Green Synthesis of Selenium Nanoparticles Using Clove and Thulasi and its Antioxidant Activity

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**Abstract:** Introduction: Plants have been explored successfully for rapid biosynthesis of metal nanoparticles such as gold, silver, selenium, MgO, CuO, and ZnO nanoparticles. The nanoparticles are used extensively in cancer drug delivery as the drugs bound with nanoparticles can penetrate deep into the organs. Particularly, an essential dietary micronutrient, selenium found in the form of Se NPs, is relatively a new member of drug nanocarriers in medicine because Se NPs exhibit strong antioxidative and anti-bacterial activity.To study the free radical scavenging activity of clove and red tea-mediated zinc oxide nanoparticles using DPPH assay and hydroxyl radical scavenging assayAntioxidant activity: DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles.Hydroxyl radical scavenging assay: All solutions were prepared freshly.1.0mL of the reaction mixture contained 100µL of 28mM of 2-deoxy-2-ribose, carcia papaya sol Fecl3 and 1.04mM EDTA,100µL H2O2(1.0mM) and 100µL ascorbic acid.After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution. Vitamin E was used as positive control. The percentage inhibition was only 65% when the concentration was 10 μl, at 20 μl it was 70%, at 30 μl it was 80%, at 40 μl it was 90% and at 50 μl it was 90%. It was observed from the spectra that the extract at 517 nm had the highest radical scavenging activity at a concentration of 50 μl (90%), which is indicative of significant antioxidant activity as potent as DPPH itself.The percentage inhibition was 50% when the concentration was 10 μl, at 20 μl it was 55%, at 30 μl it was 60%, at 40 μl it was 70% and at 50 μl it was 90%. It was observed from the spectra that the extract at 517 nm had the highest radical scavenging activity at a concentration of 50 μl (90%), which is indicative of significant antioxidant activity as potent as hydroxy radical scavenging assay. In this study, a simple, biological and low-cost approach was done for the preparation of selenium nanoparticles using Tulsi and clove extract. Thus clove and thulasi-mediated selenium nanoparticles can be subjected to various other biological activities such as antibacterial,antifungal, and cytotoxic evaluation to know the efficiency of these nanoparticles.

**Keywords:** Selenium, clove, tulsi, antioxidant activity

# Introduction

Cloves (Syzygium aromaticum) are dried aromatic unopened floral buds of an evergreen tree belonging to the family Myrtaceae, indigenous to India, Indonesia, Zanzibar, Mauritius and Ceylon.[(Chokkattu et al., 2022; Vargas-Bernal et al., 2020)](https://paperpile.com/c/p5TRzb/hZQz+l61f) They are esteemed as a flavoring agent and also used as a spice for scenting, chewing tobacco and an ingredient of betel chew. Cloves have many therapeutic uses: anti-inflammatory, antioxidant and antifungal. [(Anandhi et al., 2022; Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study, n.d.; Chokkattu et al., 2022)](https://paperpile.com/c/p5TRzb/VpDs+l61f+g5Um) The antioxidant contents of fruits and vegetables increase because of natural antioxidant consumption, which has been found to be related to reduced risk of cancer and heart diseases. Tulsi is a basil family Lamiaceae (tribe ocimeae) is native to the Indian subcontinent, China, and Southeast Asia and widespread as a cultivated plant throughout the Southeast Asian tropic Tulsi has the effect of antiseptic and analgesic properties and relieves swelling. [(Prasad, 2019)](https://paperpile.com/c/p5TRzb/fs42)Nanotechnology explores a variety of promising approaches in the field of biomedical sciences. For biogenesis of selenium (Se) nanoparticles different parts of a plant are used as they contain metabolites such as alkaloids,[(Mohapatra et al., n.d.; Pandiyan et al., 2022; Prasad, 2019)](https://paperpile.com/c/p5TRzb/fs42+zSeW+PXHY) flavonoids, phenols, proteins, and other phytochemicals which act as reducing agent to produce and stabilize nanoparticles.[(Aronson, 2005)](https://paperpile.com/c/p5TRzb/Io12) Nanotechnology is also widely practiced in medicine, agriculture, and many other technologies. [(Aparna et al., 2021; Chokkattu et al., 2023)](https://paperpile.com/c/p5TRzb/4v3D+IC4d)The nanoparticles are major drug carriers for delivering very sensitive and highly valuable drugs to complicated diseases. Selenium is the important micronutrient of our body and selenium nanoparticles for biomedical applications are very useful for the biomedical community. [(Bhonsle et al., 2022; Dhanvanth & Maheswari, 2022; Ganapathy 2021)](https://paperpile.com/c/p5TRzb/33hX+V4Z2+epr8) Se nanoparticles present lower toxicity and higher biocompatibility than organic or inorganic Se compounds, attracting the attention of the scientific community for their application as therapeutic and theranostic agents. [(Baronzio & Dieter Hager, 2008; Muthuswamy Pandian et al., 2022)](https://paperpile.com/c/p5TRzb/n7rC+dlNh) Nano-selenium has a high potential to act as antiviral, antifungal, and antibacterial. Antioxidant activity is one of the most fundamental features of Nano-selenium, which can remove harmful peroxides from the body through glutathione peroxidase and protect the membranestructure of organisms from damage. [(Mishra, 2016; Subramanian & Harikrishnan, 2023)](https://paperpile.com/c/p5TRzb/ysy0+7Anu) The aim of the study is to study the free radical scavenging activity of clove and red tea mediated zinc oxide nanoparticles using DPPH assay and hydroxyl radical scavenging assay

# Materials and methods

## PREPARATION

Plant leaf samples of clove and thulasi were collected, washed, shade-dried and powdered. Extractions of the secondary metabolites from these samples were done by soxhlet apparatus by using methanol as solvent. Twenty grams of plant leaf powder was taken in a Whatman filter paper and placed in the thimble containing 200 mL of methanol in a 500 mL Round bottom flask. The extract obtained in solution was distilled to remove solvent by distillation process for 3h. The extracts were collected and dried in glass jars at 40oC. The extracts were stored at 4oC for further analysis.Two hundred milliliters of 40mM ascorbic acid were taken in six different conical flasks. Ten milliliters of 20mM sodium selenite was taken in six test tubes separately. Contents in the test tubes were added to six conical flasks containing ascorbic acid. From these, one conical flask was used as control and remaining used for samples. One milliliter of all the above five different plant extracts was added into the remaining five conical flasks separately. After addition of the extracts, both samples and test were analyzed spectrophotometrically at regular intervals of time (0h, 2h, 4h, and 6h). By using clove and thulasi sample, selenium nanoparticles were produced by using same method which was mentioned above.Sample with1mL ascorbic acid and 10 mL of sodium selenite was added to conical flask containing 200 mL of ascorbic acid was taken. Both control and sample were incubated at room temperature for 4 hours(Rafi et al., 2024). After that solution were centrifuged at 8,000rpm for 20 min at 4oC.The pellet was water washed for 1-2 times and with absolute ethanol for 3 times. The pellet was dried overnight. Suspended the selenium nanoparticles in 0.1 M phosphate buffer saline (pH 7) by ultrasonication and centrifuged at 8,000rpm for 20 min. The pellet was dried overnight and the powder form of selenium nanoparticles were used for further analysis.

## ANTIOXIDANT ACTIVITY

## DPPH METHOD

## Antioxidant activity

DPPH assay was used to test the antioxidant activity of biogenic synthesized zinc oxide nanoparticles. Diverse concentrations (10µL,20µL,30µL,40µL,50µL) of Justicia adhatoda leaf extract interceded zinc oxide nanoparticle was mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes (Tuluwengjiang et al., 2024). Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. Ascorbic acid was used as standard. The percentage of inhibition was determined from the following equation,

% inhibition= Absorbance of control- Absorbance of test sample × 100

Absorbance of control

## HYDROXYL RADICAL SCAVENGING ASSAY

All solutions were prepared freshly.1.0mL of the reaction mixture contained 100µL of 28mM of 2-deoxy-2-ribose ( dissolved in phosphate buffer,pH 7.4), 500µL solution of various concentrations of the Carcia papaya (10µL,20µL,30µL,40µL,50µL) 200µL of 200µM Fecl3 and 1.04mM EDTA (1:1 v/v),100µL H2O2(1.0mM) and 100µL ascorbic acid(1.0mM).After an incubation period of 1 hour at 37°C the extent of deoxyribose degradation at about 532nm against the blank solution . Vitamin E was used as a positive control.

## FRAP ASSAY

## REAGENTS FOR FRAP ASSAY

a) Acetate buffer 300 mM pH 3.6: Weigh 3.1g sodium acetate trihydrate and add 16 ml of glacial acetic acid and make the volume to 1 L with distilled water. b) TPTZ (2, 4, 6-tripyridyl-s- triazine): (M.W. 312.34), 10 mM in 40 mM HCl (M.W. 36.46). c) FeCl3. 6 H2O: (M.W. 270.30), 20 mM. The working FRAP reagent was prepared by mixing a, b and c in the ratio of 10:1:1 just before testing. Standard was FeSO4. 7 H2O: 0.1 - 1.5 mM in methanol. All the regents were prepared from Merck (Germany) company.

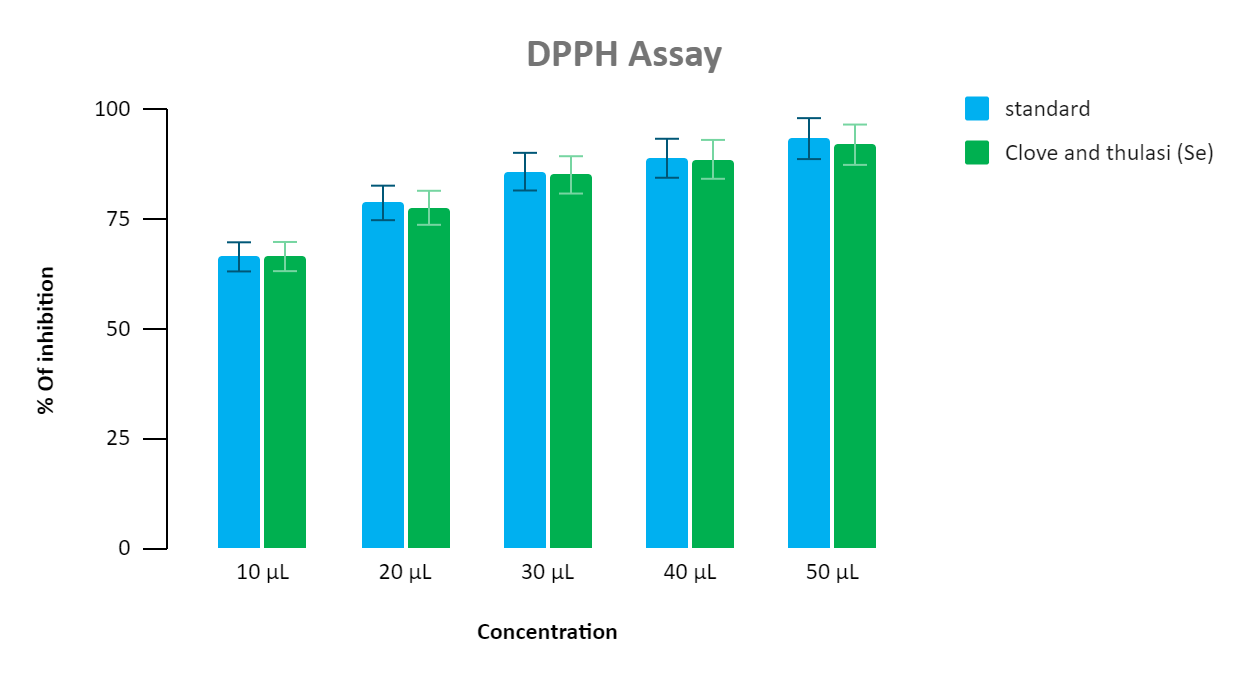
b) ProcedureFRAP solution (3.6 mL) add to distilled water (0.4 mL) and incubated at 37˚C for 5 min. Then this solution mixed with a certain concentration of the plant extract (10µL,20µL,30µL,40µL,50µL )and incubated at 37˚C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For construction of the calibration curve, five concentrations of FeSO4, 7H2O (0.1, 0.4, 0.8, 1, 1.12, 1.5 mM) were used and the absorbance values were measured as for sample solutions.

# Result

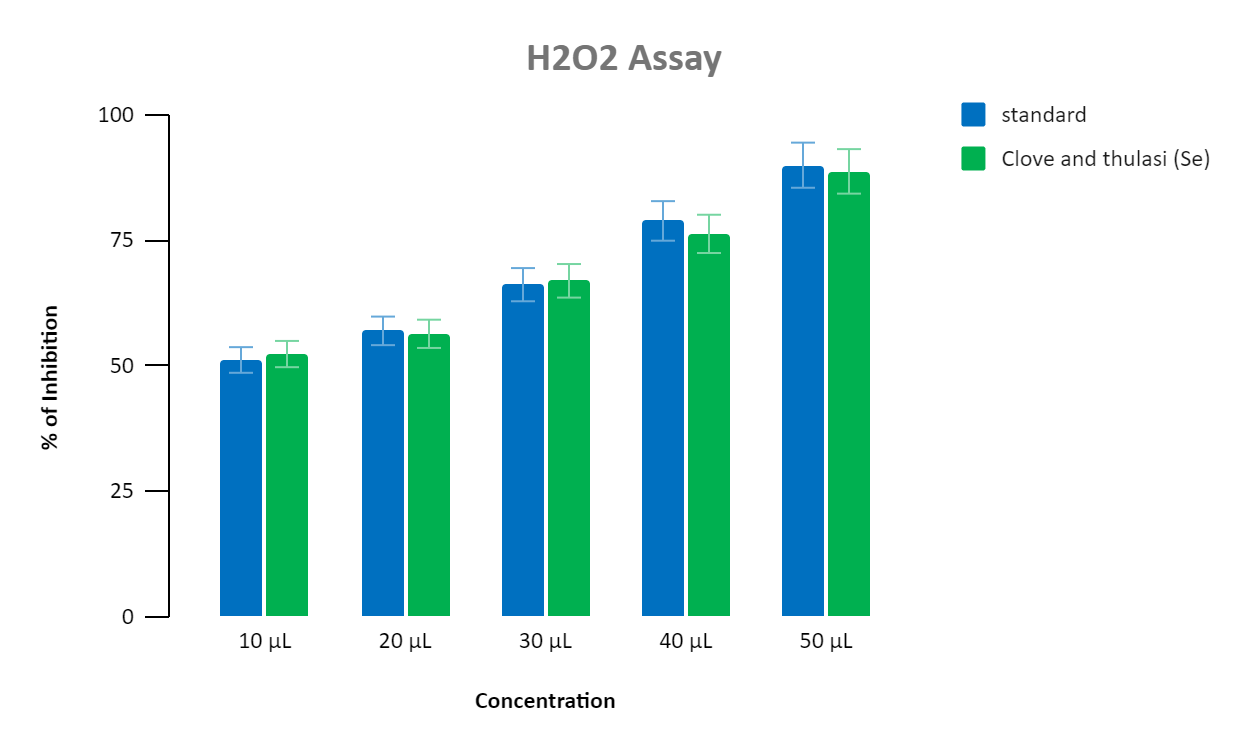
​​The percentage inhibition was only 65% when the concentration was 10 μl, at 20 μl it was 70%, at 30 μl it was 80%, at 40 μl it was 90% and at 50 μl it was 90%. It was observed from the spectra that the extract at 517 nm had highest radical scavenging activity at a concentration of 50 μl (90%), which is indicative of significant antioxidant activity as potent as DPPH itself.

The percentage inhibition was 50% when the concentration was 10 μl, at 20 μl it was 55%, at 30 μl it was 60%, at 40 μl it was 70% and at 50 μl it was 90%. It was observed from the spectra that the extract at 517 nm had highest radical scavenging activity at a concentration of 50 μl (90%), which is indicative of significant antioxidant activity as potent as hydroxy radical scavenging assay.

The result has been given in graphical representation below:



**Figure 1:** The bar chart illustrates the mean value of DPPH radical scavenging between standard sample and clove and tulsi. X axis represents the concentration. Y axis represents the percentage of inhibition . The blue color denotes the standard sample and the green color denotes clove and thulasi.



**Figure 2:** The bar chart illustrates the mean value of H2O2 radical scavenging between standard sample and clove and tulsi. X axis represents the concentration. Y axis represents the percentage of inhibition . The blue color denotes the standard sample and the green color denotes clove and thulasi.

# Discussion

The antioxidant capacities in vitro of the SeNPs were investigated, most of them can be classified into two types assays based on electron transfer (ET-based) such as DPPH, and assays based on hydrogen atom transfer (HAT-based) reactions such as hydroxyl radical scavenging assay depending upon the chemical reactions involved.[(Adel et al., 2023; Mal, 2018; Ramamurthy et al., 2022)](https://paperpile.com/c/p5TRzb/EtbD+49JU+RwSg) Among them, DPPH and lipid peroxidation were carried out in hydrophobic media[(Egbuna et al., 2022; Sreevarun et al., 2023)](https://paperpile.com/c/p5TRzb/wxjx+0rPZ). The effect of the high water solubility of the nanoparticles, led to the separation of the Se nanoparticle rich water phase from the free radical-rich lipid phase, and thus reduced the ability of Se to capture the free radicals.[(Solanki et al., 2023; Surai, 2006)](https://paperpile.com/c/p5TRzb/q2fn+yEfz) The higher values in DPPH indicated that the nanoparticles were more likely to ET-based reaction rather than HAT-based reaction [(Savita, 2022; Wadhwani et al., 2022)](https://paperpile.com/c/p5TRzb/x0Ds+GE9a). This behavior was different from that of some organic antioxidants such as rutin, which could react quickly with lipid peroxyl radicals but not nitrogen radicals. The discussion about the difference between Se and organic antioxidants was not discussed because it was beyond the scope of this work.[(Madkour, 2020; Marya et al., 2022)](https://paperpile.com/c/p5TRzb/Gx13+dA9i)

The antioxidant capacities of SeNPs were observed that the RSC% of the nanoparticles was enhanced by approximately 25% after a treatment of 30 days storage in H2O2 assay.[(Merchant et al., 2022; Zohuri, 2016)](https://paperpile.com/c/p5TRzb/KmkH+UQsI) Such enhancement was normally caused by the protection of the stabilized CS shell on the antioxidant activity of Se during storage. This effect was not significant in DPPH and lipid peroxidation (p < 0.05), which was probably due to the low level of RSC% concealing the difference between these assays. [(Cytotoxic and Antimicrobial Effects of Herbal Formulation (Ficus Benghalenis, Azadirachta Indica and Menthapiperita) Based Mouthwash, n.d.; Laghari et al., 2023)](https://paperpile.com/c/p5TRzb/0b3l+jZj0) Anyway, the use of the stabilized nanoparticles was the best choice for the following experiments. No effect of molecular weight was observed in all tests in vitro. Although the molecular weight could affect the Se release rate via the modification of the nano-carrier microstructure, the minor difference of the released Se quantities was not serious enough to disturb the antioxidant capacities in the present experimental conditions. [(Jain & Verma, 2022; Lagaron et al., 2020)](https://paperpile.com/c/p5TRzb/12o6+JqzA)

# Conclusion

In this study, a simple, biological and low-cost approach was done for the preparation of selenium nanoparticles using Tulsi and clove extract. Thus clove and thulasi mediated selenium nanoparticles can be subjected to the various other biological activities such as antibacterial, antifungal, cytotoxic evaluation to know the efficiency of these nanoparticles.

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