

Green synthesis of selenium nanoparticles using clove and Thulasi and their 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.

Aim: 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:

Antioxidant 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 a positive control.

Result and discussion: 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.

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 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. (1,2) 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. (2–4) The antioxidant contents of fruits and vegetables increase because of natural antioxidant consumption, which is related to reduced risk of cancer and heart disease. Tulsi is a member of the basil family Lamiaceae (tribe Ocimeae), is native to the Indian subcontinent, China, and Southeast Asia, and is widespread as a cultivated plant throughout the Southeast Asian tropics. Tulsi has the effect of antiseptic and analgesic properties and relieves swelling. (5)

Nanotechnology explores a variety of promising approaches in the field of biomedical sciences. For the biogenesis of selenium (Se) nanoparticles, different parts of a plant are used as they contain metabolites such as alkaloids,(5–8) flavonoids, phenols, proteins, and other phytochemicals which act as reducing agents to produce and stabilize nanoparticles. (9) Nanotechnology is also widely practiced in medicine, agriculture, and many other technologies. (10,11)The nanoparticles are major drug carriers for delivering very sensitive and highly valuable drugs to complicated diseases. Selenium is an important micronutrient of our body, and selenium nanoparticles for biomedical applications are very useful for the biomedical community. (12–14)

Se nanoparticles present lower toxicity and higher biocompatibility than organic or inorganic Se compounds, attracting the scientific community's attention for their application as therapeutic and theranostic agents. (15–17) Nano-selenium has a high potential to act as an 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 membrane structure of organisms from damage. (18,19) 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 the 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 the distillation process for 3h. The extracts were collected and dried in glass jars at 40 °C. The extracts were stored at 40C for further analysis.

Two hundred milliliters of 40 mM ascorbic acid were taken in six different conical flasks. Ten milliliters of 20 mM 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 a control, and the remaining used for samples. One milliliter of all the above five different plant extracts was added to the remaining five conical flasks separately. After the addition of the extracts, both samples and tests were analyzed spectrophotometrically at regular intervals of time (0h, 2h, 4h, and 6h). By using clove and thulasi samples, selenium nanoparticles were produced by using the same method that was mentioned above . A sample with 1 mL of ascorbic acid and 10 mL of sodium selenite was added to a conical flask, containing 200 mL of ascorbic acid was taken. Both the control and the sample were incubated at room temperature for 4 hours. After that, the solutions were centrifuged at 8,000 rpm for 20 min at 40C. The pellet was water washed 1-2 times and with absolute ethanol 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 8,000 rpm for 20 min. The pellet was dried overnight, and the powder form of selenium nanoparticles was used for further analysis.

ANTIOXIDANT ACTIVITY:

1. DPPH METHOD

Antioxidant activity

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

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

HYDROXYL RADICAL SCAVENGING ASSAY:

All solutions were prepared freshly.1.0mL of the reaction mixture contained 100 μ L of 28mM of 2-deoxy-2-ribose (dissolved in phosphate buffer,pH 7.4), 500 μ L solution of various concentrations of the *Carcia papaya* (10 μ L,20 μ L,30 μ L,40 μ L,50 μ L) 200 μ L of 200 μ M FeCl₃ and 1.04mM EDTA (1:1 v/v),100 μ L H₂O₂(1.0mM) and 100 μ L 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) FeCl₃.6H₂O: (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. The standard was FeSO₄. 7 H₂O: 0.1 - 1.5 mM in methanol. All the reagents were prepared from the Merck (Germany) company.

b) Procedure

FRAP solution (3.6 mL) is added to distilled water (0.4 mL) and incubated at 37°C for 5 min. Then this solution was mixed with a certain concentration of the plant extract (10 μ L,20 μ L,30 μ L,40 μ L,50 μ L)and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 593 nm. For the 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 μ L, at 20 μ L it was 70%, at 30 μ L it was 80%, at 40 μ L it was 90%, and at 50 μ L 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 μ L (90%), which is indicative of significant antioxidant activity as potent as DPPH itself.

The percentage inhibition was 50% when the concentration was 10 μ L, at 20 μ L it was 55%, at 30 μ L it was 60%, at 40 μ L it was 70%, and at 50 μ L 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 μ L (90%), which is indicative of significant antioxidant activity as potent as the hydroxy radical scavenging assay.

The result has been given in graphical representation below:

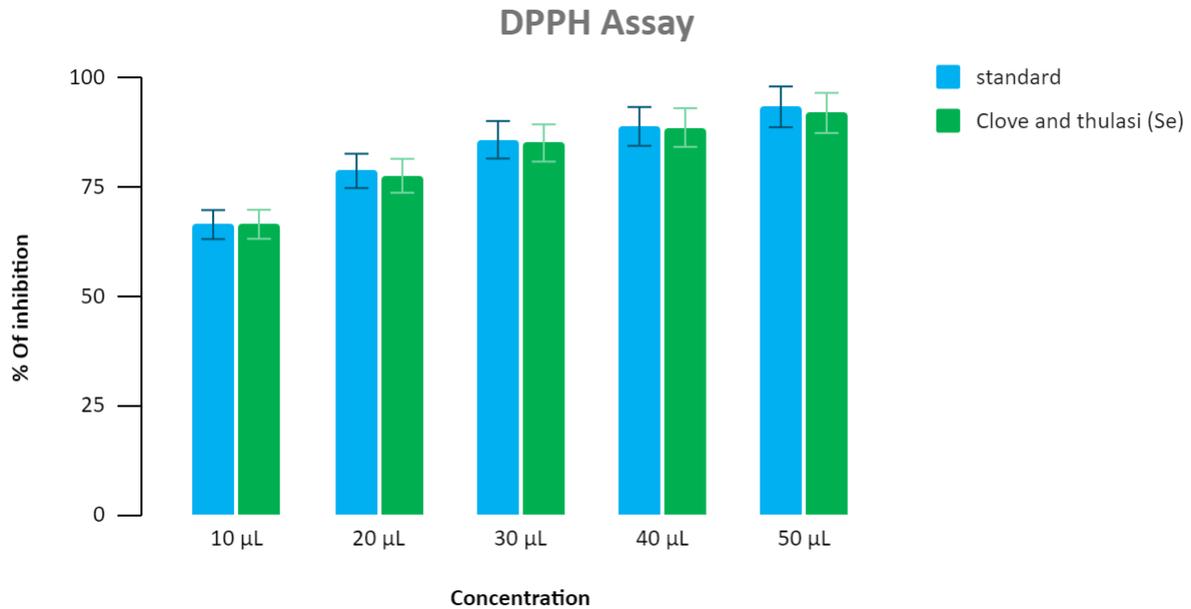


Figure 1: The bar chart illustrates the mean value of DPPH radical scavenging between the standard sample and clove and tulsii. The x-axis represents the concentration. The y-axis represents the percentage of inhibition. The blue color denotes the standard sample, and the green color denotes clove and thulasi.

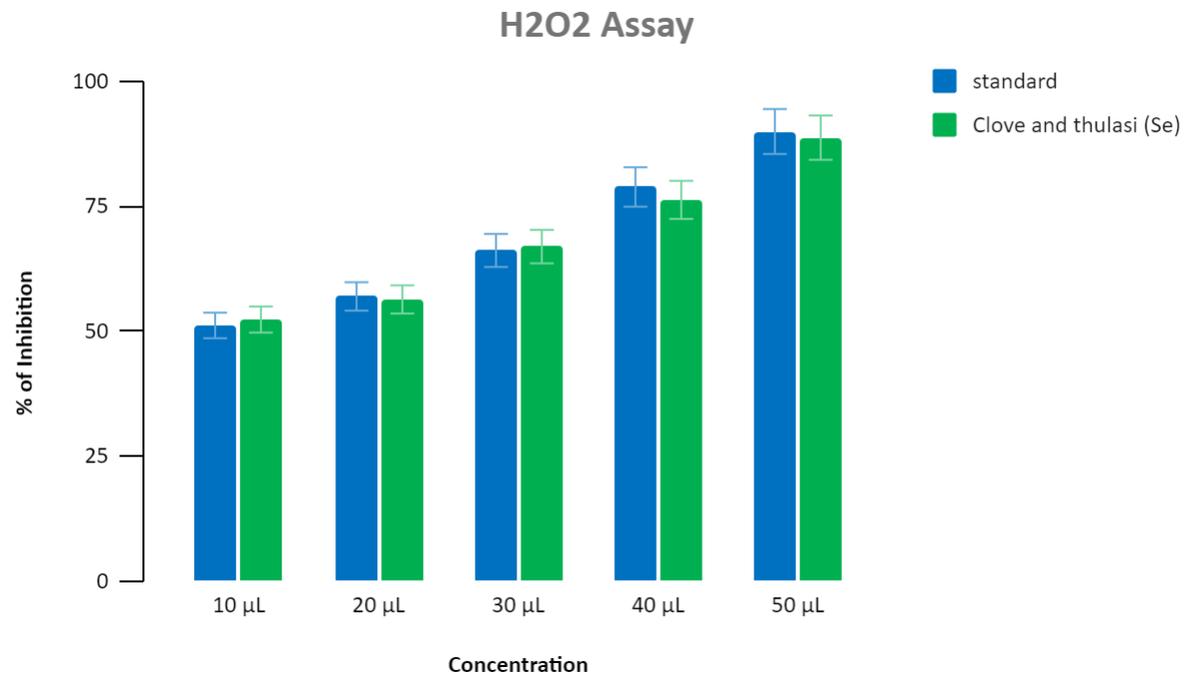


Figure 2: The bar chart illustrates the mean value of H2O2 radical scavenging between the standard sample and clove and tulsii. The x-axis represents the concentration. The y-axis represents the percentage of inhibition. The blue color denotes the standard sample, and the green color denotes clove and thulasi.

Discussion:

The antioxidant capacities in vitro of the SeNPs were investigated; most of them can be classified into two types of assays based on electron transfer (ET-based), such as DPPH, and assays based on hydrogen atom transfer (HAT-based) reaction, such as hydroxyl radical scavenging assay, depending upon the chemical reactions involved. (20–22) Among them, DPPH and lipid peroxidation were carried out in hydrophobic media(23,24). 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. (25,26) The higher values in DPPH indicated that the nanoparticles were more likely to undergo ET-based reaction rather than HAT-based reaction (27,28). This behavior was different from that of some organic antioxidants such as rutin, which could react quickly with lipid peroxy 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. (29,30)

The antioxidant capacities of SeNPs were observed, and the RSC% of the nanoparticles was enhanced by approximately 25% after a treatment of 30 days of storage in the H₂O₂ assay. (31,32) 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. (33,34) 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. (35,36)

Conclusion:

In this study, a simple, biological, and low-cost approach was used 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.

Reference:

1. Vargas-Bernal, R.; He, P.; Zhang, S. *Hybrid Nanomaterials: Flexible Electronics Materials*; BoD – Books on Demand, 2020.
2. Chokkattu, J. J.; Mary, D. J.; Shanmugam, R.; Neeharika, S. Embryonic toxicology evaluation of ginger- and clove-mediated titanium oxide nanoparticles-based dental varnish with zebrafish. *The journal of contemporary dental practice* **2022**, 23(11), 1157–1162. doi:10.5005/jp-journals-10024-3436.
3. Anandhi, P.; Tharani, M.; Rajeshkumar, S.; Lakshmi, T. Antibacterial activity of cinnamon and clove oil against wound pathogens. *Journal of population therapeutics and clinical pharmacology = Journal de la therapeutique des populations et de la pharamcologie clinique* **2022**, 28(2), e41–e46. doi:10.47750/jptcp.2022.871.
4. *Anti-Inflammatory Potential of a Mouthwash Formulated Using Clove and Ginger Mediated by Zinc Oxide Nanoparticles: An In Vitro Study*.
5. Prasad, R. *Plant Nanobionics: Volume 1, Advances in the Understanding of Nanomaterials Research and Applications*; Springer, 2019.
6. Mohapatra, S.; L, Leelavathi; I., M. A.; R., P. K.; S., R. Assessment of Antimicrobial Efficacy of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation against Oral Pathogens - An In Vitro Study. <https://www.researchgate.net/profile/Subhashree-Mohapatra-4/publication/343133648_Assessment_of_Antimicrobial_Efficacy_of_Zinc_Oxide_Nanoparticles_Synthesize_d_Using_Clove_and_Cinnamon_Formulation_against_Oral_Pathogens_-_An_In_Vitro_Study/links/6361f4a5431b1f5300621d97/Assessment-of-Antimicrobial-Efficacy-of-Zinc-Oxide-Nanoparticles-Synthesized-Using-Clove-and-Cinnamon-Formulation-against-Oral-Pathogens-An-In-Vitro-Study.pdf> Accessed 23.11.18.
7. Pandiyan, I.; Sri, S. D.; Indiran, M. A.; Rathinavelu, P. K.; Prabakar, J.; Rajeshkumar, S. Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. *Journal of conservative dentistry: JCD* **2022**, 25(3), 241–245. doi:10.4103/JCD.JCD_369_21.
8. *Green Synthesis of AgNPs from Leaves Extract of Salvia Sclarea, Their Characterization*.
9. Aronson, J. K. *Side Effects of Drugs Annual: A Worldwide Yearly Survey of New Data and Trends in Adverse Drug Reactions*; Elsevier, 2005.
10. Chokkattu, J. J.; Neeharika, S.; Rameshkrishnan, M. Applications of nanomaterials in dentistry: A review. *Journal of International Society of Preventive & Community Dentistry* **2023**, 13(1), 32–41. doi:10.4103/jispcd.JISPCD_175_22.
11. Aparna, J.; Maiti, S.; Jessy, P. Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. *Journal of conservative dentistry: JCD* **2021**, 24(6), 553–557. doi:10.4103/jcd.jcd_638_20.
12. Bhonsle, A. S. R.; Priyadarshini, R.; Kumar, R.; Sinduja, P. Antidiabetic and Cytotoxic Effect of Hexane Extract in Mucuna Pruriens. *HIV Nursing* **2022**.
13. Dhanvanth, M.; Maheswari, T. N. U. Topical herbal therapeutic formulation used in the management of oral potentially malignant disorders – A systematic review. *Journal of Indian Academy of Oral Medicine and Radiology* **2022**, 34(2), 223–227. doi:10.4103/jiaomr.jiaomr_101_21.
14. Ganapathy, D.; Professor and Head of Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai. Awareness of hazards caused by long-term usage of polyethylene terephthalate (PET) bottles. *International journal of dentistry and oral science* **2021**, 2976–2980. doi:10.19070/2377-8075-21000605.
15. Baronzio, G. F.; Dieter Hager, E. *Hyperthermia In Cancer Treatment: A Primer*; Springer Science & Business Media, 2008.
16. Muthuswamy Pandian, S.; Subramanian, A. K.; Ravikumar, P. A.; Adel, S. M. Biomaterial testing in contemporary orthodontics: Scope, protocol and testing apparatus. *Seminars in orthodontics* **2022**. doi:10.1053/j.sodo.2022.12.011.
17. *Anti Inflammatory Activity of Selenium Nanoparticles Using Clove and Thulasi Formulation*.

18. Mishra, N. K. *Catalytic Application of Nano-Gold Catalysts*; BoD – Books on Demand, 2016.
19. Subramanian, A.; Harikrishnan, S. 3D printing in orthodontics: A narrative review. *Journal of international oral health: JIOH* **2023**, 15(1), 15. doi:10.4103/jioh.jioh_83_22.
20. Mal, J. *Microbial Synthesis of Chalcogenide Nanoparticles: Combining Bioremediation and Biorecovery of Chalcogen in the Form of Chalcogenide Nanoparticles*; CRC Press, 2018.
21. Ramamurthy, S.; Thiagarajan, K.; Varghese, S.; Kumar, R.; Karthick, B. P.; Varadarajan, S.; et al. Assessing the in vitro antioxidant and anti-inflammatory activity of Moringa oleifera crude extract. *The journal of contemporary dental practice* **2022**, 23(4), 437–442. doi:10.5005/jp-journals-10024-3323.
22. Adel, S. M.; El-Harouni, N.; Vaid, N. R. White Spot lesions: State of the art biomaterials and workflows used in prevention, progression and treatment. *Seminars in orthodontics* **2023**. doi:10.1053/j.sodo.2023.01.002.
23. Egbuna, C.; Găman, M.-A.; Jeevanandam, J. *Applications of Nanotechnology in Drug Discovery and Delivery*; Elsevier, 2022.
24. Sreevarun, M.; Ajay, R.; Suganya, G.; Rakshagan, V.; Bhanuchander, V.; Suma, K. Formulation, configuration, and physical properties of dental composite resin containing a novel $2\pi + 2\pi$ photodimerized crosslinker - cinnamyl methacrylate: An in vitro research. *The journal of contemporary dental practice* **2023**, 24(6), 364–371. doi:10.5005/jp-journals-10024-3480.
25. Surai, P. F. *Selenium in Nutrition and Health*; Nottingham Trent University, 2006.
26. Solanki, L. A.; Dinesh, S. P. S.; Jain, R. K.; Balasubramaniam, A. Effects of titanium oxide coating on the antimicrobial properties, surface characteristics, and cytotoxicity of orthodontic brackets - A systematic review and meta analysis of in-vitro studies. *Journal of oral biology and craniofacial research* **2023**, 13(5), 553–562. doi:10.1016/j.jobcr.2023.05.014.
27. Savita. *Myconanotechnology: Green Chemistry for Sustainable Development*; Bentham Science Publishers, 2022.
28. Wadhvani, V.; Sivaswamy, V.; Rajaraman, V. Surface roughness and marginal adaptation of stereolithography versus digital light processing three-dimensional printed resins: An in-vitro study. *Journal of Indian Prosthodontic Society* **2022**, 22(4), 377–381. doi:10.4103/jips.jips_8_22.
29. Madkour, L. H. *Reactive Oxygen Species (ROS), Nanoparticles, and Endoplasmic Reticulum (ER) Stress-Induced Cell Death Mechanisms*; Academic Press, 2020.
30. Marya, A.; Venugopal, A.; Karobari, M. I.; Rokaya, D. White Spot lesions: A serious but often ignored complication of orthodontic treatment. *The open dentistry journal* **2022**, 16(1). doi:10.2174/18742106-v16-e2202230.
31. Zohuri, B. *Directed Energy Weapons: Physics of High Energy Lasers (HEL)*; Springer, 2016.
32. Merchant, A.; Ganapathy, D. M.; Maiti, S. Effectiveness of local and topical anesthesia during gingival retraction. *Brazilian dental science* **2022**, 25(1), e2591. doi:10.4322/bds.2022.e2591.
33. *Cytotoxic and Antimicrobial Effects of Herbal Formulation (Ficus Benghalensis, Azadirachta Indica and Menthapiperita) Based Mouthwash*.
34. Laghari, I. A.; Pandey, A. K.; Samykano, M.; Aljafari, B.; Kadirgama, K.; Sharma, K.; et al. Thermal energy harvesting of highly conductive graphene-enhanced paraffin phase change material. *Journal of thermal analysis and calorimetry* **2023**, 148(18), 9391–9402. doi:10.1007/s10973-023-12336-5.
35. Lagaron, J. M.; Torres-Giner, S.; Prieto, C. *Nanomaterials to Enhance Food Quality, Safety, and Health Impact*; MDPI, 2020.
36. Jain, R. K.; Verma, P. Visual assessment of extent of White Spot lesions in subjects treated with fixed orthodontic appliances: A retrospective study. *World journal of dentistry* **2022**, 13(3), 245–249. doi:10.5005/jp-journals-10015-2042.