



Anti inflammatory and antifungal activity of zinc oxide nanoparticle using red sandalwood extract

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ABSTRACT

Introduction : Pterocarpus santalinus is valued for its strong, dark-purple, bitter heartwood. The gentle aroma of P. santalinus heartwood is caused by the presence of terpenoids, which gives it its color and fragrance. P. santalinus heartwood dye is used as a light microscopy stain, and as a coloring agent in pharmaceutical preparations. Antibacterial, antimicrobial, and UV-blocking capabilities of ZnO NPs are outstanding compared with other metal oxides. The aim of the study is to analyse the antiinflammatory and antifungal activity of zinc oxide nanoparticles using red sandal wood extract.

Materials And Methods: A plant extract sample was taken and 100ml of distilled water was added. Heated at 50°C for 5-10 minutes. The heated solution was filtered. .0.861g of zinc oxide was measured and dissolved in 70 ml of distilled water. The mixture was transferred to extract the sample. The solution was heated and labeled. The heating mantle was used with a temperature of 50-60°C and the time taken was 6-8 minutes. The results obtained were statistically analyzed using spearman correlation analysis and using SPSS software.

Results: The sample shows an increasing absorbance rate and increase in the zone of inhibition with the increase in the concentration of the extract. It also showed an effective antifungal activity against C.albicans. The correlation analysis is statistically significant with p value<0.05.

Conclusion : From the present study, it is concluded that red sandal mediated zinc oxide nanoparticles possess anti-inflammatory and antifungal activity.

Keywords: anti-inflammatory, anti-fungal, Innovative technique, Pterocarpus santalinus, red sandalwood, Zinc oxide nanoparticle

INTRODUCTION

Natural herbal extracts of red sandalwood, botanical name *Pterocarpus santalinus*, have been used in therapeutics for centuries and are considered to have anti-diabetic, anti-inflammatory, antioxidant, and analgesic effects. 1 2In English, *Pterocarpus santalinus* L. f. (Fabaceae) is known as red sandalwood, but it also goes by other names in other languages. *Pterocarpus* is derived from the Greek words *pteron* (wing) and *karpos* (fruit), referring to the winged pod, while *santalinus* is derived from the Latin words *sandal* and *inus* (meaning similar to), referring to a plant that has characteristics similar to Indian sandalwood, *Santalum album* L.³⁴

P. santalinus (also known as African or Nepalese sandalwood and Indian sandalwood) is valued for its strong, dark-purple, bitter heartwood. The active components of Red Sandalwood, such as anthocyanins, saponins, tannins, isoflavonoids, terpenoids, and associated phenolic compounds, beta-sitosterol, and lupeol, are considered to be multifunctional in the treatment of various diseases. 5–7The gentle aroma of *P. santalinus* heartwood is caused by the presence of terpenoids, which gives it its color and fragrance. *P. santalinus* heartwood dye is used as a light microscopy stain, a coloring agent in pharmaceutical preparations, and in the food, leather, and textile industries. 7–10. Many studies have taken place using red sandalwood mediated zinc oxide nanoparticles. Various activities such as anti-diabetic, antioxidant, cytotoxicity, and anti-cancer activities have been done by many authors. The previous studies have faced challenges mainly on the method that has been adopted to synthesize the nanoparticles. The chemical method of synthesis has its disadvantages such as the distribution of the size and control of the deposit parameters but it's also a widely used technique as it is very cost-efficient, requires no chemical purification, and large-scale production of the nanoparticle can be achieved through this method. 11,12

Due to their unique physical and chemical properties, zinc oxide nanoparticles (ZnO NPS), one of the most common metal oxide nanoparticles, are widely used in a variety of fields. 13–16ZnO NPs were first used in the

rubber industry to provide wear resistance to rubber composites, increase the durability and strength of high polymers, and provide anti-aging properties, among other things. ZnO is increasingly used in personal care products, such as cosmetics and sunscreen, due to its good UV absorption properties. 17ZnO NPs also have outstanding antibacterial, antimicrobial, and UV-blocking properties. Zinc is commonly recognized as an important trace element that can be found in all body tissues, including the brain, muscle, bone, and skin. 18–20

Zinc participates in the body's metabolism and plays important roles in protein and nucleic acid synthesis, hematopoiesis, and neurogenesis as the key component of various enzyme systems. Because of the small particle size of Nano-ZnO, zinc is more readily absorbed by the body. 21As a result, nano-ZnO is widely used in food. Furthermore, the US Food and Drug Administration has listed ZnO as a “GRAS” (generally accepted as safe) substance (FDA). 22,23ZnO NPs have gotten a lot of attention in biomedical applications because of these properties. ZnO NPs, which are relatively inexpensive and less toxic than other metal oxides NPs, have a wide variety of biomedical uses, including anticancer, drug delivery, antibacterial, and diabetes treatment; anti-inflammation; wound healing; and bioimaging. 24–27.

Our team has extensive knowledge and research experience that has translated into high quality publications^{28,29–42,43–47}. This study aims to determine the anti-inflammatory and antifungal activity of red sandalwood mediated zinc oxide nanoparticles.

MATERIALS AND METHODS

Preparation of plant extract

Plant samples such as *Pterocarpus santalinus* were extracted from fresh or dried plant material. There was no bias and a random sampling method was adopted. Since the plant extract was already available in powdered form, the extract was directly prepared. 100ml of distilled water taken in measuring cylinder 1.008g of *Pterocarpus santalinus* was measured and taken using a weighing machine. (Fig.1) Then, the

weighted powder is mixed with the distilled water that is taken in a conical flask. The solution is labeled and heated by using a machine called the heating mantle. The temperature of the heating mantle was set to about 50°C and the time

taken to heat is about 5-10 minutes. The heating solution is taken out of the heating mantle when there is an appearance of small bubbles. After the heating process, the heated solution is filtered using filter paper.

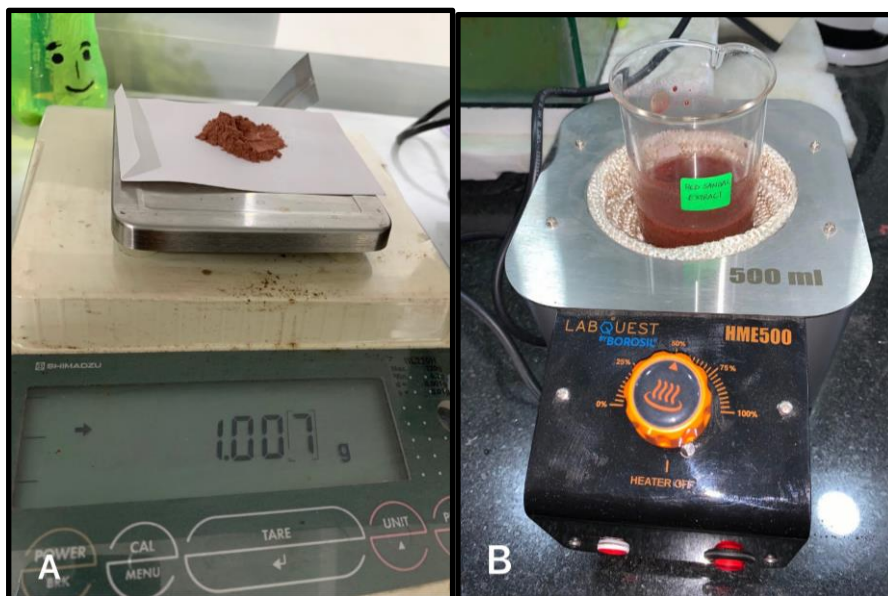


FIGURE 1: Image A depicting the measurement of red sandalwood powder and image B depicting the heating to prepare the red sandalwood extract.

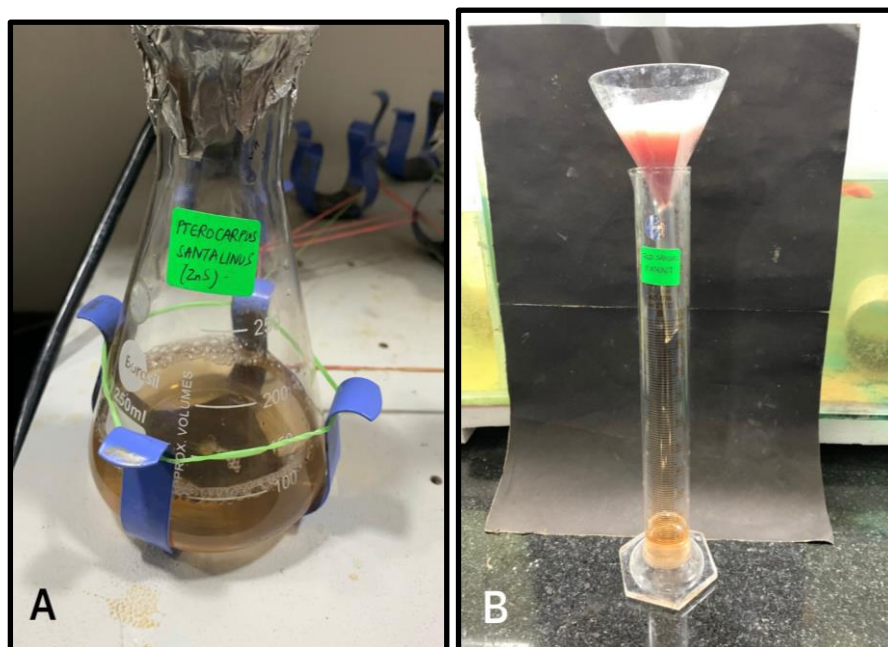


FIGURE 2: Image A depicting the extract being placed in the orbital shaker. Image B Depicting the filtration of the extract.

Preparation of zinc oxide nanoparticle

0.861g of zinc oxide was measured and dissolved in 70 ml of distilled water. (Fig.3) Now the calculated amount of zinc oxide and distilled water has been applied to the previously prepared plant extract. The solution was to be red. The

measured powder which was mixed with distilled water was taken in a conical flask. The solution was heated and labeled. The heating mantle was used with a temperature of 50-60 degrees Celsius and the time taken was 6-8 minutes. The annealed powder was used as a sample for analysis.



FIGURE 3: Image A depicts the zinc oxide powder. Image B depicts 0.861gms of zinc oxide that was measured to prepare the nanoparticle.

Anti Inflammatory Activity

Bovine serum albumin was used for the assay. 2 ml of bovine albumin was mixed with 400 microliters of zinc oxide nanoparticles in

different concentrations was used as standard and then incubated for 55°C and then the results were analyzed spectrometrically (Fig. 4).

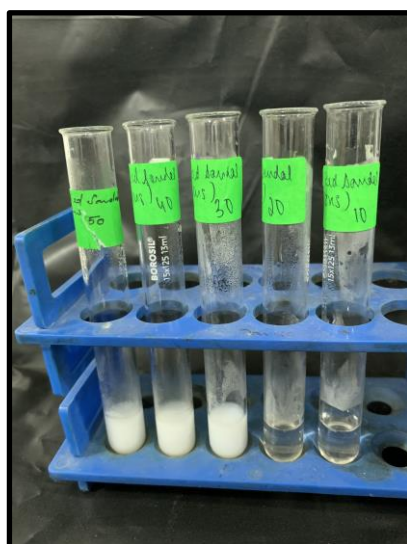


FIGURE 4: Image depicting samples with different concentrations of red sandalwood zinc oxide nanoparticles with bovine serum albumin to show anti-inflammatory activity.

Antifungal Activity

Candida albicans were used as a tested pathogen by agar well diffusion assay. (Fig. 5) Sabouraud's Dextrose Agar is used to prepare the medium. The prepared and sterilized medium was

swabbed with test organisms and nanoparticles with different concentrations were added to the well. The plates were incubated at 28° C for 48-72hours. After the incubation time, the zone of inhibition was measured.



FIGURE 5: Plate depicting Zone of inhibition of *C. albicans* with different concentrations of red sandalwood zinc oxide nanoparticle to show antifungal activity.

The results obtained were tabulated accordingly and their graphs were plotted using correlation analysis in statistical software “SPSS version 23”. The graphs thus collected were studied and

analyzed. The data collected and studied were validated by the guide and the nanoparticle researcher.

RESULTS

Anti Inflammatory

TABLE 1: The table depicts the concentration and the absorbance rate obtained for the activity.

Concentration	Absorbance
10 microlitre	0.092
20 microlitre	0.104
30 microlitre	0.195
40 microlitre	0.215
50 microlitre	0.265

Anti Fungal (C Albicans)

TABLE 2: The table depicts the concentration and the zone of inhibition obtained for the activity.

Concentration	Zone Of Inhibition
25 microlitre	9mm
50 microliter	14mm
100 microliter	20mm
standard	12mm

Anti Inflammatory

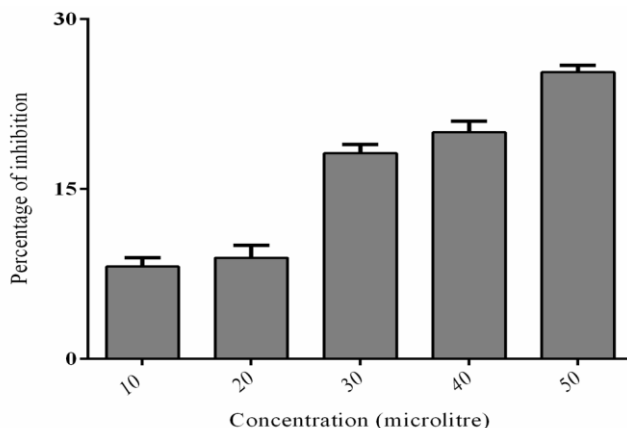


FIGURE 6: The above bar graph represents the increase in absorbance rate with an increase in concentration. X axis denotes concentration and Y axis denotes percentage of inhibition, with positive spearman correlation ($r=1$) with rise in concentration

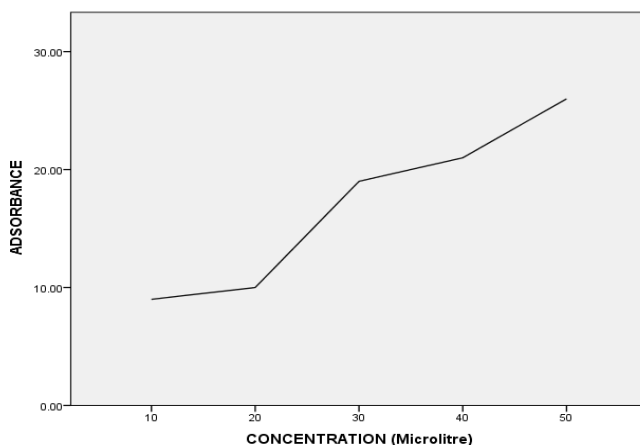


FIGURE 7: The above line graph depicts the positive correlation between the absorbance rate with an increase in the concentration of the sample.

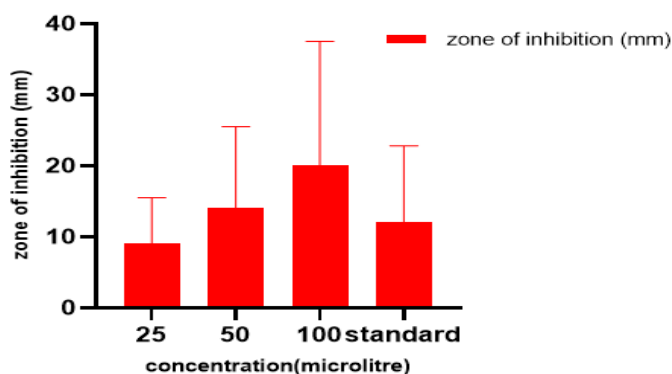


FIGURE 8: The above bar graph denotes the relation between concentration and increase in the zone of inhibition of *Candida albicans*.

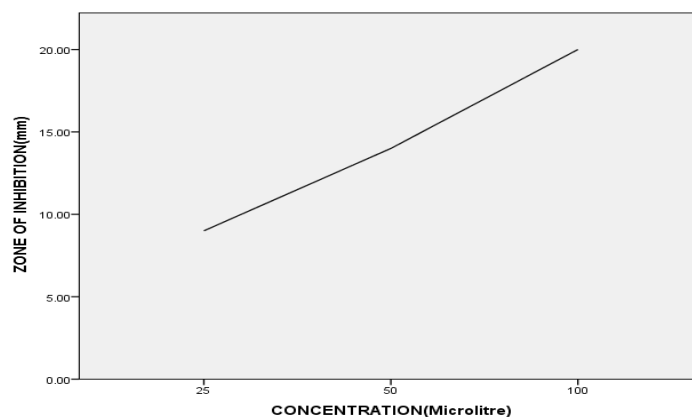


FIGURE 9: The above line graph depicts the positive correlation between the zone of inhibition with the increase in the concentration of the sample.

The test for anti-inflammatory activity was assessed using bovine serum albumin. 2 ml of the bovine albumin was added to the red sandalwood ZnO nanoparticle extract with the extract concentration ranging from 1 microlitre to 5 microlitres and the result was seen after incubation. The study showed a positive increase in the absorbance rate with the increase in the concentration of the nanoparticle extract. The test for antifungal activity was assessed using the fungal pathogen, *Candida albicans*. The test was done using Sabouraud's dextrose agar in a sterile medium and the plates were incubated for 48-72 hours at 28 degrees celsius and the zone of inhibition was obtained. The study showed positive results as the zone of inhibition of *Candida Albicans* showed a positive increase with an increase in the concentration of the extracted sample. From the results obtained from our study, it has been observed that the red sandalwood mediated zinc oxide nanoparticle has a positive effect on anti-inflammatory and antifungal activity.

DISCUSSION

Nanomaterials are particles with nanoscale dimensions, are extremely small particles with increased catalytic reactivity, thermal conductivity, nonlinear optical efficiency, and chemical stability due to their wide surface area to volume ratio.⁴⁸ Nanoparticles have been incorporated into many consumer industries, including industrial, health, food, feed, space, chemical, and cosmetics, necessitating a green

and environmentally friendly approach to their synthesis. ^{49,50} Biosynthesis of nanoparticles is a method of synthesizing nanoparticles for biomedical applications using microorganisms and plants. This method is eco-friendly, cost-effective, biocompatible, safe, and environmentally friendly. Plants, bacteria, fungi, algae, and other species are all used in green synthesis. ⁵¹ They allow the development of ZnO NPs on a large scale without the addition of impurities. Biomimetic NPs have higher catalytic activity and needless costly and toxic chemicals to manufacture. ⁵²

Similar studies have been done earlier with different fungal pathogens that have shown positive results. In the study by Saquib, because of the large-scale development, easy downstream processing, and economic viability, extracellular synthesis of NPs from the fungus is extremely useful. Fungal strains are favored over bacteria due to their higher resistance and ability to bioaccumulate metals. ^{53–55} SEM, TEM, and XRD analysis confirmed that NPs synthesized with *Candida albicans* had a similar size range of 15–25 nm. For the synthesis of ZnO NPs, *Aspergillus* species have been commonly used, and NPs synthesized from these fungal strains were mostly spherical.^{56,57} In the study by MD Jayappa, the anti-inflammatory activity of the ZnO NPs was assessed using the human red blood cells membrane stabilization method where the blood samples were collected without nonsteroidal and anti-inflammatory drugs (NSAIDs). This study showed positive

absorbance of 560 nm for the anti-inflammatory activity. 58 Several other studies have shown that the zinc oxide nanoparticle has been very effective and significant in antioxidant, anti-diabetic, and anti-cancer activities.

Other green sources for the synthesis of nanoparticles include biocompatible chemicals. It is a simple and cost-effective process that removes the production of any side products during the nucleation and synthesis of nanoparticles. With its well-dispersed existence, it results in the creation of nanoparticles of regulated shape and size 59. The limitations of this study were mainly the sample size and the errors that might've been caused in the measurement of the concentrations in the extract. In the last decade, the biosynthesis of nanoparticles using an environmentally friendly approach has been a subject of study. For the synthesis of shape and size-controlled nanoparticles, green sources act as both a stabilizing and a reducing agent. Plant-based nanoparticles have a wide range of applications in the food, pharmaceutical, and cosmetic industries, and have thus become a major research subject.60,61

CONCLUSION

From the present study, it is concluded that red sandal mediated zinc oxide nanoparticles possess anti-inflammatory and antifungal activity. In summary, this is a simple, effective biosynthetic method that could be used to substitute chemical and physical methods for large-scale ZnO-NP development. The use of such environmentally-friendly ZnO-NPs in a variety of fields, including medicine, catalysis, and drug delivery systems, makes this bioreduction method an excellent choice for large-scale synthesis.

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CONFLICT OF INTEREST

The author declares that there were no conflicts of interests in the present study.

Authorship Criteria

Ashna, Dr. R.Priyadharshini and Dr. Rajesh kumar framed the Concept, design of study or acquisition of data or analysis and interpretation of data

Drafting the article or revising was done by Dr.R.Priyadharshini and Dr.Palati sinduja

Final approval of the version to be published was done by Dr. R.Priyadharshini and Dr. Rajesh kumar

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REFERENCES

1. Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, et al. Anti-proliferative activity of *Bupleurum scrozonrifolium* in A549 human lung cancer cells in vitro and in vivo. *Cancer Letters*. 2005;222(2):183–93.
2. Tan ML, Sulaiman SF, Najimuddin N, Samian MR, Tengku Muhammad TS. Methanolic extract of *Pereskia bleo* (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. *Journal of Ethnopharmacology*. 2005;96(1-2):287–94.
3. Ramawat KG, Goyal S. The Indian Herbal Drugs Scenario in Global Perspectives. *Bioactive Molecules and Medicinal Plants*. 2008;325–47.
4. Aluri JSR. Pollination ecology of the Red Sanders *Pterocarpus santalinus* (Fabaceae), an endemic and endangered tree species. *Curr Sci*. 2002 Nov 10;83(9):23–8.
5. Bulle S, Reddy VD, Padmavathi P, Maturu P, Ch VN. Modulatory role of *Pterocarpus santalinus* against alcohol-induced liver oxidative/nitrosative damage in rats. *Biomedicine & Pharmacotherapy*. 2016;83(3):1057–63.
6. Kumar N, Seshadri TR. Triterpenoids of *Pterocarpus santalinus*: Constitution of a new lupene diol. *Phytochemistry*. 1975;14(2):521–3.
7. Warakagoda PS, Kumari DLC, Subasinghe S. In vitro shoot tip culture of Red Sandalwood (*Pterocarpus santalinus* L.). *Proceedings of*

- International Forestry and Environment Symposium. 2013;2(1):28–32.
8. Kumar N, Ravindranath B, Seshadri TR. Terpenoids of *Pterocarpus santalinus* heartwood. *Phytochemistry*. 1974;13(3):633–6.
 9. Brundha MP. A Comparative Study-The Role of Skin and Nerve Biopsy in Hansen's Disease. *Res J Pharm Biol Chem Sci*. 2015;7(10):837.
 10. Harsha L, Brundha MP. Prevalence of Dental Developmental Anomalies among Men and Women and its Psychological Effect in a Given Population. *Journal of Pharmaceutical Sciences and Research; Cuddalore*. 2017 Jun 20;9(6):869–73.
 11. Modan EM, University of Pitești, Romania, Plăiașu AG, University of Pitești, Romania. Advantages and Disadvantages of Chemical Methods in the Elaboration of Nanomaterials. *The Annals of "Dunarea de Jos" University of Galati Fascicle IX, Metallurgy and Materials Science*. 2020;43(1):53–60.
 12. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine--A Literature Review. *Research Journal of Pharmacy and Technology*. 2019;12(8):4052–6.
 13. Jiang J, Pi J, Cai J. The Advancing of Zinc Oxide Nanoparticles for Biomedical Applications. *Bioinorganic Chemistry and Applications*. 2018;2018(3):1–18.
 14. Wu D, Chen Z, Cai K, Zhuo D, Chen J, Jiang B. Investigation into the antibacterial activity of monodisperse BSA-conjugated zinc oxide nanoparticles. *Current Applied Physics*. 2014;14(11):1470–5.
 15. Rajeshkumar S, Sandhiya D. Biomedical Applications of Zinc Oxide Nanoparticles Synthesized Using Eco-friendly Method. *Nanoparticles and their Biomedical Applications*. 2020;5(2):65–93.
 16. Rajeshkumar S, Lakshmi T, Naik P. Recent advances and biomedical applications of zinc oxide nanoparticles. *Green Synthesis, Characterization and Applications of Nanoparticles*. 2019;6(2):445–57.
 17. Mirzaei H, Darroudi M. Zinc oxide nanoparticles: Biological synthesis and biomedical applications [Internet]. Vol. 43, *Ceramics International*. 2017. p. 907–14.
 18. Zhang Y, Nayak T, Hong H, Cai W. Biomedical Applications of Zinc Oxide Nanomaterials. *Current Molecular Medicine*. 2013;13(10):1633–45.
 19. Agarwal H, Nakara A, Menon S, Shanmugam VK. Microbe-mediated Synthesis of Zinc Oxide Nanoparticles and Its Biomedical Applications. *Microbial Nanotechnology*. 2020;3(2):162–77.
 20. Barui AK, Kotcherlakota R, Patra CR. Biomedical applications of zinc oxide nanoparticles. *Inorganic Frameworks as Smart Nanomedicines*. 2018;8(2):239–78.
 21. Ruzskiewicz JA, Pinkas A, Ferrer B, Peres TV, Tsatsakis A, Aschner M. Neurotoxic effect of active ingredients in sunscreen products, a contemporary review. *Toxicology Reports*. 2017;4(2):245–59.
 22. Zhang ZY, Xiong HM. Photoluminescent ZnO Nanoparticles and Their Biological Applications. *Materials*. 2015;8(6):3101–27.
 23. Zhang HJ, Xiong HM. Biological Applications of ZnO Nanoparticles. *Current Molecular Imaging*. 2013;2(2):177–92.
 24. Kim S, Lee SY, Cho HJ. Doxorubicin-Wrapped Zinc Oxide Nanoclusters for the Therapy of Colorectal Adenocarcinoma. *Nanomaterials*. 2017;7(11):354.
 25. Kim S, Lee SY, Cho HJ. Berberine and zinc oxide-based nanoparticles for the chemophotothermal therapy of lung adenocarcinoma. *Biochemical and Biophysical Research Communications*. 2018;501(3):765–70.
 26. Xiong HM. ZnO Nanoparticles Applied to Bioimaging and Drug Delivery. *Advanced Materials*. 2013;25(37):5329–35.
 27. Xiong HM. ChemInform Abstract: ZnO Nanoparticles Applied to Bioimaging and Drug Delivery. *ChemInform*. 2013;44(49):82–86.
 28. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. *Am J Cancer Res*. 2020 Aug 1;10(8):2546–54.
 29. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. *Hypertens Res*. 2020 Dec;43(12):1459–61.
 30. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in *Streptococcus* sp., using computational approach. *Journal of King Saud University - Science*. 2020 Jan 1;32(1):904–9.
 31. Smiline Girija AS. Delineating the Immuno-Dominant Antigenic Vaccine Peptides Against *gacS*-Sensor Kinase in *Acinetobacter baumannii*: An in silico Investigational Approach. *Front Microbiol*. 2020 Sep 8;11:2078.
 32. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of *csgA* gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University - Science*. 2020 Dec 1;32(8):3380–7.
 33. Girija ASS. Fox3+ CD25+ CD4+ T-regulatory cells may transform the nCoV's final destiny to CNS! *J Med Virol*. 2020 Sep 3;2(1):45–48
 34. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated

- gene alterations, and molecular pathways. *Environ Sci Pollut Res Int.* 2021 Jun;28(21):27162–73.
35. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol.* 2021 Feb;122:105030.
 36. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences [Internet].* 2020;82(2):55-59
 37. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β -lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection. *Acta Microbiol Immunol Hung.* 2019 Dec 9;67(1):49–55.
 38. Priyadharsini JV, Paramasivam A. RNA editors: key regulators of viral response in cancer patients. *Epigenomics.* 2021 Feb;13(3):165–7.
 39. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koenigii* bio-compounds: An in-silico approach. *Acta Virol.* 2020;64(1):93–9.
 40. Girija As S, Priyadharsini J V, A P. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). *Acta Clin Belg.* 2021 Apr;76(2):106–12.
 41. Anchana SR, Girija SAS, Gunasekaran S, Priyadharsini VJ. Detection of *csgA* gene in carbapenem-resistant *Acinetobacter baumannii* strains and targeting with *Ocimum sanctum* biocompounds. *Iran J Basic Med Sci.* 2021 May;24(5):690–8.
 42. Girija ASS, Shoba G, Priyadharsini JV. Accessing the T-Cell and B-Cell Immuno-Dominant Peptides from *A.baumannii* Biofilm Associated Protein (bap) as Vaccine Candidates: A Computational Approach. *Int J Pept Res Ther.* 2021 Mar 1;27(1):37–45.
 43. Arvind P TR, Jain RK. Skeletally anchored forsores fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. *Orthod Craniofac Res.* 2021 Feb;24(1):52–61.
 44. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another *Choluteca* Bridge! *Semin Orthod.* 2021 Mar 1;27(1):53–6.
 45. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. *Clin Oral Investig.* 2019 Sep;23(9):3543–50.
 46. Varghese SS, Ramesh A, Veeraiyan DN. Blended Module-Based Teaching in Biostatistics and Research Methodology: A Retrospective Study with Postgraduate Dental Students. *J Dent Educ.* 2019 Apr;83(4):445–50.
 47. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of *Streptococcus mutans*, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial [Internet]. *Clinical Oral Investigations.* 2020 Dec;2(2). p. 3275–80.
 48. Khan ST, Musarrat J, Al-Khedhairi AA. Countering drug resistance, infectious diseases, and sepsis using metal and metal oxides nanoparticles: Current status. *Colloids and Surfaces B: Biointerfaces.* 2016;146(3):70–83.
 49. Vidya. C, Manjunatha. C, Chandraprabha. MN, Rajshekar M, Antony Raj M A. Hazard free green synthesis of ZnO nano-photo-catalyst using *Artocarpus Heterophyllus* leaf extract for the degradation of Congo red dye in water treatment applications. *Journal of Environmental Chemical Engineering.* 2017;5(4):3172–80.
 50. Hiremath S, Vidya C, Antonyraj MAL, Chandraprabha MN, Seemashri S, Shetty B, et al. Photocatalytic Degradation Study of Rhodamine-B by Green Synthesized Nano TiO₂. *Asian Journal of Chemistry.* 2017;29(1):221–5.
 51. Elumalai K, Velmurugan S, Ravi S, Kathiravan V, Ashokkumar S. Retraction notice to “Green synthesis of Zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity” *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 2019;206(3):651.
 52. Elumalai K, Velmurugan S, Ravi S, Kathiravan V, Ashokkumar S. RETRACTED: Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy.* 2015;143(2):158–64.
 53. Pavani KV, Sunil Kumar N, Sangameswaran BB. Synthesis of Lead Nanoparticles by *Aspergillus* Species. *Polish Journal of Microbiology.* 2012;61(1):61–3.
 54. Kumar RR, Priyadharsani KP, Thamaraiselvi K. Mycogenic synthesis of silver nanoparticles by the Japanese environmental isolate *Aspergillus tamarii*. *Journal of Nanoparticle Research.* 2012;14(5).
 55. Saquib Q. *Green Synthesis of Nanoparticles: Applications and Prospects.* Springer Nature;
 56. Hoffmann MR, Martin ST, Choi W, Bahnemann DW. Environmental Applications of Semiconductor Photocatalysis. *Chemical Reviews.* 1995;95(1):69–96.
 57. Shamsuzzaman, Shamsuzzaman, Mashrai A, Khanam H, Aljawfi RN. Biological synthesis of ZnO nanoparticles using *C. albicans* and studying their catalytic performance in the synthesis of

- steroidal pyrazolines. *Arabian Journal of Chemistry*. 2017;10:S1530–6.
58. Jayappa MD, Ramaiah CK, Kumar MAP, Suresh D, Prabhu A, Devasya RP, et al. Green synthesis of zinc oxide nanoparticles from the leaf, stem and in vitro grown callus of *Mussaenda frondosa* L.: characterization and their applications. *Applied Nanoscience*. 2020;10(8):3057–74.
59. Nagarajan S, Kuppusamy KA. Extracellular synthesis of zinc oxide nanoparticle using seaweeds of gulf of Mannar, India. *Journal of Nanobiotechnology*. 2013;11(1):39.
60. Anita S, Ramachandran T, Rajendran R, Koushik CV, Mahalakshmi M. A study of the antimicrobial property of encapsulated copper oxide nanoparticles on cotton fabric. *Textile Research Journal*. 2011;81(10):1081–8.
61. Gharagozlou M, Baradaran Z, Bayati R. A green chemical method for synthesis of ZnO nanoparticles from solid-state decomposition of Schiff-bases derived from amino acid alanine complexes. *Ceramics International*. 2015;41(7):8382–7.