

How to Cite:

Abirami, S., George, A. M., & Rajeshkumar, S. (2022). Efficacy of biosynthetically developed selenium nanoparticles using plant extracts of clove and cardamom. *International Journal of Health Sciences*, 6(S5), 9799–6811. <https://doi.org/10.53730/ijhs.v6nS5.11552>

Efficacy of biosynthetically developed selenium nanoparticles using plant extracts of clove and cardamom

S. Abirami

Post graduate, Department of Orthodontics and Dentofacial Orthopaedics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai.
Corresponding author email: varshaabi77@gmail.com

Dr. Ashwin Mathew George

Professor, Department of Orthodontics and Dentofacial Orthopaedics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai.
Email: ashwingeorge90@yahoo.com

S Rajeshkumar

Professor, Nanobiomedicine Lab, Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science Saveetha University, Chennai, India.
Email: rajeshkumars.sdc@saveetha.com

Abstract--Background: The green approachable metal nanoparticles are treated to be an eco-friendly path and cost-effectiveness. In this present study, nano selenium was synthesized profitably by *clove and cardamom*. Methods: Green synthesis of selenium nanoparticles was preliminarily confirmed by color changing from dark orange to red color in the reaction mixture. The resulting constructed nanoparticles were characterized by using ultraviolet spectroscopy absorbance around 450 nm. Characterization using HRTEM (High Resolution-Transmission Electron Microscope) to identify its size and shape. The organisms used to assess the antimicrobial efficacy of *clove and cardamom* reinforced selenium nanoparticles were *Streptococcus mutans*, *Staphylococcus aureus* and *Lactobacillus* species and *Candida albicans* using Agar well diffusion method at 25 μ L, 50 μ L and 100 μ L. In addition, we have analyzed its anti-inflammatory and cytotoxicity activity. Results: UV-VIS spectroscopic analysis which showed a peak at 450 nm of the visible spectrum by reduction of selenium salts to Se NP's. TEM spherical shape was of size 5 -20 nm. Good antimicrobial activity was noted against *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus* species and *Candida*

albicans. The mean zone of inhibition was found to be increased as the concentrations of Se NPs increased. Minimal cytotoxicity was observed. Good antiinflammatory activity was recorded. Conclusion: Clove and cardamom-mediated selenium nanoparticles have very few cytotoxic effects. It also has the potential to serve as a good anti-inflammatory and antioxidant agent.

Keywords---clove and cardamom, Selenium nanoparticles, antibacterial, anti inflammatory, cytotoxicity, TEM.

Introduction

The field of nanotechnology provides promising outcomes for many beneficial effects in dentistry. In addition to an infinite innovative number of applications in other fields, recently it emerges as a contemporary surge in the field of medicine and dentistry(1,2). The rationale for this may be the ability to emulate the nanostructure and the nanosized organic components of tooth, as well as the intrinsic features of nanomaterials. Such characteristics would be an advantage in treating dental infections as it facilitates close interactions with microbial membranes(3–5).

Microorganisms are constantly invading the oral cavity, which offers a wide range of habitats for their colonization, such as teeth, gingival sulcus, connected gingiva, tongue, cheek, lip, and hard and soft palates (6,7). Teeth, unlike other parts of the body, do not normally shed and hence provide prospects for plaque biofilm formation(8). Plaque biofilm—a significant source of Periodontal and Endodontic lesions producing a complex community of oral microorganisms. These microorganisms promotes infection by amplification of conditional microbes together with their virulence factors, which alters the normal oral microbial balance(9,10). Treatments like dental restorations, removable prostheses, and fixed orthodontic braces may encourage the growth of biofilm, which in turn can lead to dental infections such as dental caries and other periodontal problems(11–13). Thus dental procedures should include complete eradication of bacteria, however, this is not feasible owing to the intricate anatomy of the tooth and the rising incidence of resistant strains(14,15). Long-term use of antibiotics may lead to the development of antibiotic resistance(16). Thus, it is imperative that the substitutes be designed as nontoxic, noninvasive with antioxidant and antibacterial activities that do not cause antimicrobial resistance or have any detrimental consequences for human health.

Recently metallic nanoparticles such as silver, gold, copper oxide, and zinc oxide have been widely synthesized in recent decades using either Physico-chemical or green chemistry techniques(17,18). High temperatures, hazardous chemicals, and an acidic pH are required for Physico-chemical procedures, which are exceedingly hazardous and toxic for biological applications(19). Whereas green synthesis is able to produce nanoparticles that are pure, safe, eco-friendly, economical, and non-toxic by utilizing high-energy renewable resources(20,21). Selenium nanoparticles have recently emerged as one of the least hazardous and most stable of all nanomaterials(22). The synthesis of selenoproteins makes selenium a

potent antioxidant and anti-inflammatory activity(23,24). In addition, several studies have shown that it possesses potent antibacterial, anticancer(25), and immunomodulatory activities, as well as anti-biofilm properties(26). Biosynthesis refers to the employment of plant extracts that have the unique property of reducing as well as capping agents for the synthesis of stable metallic nanoparticles((27–29) . But no research has been conducted as far as green synthesis of selenium nanoparticles is concerned using plant extracts of clove and cardamom.

Syzygium aromaticum (clove), native to the Maluku Islands in eastern Indonesia that belongs to the Mirtaceae family(30).It is valuable as a spice and has antimicrobial, antifungal, antioxidant, and anti-diabetic properties(31). cardamom (*Elettaria cardamomum*), a perennial aromatic plant native to southern India, Sri Lanka, Tanzania, and Guatemala with antioxidant, anti-inflammatory, anti-cancer, and antimicrobial properties is traditionally used as a culinary ingredient. Cardamom oil has been shown to have antibacterial activity against *Streptococcus mutans* in oral infections (32). While clove and cardamom-mediated nanoparticles have been studied previously (33), none of the experiments have been used to synthesize selenium nanoparticles.

In this study, we are using plant extract of *clove and cardamom* for the synthesis of selenium nanoparticles at room temperature. Selenium nanoparticles synthesis was identified by color change and UV- visible spectroscopy (UV-vis). Selenium nanoparticles were studied using high-resolution transmission electron microscopy to determine their structure and composition (HRTEM). In addition, the antibacterial, anti-inflammatory, and biocompatibility properties of SeNPs were evaluated in this study.

Aim

The aim of the current study was to validate the application of green synthesized nanoparticles by synthesizing and analyzing their activities.

1. Green synthesis of Selenium nanoparticles by using clove and cardamom as plant extracts.
2. Analyze anti-bacterial, anti-inflammatory activity, and biocompatibility properties of the synthesized nanoparticles.
3. Characterization using HRTEM (High Resolution- Transmission Electron Microscope) to identify its size and shape.

Materials and Methods

Materials:

50 g of dried cardamom and clove were collected.

Preparation of plant extract (figure 1):

Cardamom and clove were collected for extract preparation and dried cardamom and clove were ground to powder by using an electric mixer.

Clove and cardamom extract:

0.5g dried powder of clove and cardamom were taken in 50 ml of distilled water and kept in a heating mantle, boiled at 60 -80 degrees for 5 -10 min. Red-colored and light green colored solutions were formed respectively.

The obtained solution of clove and cardamom extract was cooled and filtered through Whatman No.1 filter paper. 30 ml of each filtrate was mixed together and boiled for 1 min and the filtrate was stored and further used to prepare selenium nanoparticles.

Green synthesis of Selenium (Se) nanoparticles (figure 2)

0.346 g (20 MM) salt of sodium selenite was dissolved in 60 ml of distilled water to prepare 60 ml of selenium solution. 40 ml of cardamom and clove extract were added to 60 ml of selenium precursor solution and the mixture was continuously stirred using a magnetic stirrer at 340–350 °C also kept overnight on an orbital shaker till a color change in the mixture was observed. For three days, the color change was observed at an hourly interval. At the end of the third day, light-colored orange-red colored was changed to the dark orange-red colored solution. To verify nanoparticle synthesis, UV spectroscopy was used to check for the synthesis of nanoparticles from the prepared selenium-containing clove and cardamom extract. In a centrifuge, it was then centrifuged using Lark refrigerated centrifuge for 10 min at 10,000 rpm. After that, the selenium nanoparticle pellets were collected for antimicrobial, anti-inflammatory, and cytotoxicity testing.

Characterization of synthesized Selenium Nanoparticles (TEM) (figure 3)

UV-vis spectroscopy is used to characterize the solution after the nanoparticles have been synthesized. The UV-vis absorption peak of the synthesized selenium NP's was recorded using UV -Vis Spectroscopy. 3 mL of the solution was placed in a cuvette and scanned in a double beam UV-visible spectrophotometer (ELICO SL 210 UV-Vis spectrophotometer) from 300 to 700 nm wavelengths, with the results graphically recorded. The size and shape of Selenium (Se) nanoparticles were obtained using Transmission electron microscopic analysis (TEM).

Antimicrobial activity of Selenium Nanoparticles (Agar well diffusion method) (Figure 4)

The antimicrobial efficiency of Selenium nanoparticles was assessed using the agar well diffusion method. The Mueller Hinton agar was prepared and sterilized in an autoclave for 15-20 minutes at 121 degrees Celsius. The sterile MHA media was prepared on the surface of sterile Petri plates and allowed to solidify. *Streptococcus mutans*, *Staphylococcus aureus*, and *Lactobacillus* species were swabbed with sterile cotton buds after solidification. A T-shaped well cutter was used to create the wells. Among four wells per plate 3 wells were loaded with clove-cardamom selenium nanoparticles solution in the concentration range of **25** µl, **50** µl, **100** µl, and the fourth well was loaded with a standard antibiotic (Amoxyrite) and then the plates were incubated at 37 degrees Celsius for 24 hours. Rose Bengal Agar was prepared as the medium for *Candida albicans* and inoculated plates were incubated at 37 degrees Celsius for 48 hours. After

incubation, the plates were observed and measured for the zone of inhibition around the nanoparticle-loaded wells.

Cytotoxic Effect (Brine shrimp assay)(figure 5):

Setup preparation: The artemia tank was filled with 6 liters of distilled water. To those 50 grams of iodine-free salt was added and mixed well using a spatula. 2 capsules containing 15 grams of Brine Shrimp eggs were added to the tank and left undisturbed for 5 minutes for proper soaking in saltwater. After that, the airline tip was placed inside the artemia tank and the aeration level was increased to maximum level. After 24 hours of incubation, the nauplii hatch out from the brine shrimp eggs and are observed using a stereomicroscope. The cytotoxicity Selenium (Se) nanoparticles reinforced with *clove and cardamom* extract was assessed using Brine shrimp assay. 12 well ELISA plates were taken and to each plate, 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Selenium (Se) nanoparticles reinforced with *clove and cardamom* were added to each well at different concentrations (3 μ L, 6 μ L, 12 μ L, 24 μ L, 50 μ L) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

$$\% \text{ death} = \frac{\text{Number of dead nauplii}}{\text{Number of dead nauplii} - \text{number of live nauplii}} \times 100$$

Anti-Inflammatory Activity (Protein Denaturation Assay)(figure 6)

Bovine serum albumin (BSA) was used as a reagent for the assay. BSA makes up approximately 60% of all proteins in animal serum. It's commonly used in culture, particularly when protein supplementation is necessary and the other components of serum are unwanted. BSA undergoes denaturation on heating and starts expressing antigens associated with Type III hypersensitivity reaction which are related to a disease such as rheumatoid arthritis, glomerulonephritis, serum sickness, and systemic lupus erythematosus. 2 ml of 1% bovine albumin fraction was mixed with 400 μ l of plant crude extract in different concentration (500–100 μ g/mL), and the pH of the reaction mixture was adjusted to 6.8 using 1N HCl. The reaction mixture was incubated at room temperature for 20 min and then heated at 55°C for 20 min in a water bath. The mixture was cooled to room temperature, and the absorbance value was recorded at 660 nm. An equal amount of Se nanoparticles reinforced with *clove and cardamom* was replaced with dimethyl sulfoxide for control. Diclofenac sodium in different concentrations was used as standard. The experiment was performed in triplicate.

Test Group

10 μ L, 20 μ L, 30 μ L, 40 μ L, and 50 μ L of Selenium (Se) nanoparticles reinforced with *clove and cardamom* was taken in 5 test tubes respectively. To each test tube, 2 ml of 1% Bovine Serum Albumin (BSA) was added. 390 μ L, 380 μ L, 370 μ L, 360 μ L and 350 μ L of distilled water was added to the test tube containing 10 μ L, 20 μ L, 30 μ L, 40 μ L and 50 μ L of Se nanoparticles respectively.

Control Group

2 mL of Dimethyl Sulphoxide (DMSO) was added to 2 mL of BSA solution.

Standard Group:

10 μ L, 20 μ L, 30 μ L, 40 μ L and 50 μ L of Diclofenac Sodium was taken in 5 test tubes respectively. To each test tube 2 mL of 1% Bovine Serum Albumin (BSA) was added. The test tubes were incubated at room temperature for 10 minutes. Then they were incubated in a water bath at 55 oC for around 10 minutes. Absorbance was measured at 660 nm in UV Spectrophotometer

% Inhibition was calculated using the following formula:

$$\% \text{ Of inhibition} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

Results & Discussion

Synthesis of Se NPS: The *clove and cardamom* extract when mixed with Selenium solution showed a color change from light orange red-colored to dark orange-red colored solution. This color change indicated the formation of Selenium Nanoparticles (Se NP's) (Figure 1 and 2).

UV-vis spectroscopy: UV-VIS spectroscopic analysis which showed a peak at 450 nm of the visible spectrum by reduction of selenium salts to Se NP's (Figure 3).

Transmission electron microscope: TEM analysis revealed the spherical shape of Se NPs with a size range from 5 -20 nm (Figure 3) which was similar to the results of the study.

Antimicrobial activity of Se NPs:

The inhibition zones for AuNP's at various concentrations for each of these organisms - *S. aureus*, *S. mutans*, *Lactobacillus species*, and *C. Albicans* are depicted in Figure 4.

Cytotoxicity of clove and cardamom reinforced Se NPS:

Table 1 depicts the cytotoxicity of Se nanoparticles reinforced with *clove and cardamom* extract. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased. Hence, it has low cytotoxicity activity.

Anti-inflammatory property of Selenium (Se) nanoparticles reinforced with clove and cardamom:

It was found that the values for the anti-inflammatory property of nanoparticles were closer to the standard values at 10 μ L, 20 μ L,30 μ L, 40 μ L, 50 μ L concentrations, hence it has good anti-inflammatory activity. The synthesis of nanoparticles has progressed quickly especially for medical purposes (34). Green nanotechnology using plants is an evolving eco-friendly alternative to earlier physio-chemical methods that need toxic chemicals for stability. Another distinct advantage of the green chemistry approach is comparatively cost efficient (35). Any new methods of nano-synthesis have to necessarily undergo stringent tests to

validate its cytotoxicity and characterization to justify its physical properties. In the current study, *clove and cardamom* were incorporated in selenium nanoparticles and their antimicrobial, cytotoxicity, and anti-inflammatory activity was evaluated.

In the present study, Cytotoxicity was evaluated by using a Brine shrimp assay. In this study the naupliis was subjected at 3 μ L, 6 μ L, 12 μ L, and 24 μ L concentration of green synthesized Selenium nanoparticles. The results of the brine shrimp assay showed that 80% of nauplii was able to survive at all concentration. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased. This clearly defines the extent of concentration that can be safely administered when using green synthesized nanoparticles. Since less than 50% of death of nauplii is considered to have low toxicity, it is considered as low cytotoxicity activity.

The antimicrobial activity of Se NPs was assessed using the nutrient agar well diffusion method against oral pathogens like *S. aureus*, *S. mutans*, *Lactobacillus sp.*, and *C. Albicans*. In this method, the Zone of inhibition test also called as Kirby-Bauer Test, a qualitative method was used clinically to measure the antibacterial activity of the Se NPs. The size of the zone of inhibition has related to the amount of antimicrobial activity present in the sample or product – antimicrobial potency depends on the size of the zone. Se NP's showed excellent antimicrobial activity as antibiotic potential against *Candida albicans* with 10mm, 10mm, 13mm at 25 μ L, 50 μ L, 100 μ L respectively. Good antimicrobial activity has been observed against *Streptococcus mutans*, *Lactobacillus* species and *Staphylococcus aureus*. The antimicrobial activity increased as the concentration was increased. Good anti-inflammatory activity was observed. Based on the findings of the study we can say that reinforcing selenium nanoparticles with clove and cardamom has a synergistic effect and can be used as an alternative to commercially available anti-inflammatory and antioxidant agents.

Limitations

The study was conducted in vitro, so it cannot be assumed that the results of cytotoxicity, anti-inflammatory activity and antioxidant activity could be translated into clinical effectiveness.

Recommendations

- This product can be incorporated into mouthwash which could greatly improve its efficiency.
- To validate the results of this study and determine its feasibility in in vivo studies.

Conclusion

From the above study findings, *clove and cardamom-mediated* selenium nanoparticles have less cytotoxic effects. It also has the potential to serve as a good anti-inflammatory and antimicrobial activity. Future studies are to be conducted after incorporating Se nanoparticles reinforced with *clove and*

cardamom into dentifrices or mouthwashes and various other applications in orthodontics and different branches of dentistry.

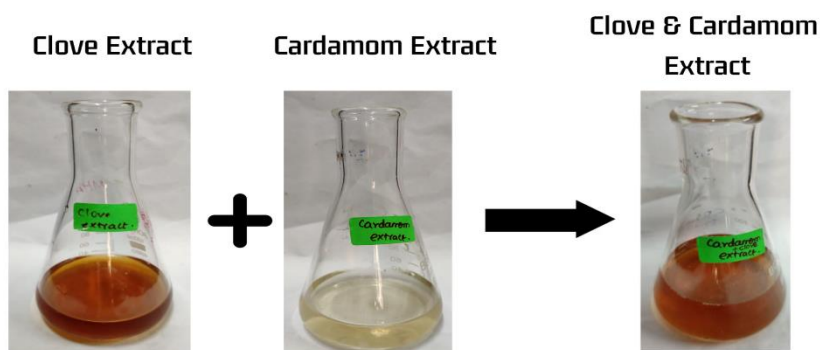


Figure 1: Preparation of plant extract: 0.5 g of dried powder of clove and cardamom was taken in 50 ml of distilled water and kept in a heating mantle at 60 -80 degree for 5 -10 min and red colored and light green colored solution was formed respectively. Filtration was done using Whatman filter paper no.1.30 ml of each filtrate was mixed together and boiled for 1 min and filtrate was stored.

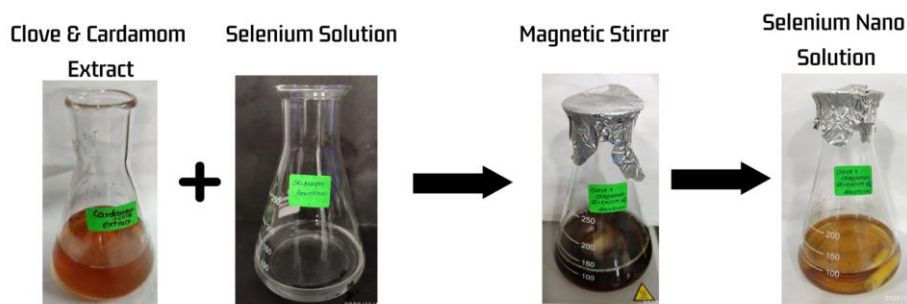


Figure 2: Preparation of Selenium (Se) nanoparticles and visual observation: 60 ml of cardamom and clove extract were added to 40 ml of Selenium precursor solution and mixture was continuously stirred using magnetic stirrer at 340–350 °C and also kept overnight on an orbital shaker till color change was observed from light orange red colored to dark orange red colored solution indicating the formation of Se nanoparticles.

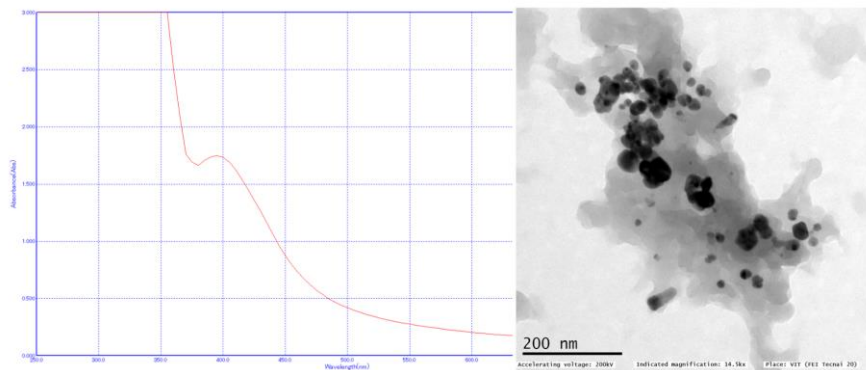


Figure 3 Left : UV-vis spectroscopy : SPR peak at 450nm ; Right:TEM analysis ,5-20 nm spherical Se nanoparticle.

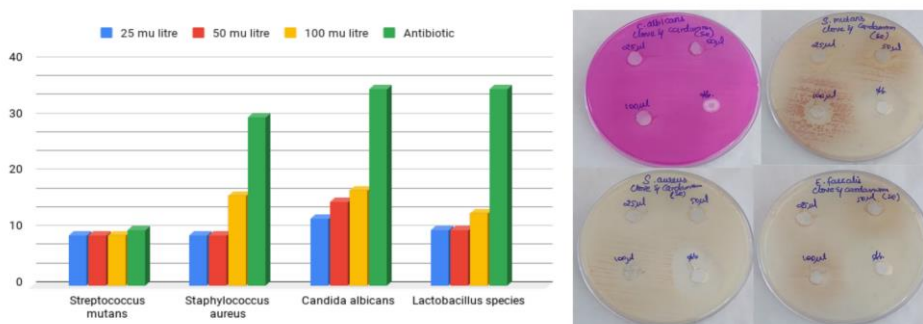


Figure 4: depicts the various zones of inhibition for the Se NP's at different concentrations of the nanoparticles for different oral pathogens.

- (a) *Streptococcus mutans*
- (b) *Staphylococcus aureus*
- (c) *Lactobacillus species*
- (d) *Candida albicans*

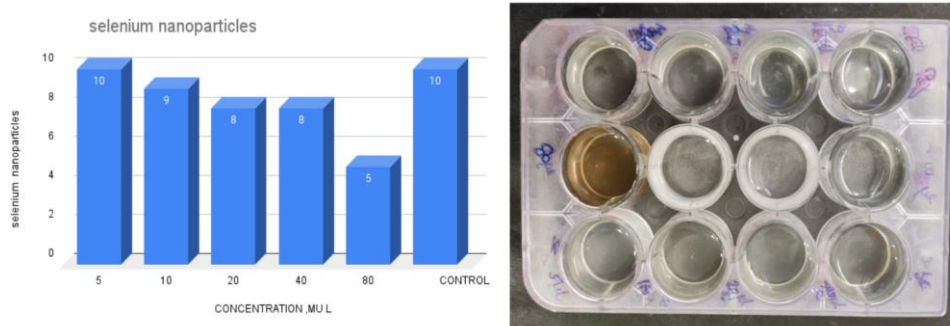


Figure 5: cytotoxicity - Brine Shrimp lethality assay of clove and cardamom mediated Se nanoparticles

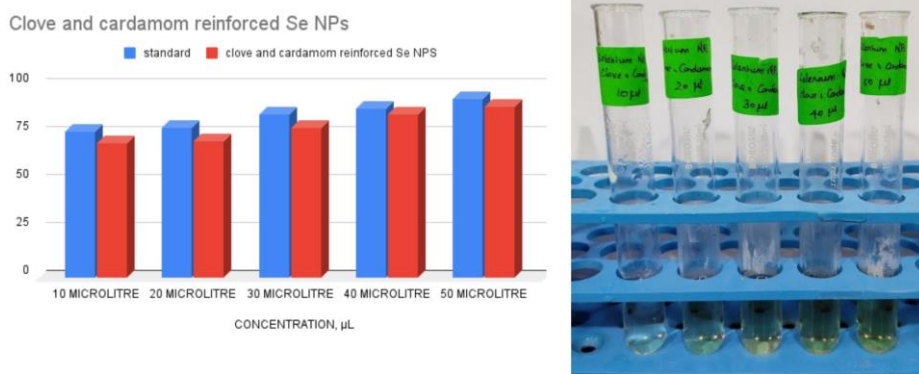


Figure 6 depicts the anti-inflammatory property of Se nanoparticles reinforced with *clove and cardamom* at various concentrations compared with the standard values

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