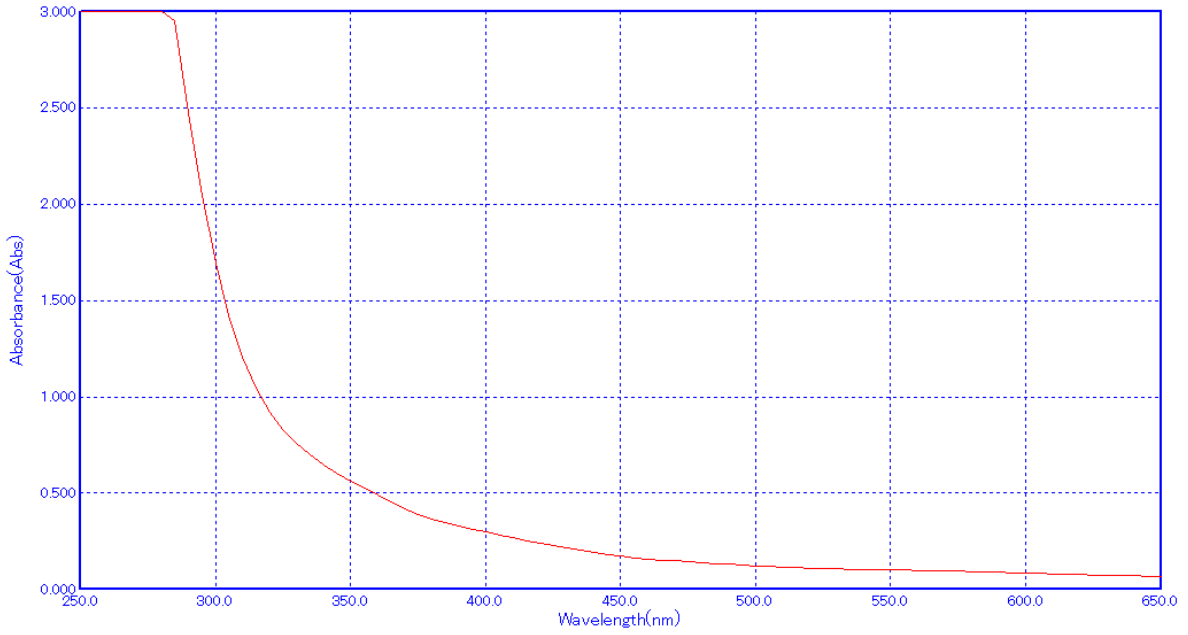


Figure 2: UV –vis spectroscopy

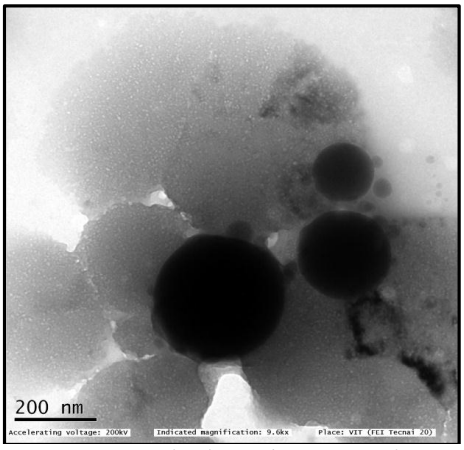


Figure 3: Transmission electron microscopy
Spherical shaped nanoparticles with the size of 25 to 100 nm

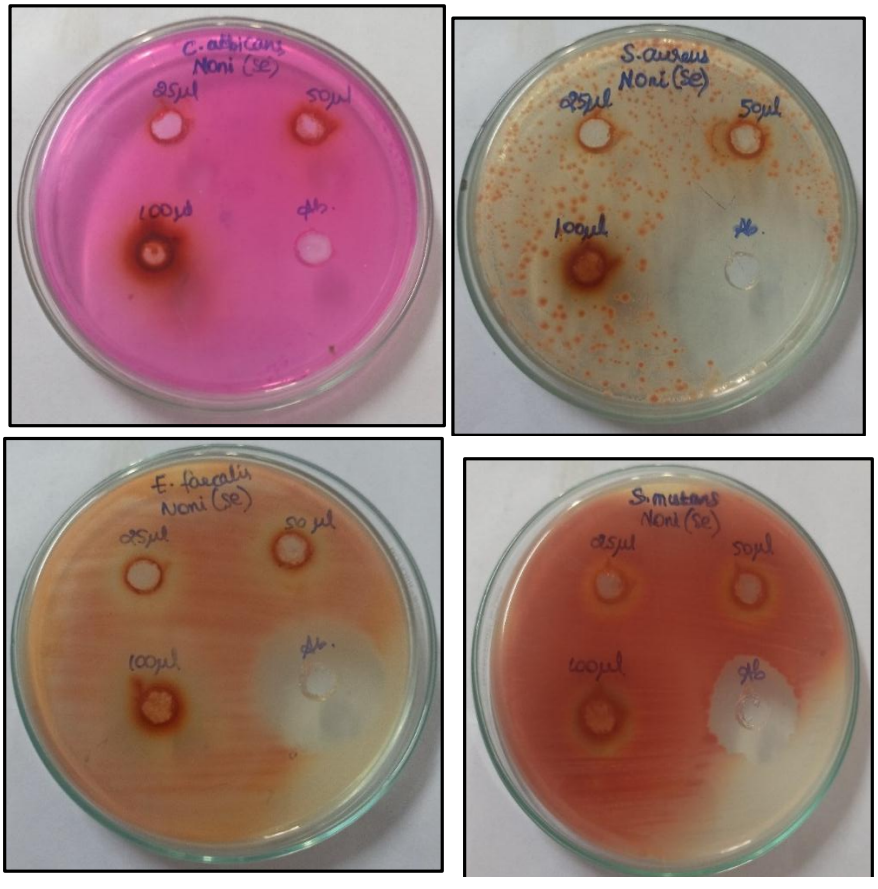


Figure 4: Antimicrobial activity of SeNP against S.mutans, S.aureus, E.faecalis and C.albicans

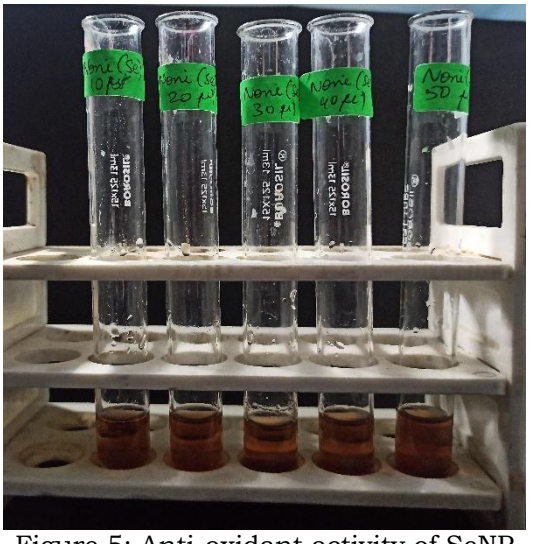
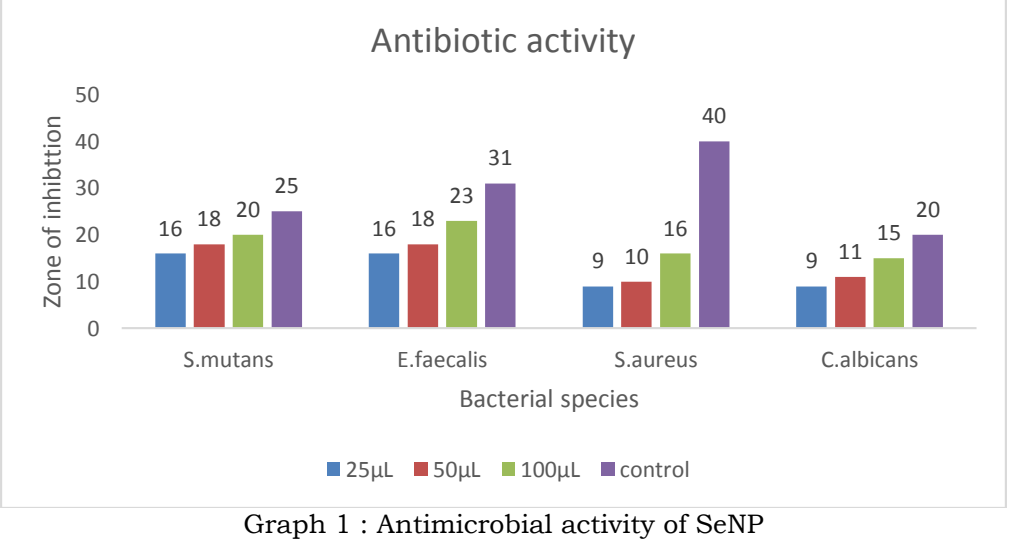
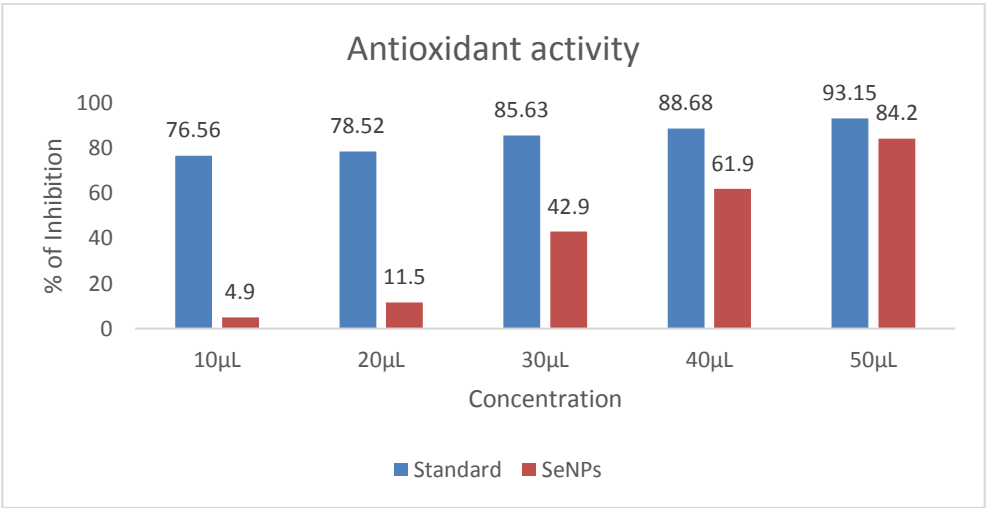


Figure 5: Anti-oxidant activity of SeNP



Graph 1 : Antimicrobial activity of SeNP



Graph 2 : Antioxidant activity of SeNP

The synthesis of SeNPs were confirmed with colour change (figure 1) and UV vis spectral findings(figure 2). The size of the selenium nanoparticles was found to be Spherical shaped nanoparticles with the size of 25 to 100 nm by Transmission Electron Microscopy(figure 3). Antimicrobial efficacy of different concentrations of SeNPs are presented in Figure. The mean zone of inhibition (ZOI) was found to increase as the concentration of NPs increased, however the maximum was found for ampicillin/cycloheximide. 100 µL concentration of SeNPs produced ZOI almost that of ampicillin/cycloheximide, but 25 µL and 50 µL concentrations were not as effective as ampicillin/cycloheximide. Among the microorganisms used, S.mutans , E.faecalis and C.albicans exhibited almost the same activity as the control at 100 µL concentration (figure 4 and graph 1). DDPH assay found biogenic SeNPs to possess effective antioxidant properties and its equivalent when compared to ascorbic acid at 50 µLconcentrations tested (figure 5 and graph 2).

Discussion

As the selenium nanoparticles (SeNPs) possess antimicrobial and anticancer properties, they can be used as nanomedicines(12). However, there is a very narrow margin between activity and toxicity(13). Compared with inorganic selenium and organic selenium, selenium nanoparticles (SeNPs) are found to show good bio availability, higher biological activity and lower toxicity. Recent studies have shown that selenium nanoparticles indicated potential bioactivities, especially antitumor properties(14). In our study 100 µL concentration of SeNPs produced ZOI almost that of ampicillin/cycloheximide, but 25 µL and 50 µL concentrations were not as effective as ampicillin/cycloheximide. Only limited evidence exits about the antimicrobial efficacy of biogenic SeNPs. But similar findings were found in a study conducted in which the 100, 100, 250 and 100 µg/mL selenium nanoparticles were found to inhibit 99 % growth of Pseudomonas

aeruginosa, Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes, respectively(15) The effectiveness of Triphala conjugated 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 (1).

Morinda citrifolia which has phytochemicals deacetylasperulosidic acid and asperulosidic acid are the major constituents of noni fruit which possess antibacterial activity (16) The extracts of noni fruit and leaves were found to exhibit moderate antibacterial activity against S. aureus and Proteus vulgaris compared to standard levofloxacin in a study conducted by P Selvam et al and Jian Yang et al (17) Noni fruit extract (NFE) for its antimicrobial activity is even researched to use as a natural sanitizer for fresh cut produce (18)

The efficiency of Theobroma cacao L. bean shell extract as a antioxidant agent in the green microwave-assisted synthesis of Se nanoparticles has been proven (19). Similar findings have been found in many studies proving the antioxidant property of SeNP with different combinations (20–22). Similarly noni fruit extract also exhibit antioxidant properties(23,24) The synthesised biogenic SeNPs holds great potential as a antioxidant and could be used effectively in a myriad of application

Conclusion

This study proves that the synthesized biogenic SeNPs shows great potential as an oral antimicrobial agent with potent antioxidant activity. Further animal studies/in-vivo research is however necessary to establish the above findings and its relevance in improving oral health of the masses. Biogenic SeNPs is eco-friendly, inexpensive and can be developed into dental varnish/mouthwash or localized drug delivery system to treat conditions like oral submucous fibrosis due to its potent antioxidant activity.

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