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Synthesis, Characterization and Antimicrobial Activity of Selenium Nanoparticles with *Clitoria Ternatea* on Oral Pathogens

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***Abstract*---**Background: Selenium nanoparticles have been studied for possible therapeutic effects in a variety of oxidative stress and inflammation-mediated diseases such as arthritis, cancer, diabetes, nephropathy, as well as antimicrobial properties. *Clitoria ternatea* is a perennial herbaceous plant which has been documented for its antimicrobial properties that are beneficial to human health. Therefore, this study was designed to synthesize, characterize and investigate the synergistic antimicrobial activity of selenium nanoparticles with *Clitoria ternatea* on oral pathogens. Materials and method: Green synthesis of selenium nanoparticles was achieved by a simple biological procedure using the reducing power of *Clitoria ternatea* flower extract. The synthesized nanoparticles were characterized using UV-vis-spectroscopy and Transmission Electron Microscopy. The antimicrobial activity was assessed using agar well diffusion method against strains of *S.aureus, S.mutans, E.faecalis and C.albicans*. Results: The synthesised nanoparticles showed potential antimicrobial activity against strains of gram positive bacteria especially against S.aureus where 35 mm of inhibitory activity was observed. They also showed enhanced antifungal property as compared to the control. Conclusion: *Clitoria ternatea* extract had many natural bioactive compounds that could be used to make selenium nanoparticles without the use of any chemical reducing agents, also with their enhanced activity against *S.aureus* and *C.albicans* they can be used for as oral delivery agents.

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Introduction

Nanotechnology is an emerging field of science which focuses on synthesis, characterization followed by application of elements, materials on a nano scale (1). Nano materials have numerous applications in almost all areas of utilities like food industry, manufacturing industries, pharmaceuticals, health care. Nano particles are basically measured to be as 109 meter in length, in at least one dimension; however, they are commonly defined to be of diameter in the range of 1 to 100 nm (2). Owing to their size, they can be used in both in vivo and in vitro biomedical research. Nanoparticles can be found as byproducts of industrial processes, can be manufactured by humans to have certain required properties for desired applications and they are also available naturally in the environment in the bodies of organisms, insects, plants, animals and human bodies (3,4).

In recent times, nanoparticles have been used in multiple dental applications, such as dental implants, dental restorative materials, teeth whitening agents, anti-sensitivity agents, polishing of enamel surfaces, re-mineralizing agents and anti caries agents (5). This shows, nano technology is going to hold its essential part in clinical dental practice as they have reduced toxicity, enhanced bioactivity and are environment friendly (6)

Metal nanoparticles of Ag, Au, Ce, Fe, Se, Si, Ti and Zn have a special place in the area of nanotechnology as they offer a unique opportunity as therapeutic agents. Among these, selenium nanoparticles are one of the most extensively studied. Among these nanoparticles, selenium nanoparticles (SeNPs) are one of the most extensively studied, as well as have been proved to have vital prospects in the field of medicine (3). SeNPs exhibit attractive anticancer activity and reduced toxicity concerns compared to different Se species. SeNPs have been used in many disease conditions including cancer, diabetes, inflammatory disorders, liver fibrosis, and drug induced toxicities (7–9). Also, selenium has been effectively tested against bacteria like E.Coli, S. aureus and C.albicans (10,11).

However, the growing problem of chemical contamination from nanoparticle synthesis has raised the demand for eco-friendly alternatives to all known chemical and physical processes. Consequently, nanotechnology has found a suitable answer in the incorporation of green chemistry principles, giving rise to what is called green nanotechnology, which pursues nanoparticle reactions that are environmentally friendly, cost-effective, and safe for both the environment and the patients (12). Therefore, various studies have been conducted to demonstrate the effectiveness of green Nano-technology against pathogens (13–15).

Clitoria ternatea, commonly known as “blue pea” is one such perennial herb which has immense properties in its leaves, seeds, fruits, bark, and flowers to be used in medicinal purposes (16). It has been used for centuries as a memory enhancer, anti-stress, anxiolytic, antidepressant, anticonvulsant, tranquilizer and sedative agent, and it has been documented that triterpenoids, flavonol

glycosides, anthocyanins and steroids found in the plant are responsible for all these therapeutic uses (17). Leaf and root extracts of *Clitoria ternatea* has been shown to have an effective and powerful antimicrobial properties against Escherichia coli, Staphylococcus aureus and Vibrio cholera (18). Previous in vivo and in vitro ethanolic extracts from vivid blue flowers of C. ternatea L. showed the best antibacterial activity against *B. subtilis* (19).

Several studies have demonstrated the synergistic effect of Cl. Ternatea with zinc, silver, copper nanoparticles however the effect of selenium nanoparticle with Clitoria ternatea has not been explored. Therefore, the aim of the study is to synthesize, characterize and assess the anti- microbial properties of selenium nanoparticles with Clitoria ternatea on S. mutans, S.aureus, E.faecalis and C.albicans.

Materials and method

Study design

In-vitro study.

Biogenic synthesis of selenium nanoparticles

Dried flowers of *Clitoria ternatea* was obtained from dedicated ayurvedic pharmacy and inspected for purity and phytochemical composition prior to use. Aqueous extract of *Clitoria ternatea* flowers were prepared by grinding the dried flowers to fine powder and boiling 10 gm of *Clitoria ternatea* flower powder in 100 mL of double distilled water in a water broth at 55-60 degrees for ten minutes to obtain 1% of the *Clitoria ternatea* extract. Whatmann number 1 filter paper was used to filter the solution following boiling, and the obtained filtrate was used of nanoparticle synthesis. 50 ml of the flower extract was mixed with 30 mM sodium selenium and 50 ml distilled water. The solution was then placed 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 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 ^{0}C for 2 hours and stored in air-tight containers until further analysis.

Characterization of Selenium Nanoparticles

Visual observation of colour change in solution is one of the characteristic features suggesting reduction of metal salts into nanoparticles. The solution was observed until change in colour was evident suggestive of NP synthesis (Fig 1). UV-vis spectrophotometric analysis was used to confirm SeNPs synthesis by sampling 2 ml aliquots of the prepared solution at periodic intervals using 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 12, 24, and 48 hours.

Figure 1 : Colour change observed

Microorganisms to be tested for: *S. aureus, S. mutans, E. faecalis, C. albicans*.

Anti-microbial activity of Selenium nanoparticles with Clitoria ternatea

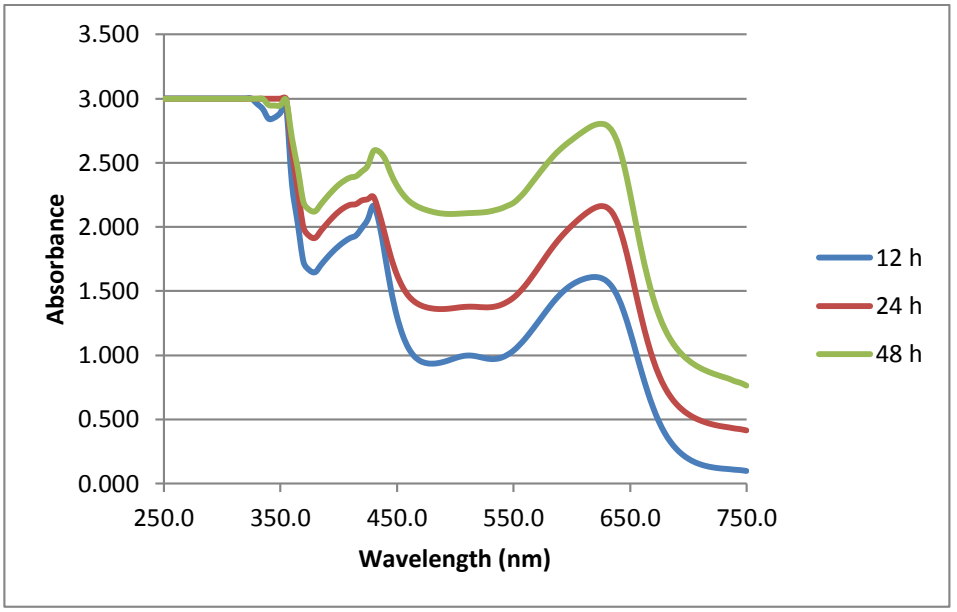
Agar well diffusion method was used to determine the antibacterial activity of different concentrations of Selenium NP with Clitoria ternatea against oral pathogens such as S. mutans, E. faecalis, S. aureus and C.albicans. Secondary cultures of microbial suspension were dispersed evenly on the surface 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. The plates were then incubated at 37 °C for 24 h to 48 h. Commercial antibiotic amoxicillin (50mg/ml) was used as positive control for S. mutans, E. faecalis, S. aureus. The zone of inhibition (mm) was recorded for each plate and compared with control. All the tests were replicated in triplicate for analysis.

Results

Characterization of the Cl. Ternatea extract mediated selenium nanoparticle:
The synthesized biogenic selenium nanoparticles were confirmed using UV spectroscopy with changes in absorption band beginning at 250 nm until 750 nm. The maximum peak was at 635 nm, and the absorption band gradually increased from 1.3-2.9 suggestive of nanoparticle reduction (Graph 1). Surface morphology of the synthesized Se-NP assessed using TEM was found to be amorphous at 200nm resolution, and at 50 nm spherical and rod shaped were revealed with the size of synthesized selenium nanoparticles ranging from 11-106 nm.

Antimicrobial activity

Agar well diffusion method was used to determine the antimicrobial activity of different concentrations of selenium nanoparticles with *Clitoria ternatea* against strains of *S.mutans*, *S.aureus, E. faecalis, C.albicans*. It was observed that at all concentrations the sample reduced the acid production by the various pathogens. The minimum zone of inhibition (ZOI) was found to increase with every concentration (Figure 2). At 25 ml the highest ZOI was observed in *S. mutans* (15mm), at 50 ml, the ZOI was observed highest for *C.albicans* (25 mm). At 100 ml the ZOI was observed at 35 mm for *S aureus*, it was higher than the control ampicillin, thus showing its antimicrobial potential. Even for *C.albicans*, the ZOI was higher (30 mm) as compared to the control used (18 mm).



Graph 1 : Uv vis spectroscopy graph of the synthesized selenium nanoparticle

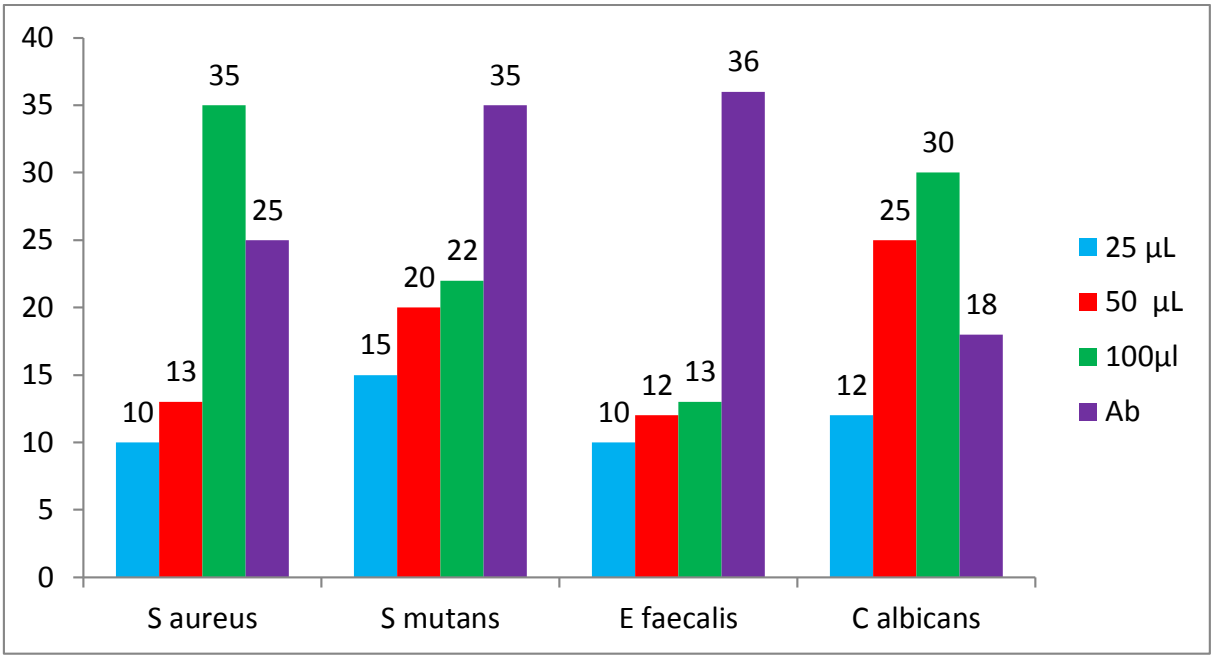


Figure 2: Antimicrobial activity of *Clitoria ternatea* extract mediated selenium nanoparticles.

Discussion

Nanoparticles are making their way to be a promising alternative to peroral drug delivery (20). Selenium nanoparticles have been identified as a pleiotropic agent with multiple potentials for biotherapy (21). However, the synthesis of these nanoparticles in physical, chemical, mechano-chemical ways increase the toxicity, as well as prove to be harmful for the environment (22). Therefore, the green synthesis of nanoparticles makes the process economically safe, simple to use without the toxicities. The plant phytochemicals are used to stabilize the nanoparticles (12).

The characterization for the selenium nanoparticles was done using UV vis spectroscopy and TEM. Uv vis spectroscopy is the technique to identify some functional groups in molecules and secondly, it can be used for assaying (23) and the peaks observed in the graph represents a restricted region of a spectrum, possibly a single wavelength which is absorbed or emitted. In this current study, UV spectroscopy peaks were observed at 650 nm confirming the synthesis, similar to various other studies (24) (25). Studies have reported where peaks were observed at 280 nm (26). The findings from the TEM analysis was also observed to be similar to those of other studies (27,28).

Selenium nanoparticles have been extensively used in oral health for preventing dental caries (29–31), inhibiting biofilm formation (32). In the present study, SeNPs with *C. ternatea* at 0.1 mg/µl was found to be more effective compared to 0.05 mg/µl of commercial ampicillin against strains of *S.aureus* as well as *C.albicans*. Similar good antibacterial and antifungal activity were reflected in

other studies as well (33,34). Contrasting antifungal findings were reported in a study by (28).

Earlier, green synthesis of selenium nanoparticles has been conducted using various other herbs, leaves such as *Withania somnifera* (35), *Allium sativum* (36), *Ocimum tenuiflorum* (37), *Moringa oleifera* (38) but to the best of our knowledge this is the first study to be conducted using *Clitoria ternatea* extract mediated selenium nanoparticles. The strengths of this study include the use of green chemistry to synthesize ZnO-NP and holistic assessment of antimicrobial activity. Future studies in a simulated oral environment are required to validate the findings of this in vitro study.

Conclusion

Green synthesized clitoria ternatea extract mediated selenium nanoparticles exhibited significant antibacterial activity against *S. Aureus* and *C.albicans* and effectively inhibited its biofilm formation and acid production at par with commercial ampicillin. Thus, it holds great potential as an oral agent to inhibit *S. Aureus*, *C.albicans* and is relatively safe for biological applications.

Conflict of interest: Nil

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