



Controlling Of Oral Pathogens Using Zinc Oxide Nanoparticles Synthesised Using Ginger Oleoresin

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ABSTRACT

Introduction: The major components present in ginger are alpha-zingiberene, gingerols and shogaols. Ginger oleoresin are used as aromatic, carminative, stomachic and stimulant. Zinc oxide nanoparticles have a diameter less than 100 nm. Zinc oxide nanoparticles are used due to its biosensor, cosmetics, storage, optical devices, window materials for displays, solar cells, and drug-delivery properties.

Materials And Methods: Ginger oleoresin was purchased and extract was prepared by dissolving in distilled water. Zinc oxide was added and it was characterised by UV visible spectroscopy method. Antibacterial and antifungal potential of the extract assessed by agar well diffusion method.

Results: Zinc oxide nanoparticles synthesised using ginger oleoresin shows antimicrobial activity. Dose dependent antimicrobial activity was observed. The prepared extract showed the highest zone of inhibition, 25 mm at 100µl against S.aureus and E.faecalis showed a maximum zone of inhibition of 24 mm at 100µl.

Conclusion: Ginger oleoresin mediated zinc oxide nanoparticles showed a potent antimicrobial activity against C.albicans, S.mutans, S.aureus and E.faecalis. Hence it may be formulated to treat infections caused by such organisms after further studies.

KEYWORDS:

Ginger oleoresin; zinc oxide nanoparticles; oral pathogens; antimicrobial; green synthesis,ecofriendly.

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INTRODUCTION

Ginger (*Zingiber officinale*) is a flowering plant, belonging to the family 'Zingiberaceae'. It is widely used as a spice and for traditional medicine. Ginger is a herbaceous perennial, which is about one meter tall bearing narrow leaf blades. Ginger has abundant antioxidants, helping in preventing the stress and damage to the DNA (1). The major components present in ginger are alpha-zingiberene, gingerols and shogaols. With deep processing of ginger, Ginger essential oils and ginger oleoresin are obtained, which have high value in the food

industry. Ginger has anti-platelet, anti-inflammatory, anti-tumorigenic, anti-diabetic, anti-clotting, analgesic components and it is used to treat vomiting, pain, cold symptoms, diarrhoea, aids digestion. Ginger has anti-cancer properties and is used to treat pancreatic, gastrointestinal, ovarian, breast cancers (2). Adverse effects of ginger include heartburn, diarrhea, burping, and general stomach discomfort. Oleoresin is defined as a natural or artificial mixture of essential oils and a resin. Ginger oleoresin are used as a flavouring agent and its bioactive components are well known for their antimicrobial and antioxidant properties (3).

Nanoparticle is defined as a nano-scale particle. Nanoparticles are used in the manufacture of scratch proof eyeglasses, crack-resistant paints, anti-graffiti coatings for walls, transparent sunscreens, stain-repellent fabrics, self-cleaning windows and ceramic coatings for solar cells (4). Zinc oxide is defined as an inorganic metal oxide nanoparticle that exhibits tremendous antibacterial activity. Zinc oxide nanoparticles are nanoparticles of zinc oxide with diameter less than 100 nm. Zinc oxide nanoparticles are majorly used in food industries, agriculture, pharmaceutical and cosmetic industries due to its high antibacterial effect (5),(6). ZnO which is formed on graphene oxide exhibits high capacity and also demonstrates the high rate performance and cycling stability due to using nanoparticles. Recently Zinc oxide nanoparticles are emerging as an leading photocatalyst for organic dye degradation due to its non toxicity, low cost and high photochemical reactivity. Zinc oxide nanoparticles are known for their large bandwidth, exciting binding level, antifungal, anti-diabetic, anti inflammatory, anti oxidant and optic properties (7).

Oral pathogens develop in the oral cavity which develop mainly due to poor oral hygiene. Oral bacteria exhibit a high specific adherence mechanism and result in colonising and causing disease in the oral cavity (8). Dental caries, periodontal diseases and streptococcal pharyngitis are the common oral infections caused by oral pathogens. Streptococcus, Actinomyces, Herpes simplex virus type-1 are some of the most common diseases causing oral pathogens. Bacteria accumulate in the oral cavity both on the hard and soft oral tissues in biofilms. Bacteria occupy the ecological niche provided by both the tooth surface and gingival epithelium (9). Oral pathogens invade the body to affect cardiac health and cognitive function. The aim of the study was to estimate the controlling of oral pathogens using zinc oxide nanoparticles synthesised using ginger oleoresins.

MATERIALS AND METHODS

Study Setting

The study was conducted in the Nanobiomedicine lab in Saveetha dental college and hospitals during the year 2020-2021. The ethical clearance was obtained for the present study (IHEC/SDC/UG-1914/21/128). Ginger oleoresin was obtained from Synthite Industries Pvt Ltd, Kerala.

Preparation of plant extract

Then 100ml of distilled water was measured using a measuring cylinder and 1g of ginger oleoresin was measured. Then the solution measured is dissolved in distilled water and is taken in a conical flask. The solution is labeled and is subjected to heating using a heating mantle. The mixture was heated for 5 to 10 minutes at 50-60 degrees Celsius. The heating is stopped with the presence of small bubbles. After heating, the solution is filtered with the help of filter paper (Figure 1).

Synthesis of Zinc oxide nanoparticles

0.861 g of anhydrous zinc oxide was measured and was dissolved in 70 ml of distilled water. The measured solution of zinc oxide and distilled water will be added to the previously prepared 30ml of plant extract. The solution was found to be pale red. Then a piece of foil paper was clogged to extract the solution. A uniform dispersion for the synthesis process was initiated using an orbital shaker and intermittent color variation was observed. The product was then dried and heated up. The annealed powder was used as a sample for analysis. Zinc oxide nanoparticles are characterized by UV visible spectroscopy, X-ray diffraction, field emission scanning electron microscopy, and Fourier transforms infrared spectroscopy.

ANTIMICROBIAL ACTIVITY

Antibacterial activity

Antibacterial activity of respective nanoparticles against the strain *S. aureus*, *S. mutans* and *E. coli*. MHA agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and let them stabilize for solidification. The wells were cut using the well cutter and the test organisms were swabbed. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the incubation time the zone of inhibition was measured.

Antifungal activity

C. albicans was used for evaluating the antifungal activity by agar well diffusion assay. Sabouraud's Dextrose Agar is used to prepare the medium. The prepared and sterilized medium was swabbed with test organisms and nanoparticles with different concentration were added to the wells. The plates were incubated at 28° C for 48 hours. After the incubation time the zone of inhibition was measured.

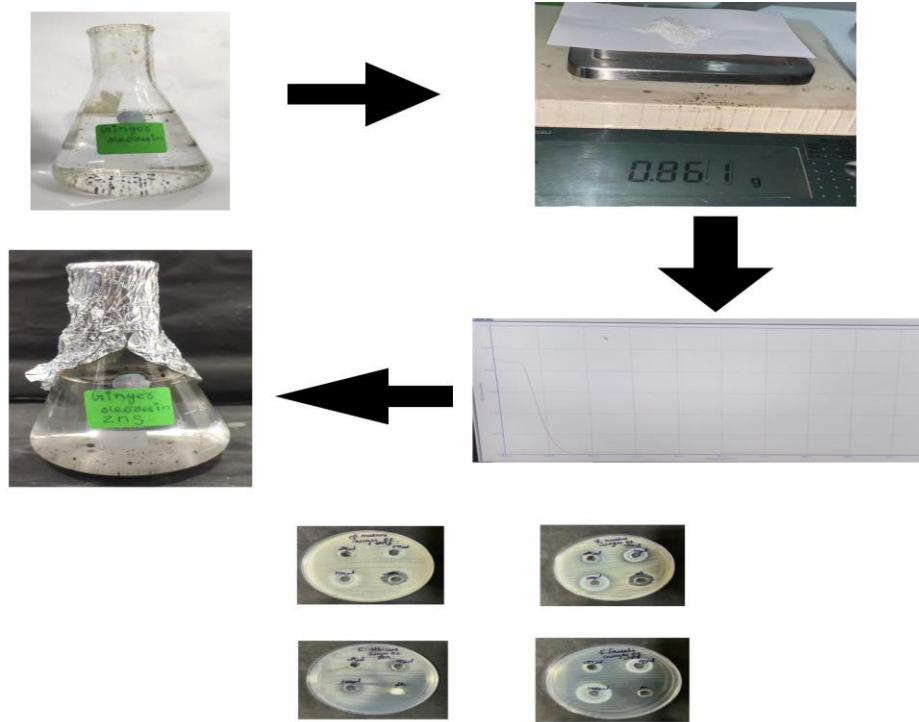


Fig.1: The figure depicts the preparation of the extract mediated zinc oxide and assessment of antimicrobial activity.

RESULTS

Figure 3 demonstrates the zone of inhibition of antibacterial activity of *E.faecalis*, *S.mutans*, *S.aureus* in ginger oleoresin mediated zinc oxide nanoparticles at 25µl, 50µl, 100µl and control. Figure 4 illustrates the zone of inhibition of antifungal

activity of *C.albicans* at 25µl, 50µl, 100µl and control in the extract. The prepared extract showed the highest zone of inhibition , 25mm at 100µl against *S.aureus* and *E.faecalis* showed a maximum zone of inhibition of 24mm at 100µl demonstrating that the zinc oxide nanoparticles synthesised using ginger oleoresin can be used in controlling pathogens.

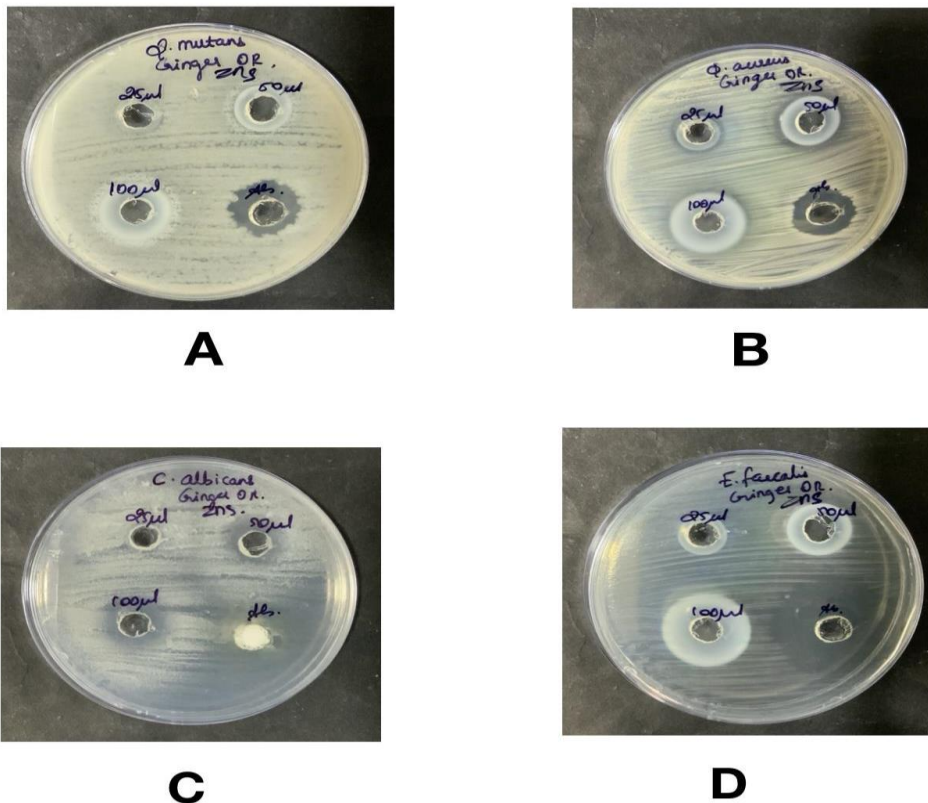


Fig.2: The picture illustrates the zone of inhibition of *S.mutans* (A), *S.aureus* (B), *C.albicans* (C) and *E.faecalis* (D) and control.

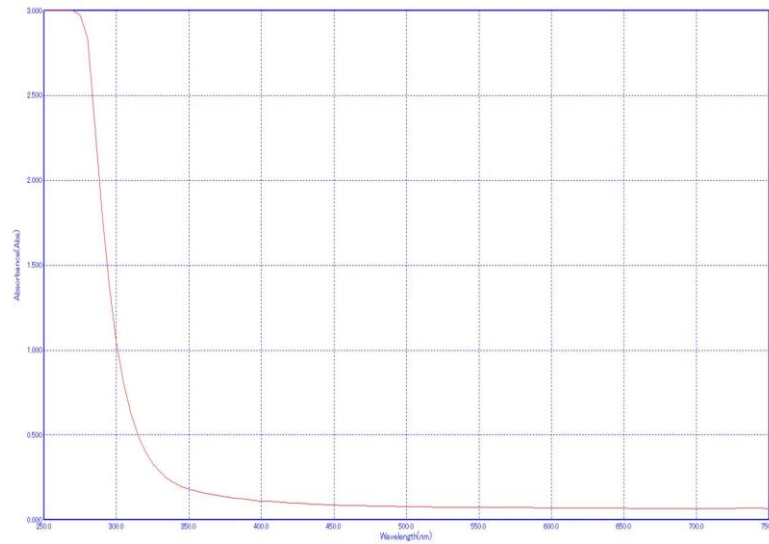


Fig.3: The figure illustrates the UV spectroscopy of ginger oleoresin mediated zinc oxide nanoparticles extract. Highest peak is seen at 270 nm.

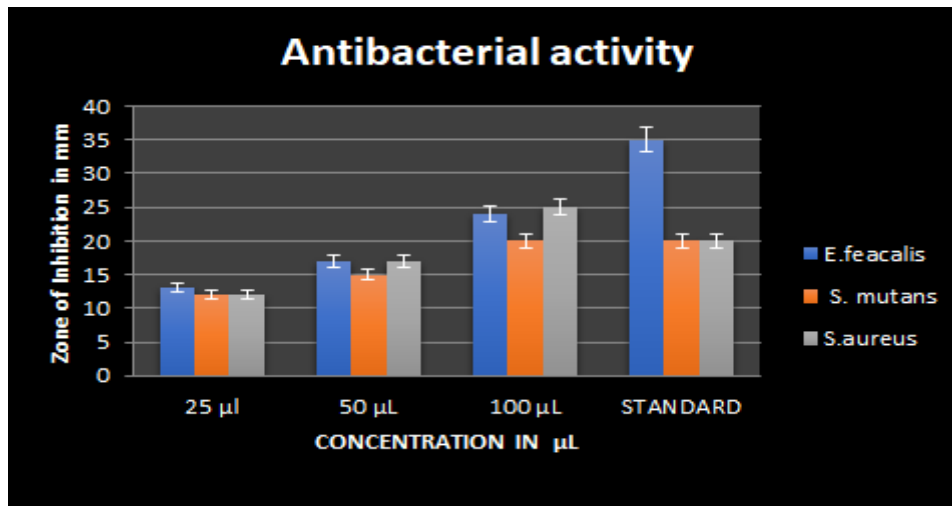


Fig.4: The graph depicts the antibacterial activity of E.faecalis, S.mutans, and S.aureus in ginger oleoresin mediated zinc oxide nanoparticles at 25µl, 50µl, 100µl concentration and at control. Blue colour represents E.faecalis, orange colour represents S.mutans and grey colour represents S.aureus. X-axis demonstrates concentration of ginger oleoresin mediated zinc oxide extract in µl and Y-axis represent the zone of inhibition produced by different bacterias.(n=3 with mean ± SD)

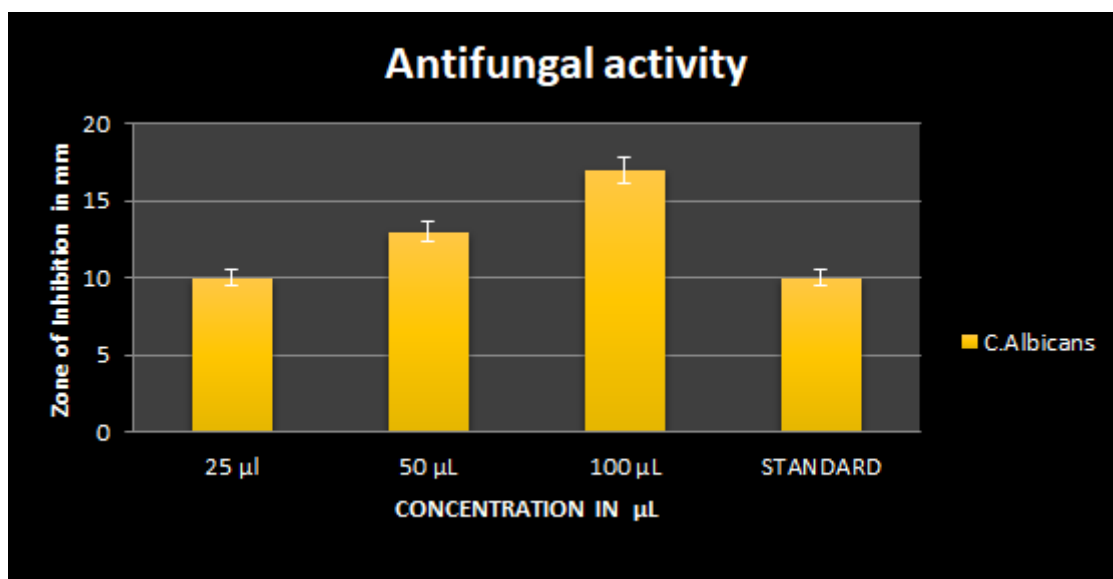


Fig.5: The graph depicts the antifungal activity of C.albicans in ginger oleoresin mediated zinc oxide nanoparticles at 25µl, 50µl, 100µl concentration and at control. Yellow colour represents C.albicans. X-axis demonstrates the concentration of ginger oleoresin mediated zinc oxide extract in µl and Y-axis represents the zone of inhibition of different bacterias. (n=3 with mean ± SD)

DISCUSSION

A study by Sanjiv Singh states that Zinc oxide nanoparticles are the most common nanoparticles used. It has a broad range of applications like personal care products, sensors, antibacterial creams, and biomedical applications. Organism-dependent cellular uptake, generation of reactive oxygen species, dissolution, and induced inflammatory response are governed by ZnO toxicity. Zinc oxide nanoparticles can act as an anticancer agent and it alters the apoptotic protein expression (10). Zinc oxide nanoparticles are of high use in the field of biomedical applications.

The study carried out by Umar Huzaifa, Doga, and Nahit reveals that zinc oxide nanoparticles exhibit antimicrobial activity against various gram-negative and gram-positive bacterial pathogens. ZnO nanoparticles' presence was confirmed by X-ray diffraction and Fourier transform infrared analysis. Further, Zinc oxide nanoparticles exhibited cytotoxic activity against strongly and weakly metastatic breast cancer cell lines (11). Zinc oxide nanoparticles are used in the manufacture of disinfectant, anti-inflammatory, and antidiabetic agents. Zinc oxide nanoparticles are used in the field of agriculture, medicine, and textile industries due to its antibacterial activity (12).

On comparing the work of Abdulrazzak, Yaseen, Maha, Mohammed demonstrates that zinc oxide nanoparticles using aqueous extract of *Deverra tortuosa* the zinc oxide nanoparticle was analyzed using Rigol ultra 3660 UV vis spectroscopy, and cytotoxicity was evaluated using MTT colorimetric assay with 96 well plates under a sterile condition. Zinc nanoparticle's presence was assessed by UV spectrophotometric analysis, FTIR spectroscopic analysis, X-ray diffraction pattern. The most common application of zinc oxide is in sunscreen. The zinc oxide formed on graphene oxide shows high capacity and high rate performance and cycling stability due to the incorporation of nanoparticles. Ginger oleoresin has volatile oils which are rich in terpenes, esters, aldehydes, ketones, alcohols and phenols. Terpenes, phenols and aldehydes exhibit broad antimicrobial spectrum and bacteriostatic and bactericidal properties which is the reason for ginger oleoresin to exhibit antimicrobial properties. Previously our team has conducted numerous studies including other oleoresins (13)-(14). Our team has extensive knowledge and research experience that has translated into high quality publications (15-19)(20)

The limitation of the study includes small sample size and only 4 test oral pathogens were used. The future scope of the study is many more properties of ginger oleoresin assessed to be used in the betterment of the oral cavity. Various nanoformulations like mouthwash, toothpaste, and oral gels can be developed which are economical, productive and safe.

CONCLUSION

The results show that zinc oxide nanoparticles synthesized by ginger oleoresin exhibited high antibacterial and antifungal properties(21-30). Thus ginger oleoresin can be used to treat

gingivitis, plaque, oral thrush, denture stomatitis can be treated.

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CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

REFERENCES

1. Rashmi KJ, Tiwari R. Pharmacotherapeutic Properties of Ginger and its use in Diseases of the Oral Cavity: A Narrative Review. *Journal of Advanced Oral Research*. 2016 May 1;7(2):1-6.
2. Weli TA, Mohammed AT. Effect of ginger extract on mutans streptococci in comparison to chlorhexidine gluconate. *J Baghdad Coll Dent*. 2013 Jun;25(2):179-84.
3. Patel RV, Thaker VT, Patel VK. Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. *Asian Pac J Trop Biomed*. 2011 Sep 1;1(1, Supplement):S58-61.
4. Rangeela M, Rajeshkumar S, Lakshmi T, Roy A. Anti-inflammatory activity of zinc oxide nanoparticles prepared using amla fruits. *Drug Invention Today [Internet]*. 2019;11(10). Available from: <http://search.ebscohost.com/login.aspx?direct=true&profile=ehost&scope=site&authtype=crawler&jrnl=09757619&AN=139166396&h=V1lovsfBddoWGGWRlEyRNep1FNEuhecsMHUNuAw9IK3zxMAy vEBa9mrr1%2FtDh2VPnDDwVe3nJ%2FtqCPSD8Hx17A%3D%3D&crl=c>
5. Sunar S, Rajeshkumar S, Roy A, Lakshmi T. Preparation of herbal formulation and it's application on nanoparticles synthesis and antibacterial activity. *Int J Life Sci Pharma Res*. 2019 Jul 19;10(3):2177-80.
6. Rónavári A, Igaz N, Adamecz DI, Szerencsés B, Molnar C, Kónya Z, et al. Green Silver and Gold Nanoparticles: Biological Synthesis Approaches and Potentials for Biomedical Applications. *Molecules [Internet]*. 2021 Feb 5;26(4). Available from: <http://dx.doi.org/10.3390/molecules26040844>
7. Kowshihan P, Roy A, Rajeshkumar S, Lakshmi T. ANTIFUNGAL AND ANTIBACTERIAL EFFECTS OF CORIANDER OLEORESIN MEDIATED SILVER NANOPARTICLES. *PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY*. 2020;58-65.
8. Meyer DH, Fives-Taylor PM. Oral pathogens: from dental plaque to cardiac disease. *Curr Opin Microbiol*. 1998 Feb;1(1):88-95.
9. Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T. A comparison of the antibacterial efficacies of essential oils against oral pathogens. *Oral Microbiol Immunol*. 2004

- Feb;19(1):61-4.
10. Singh S. Zinc oxide nanoparticles impacts: cytotoxicity, genotoxicity, developmental toxicity, and neurotoxicity. *Toxicol Mech Methods*. 2019 May;29(4):300-11.
 11. Umar H, Kavaz D, Rizaner N. Biosynthesis of zinc oxide nanoparticles using *Albizia lebeck* stem bark, and evaluation of its antimicrobial, antioxidant, and cytotoxic activities on human breast cancer cell lines. *Int J Nanomedicine*. 2019;14:87-100.
 12. Liufu S, Xiao H, Li Y. Investigation of PEG adsorption on the surface of zinc oxide nanoparticles. *Powder Technol [Internet]*. 2004; Available from: <https://www.sciencedirect.com/science/article/pii/S003259100400227X>
 13. Paul RP, Roy A, Aafreen M. M, Shanmugam R. Antibacterial Activity of White Pepper Oleoresin Mediated Silver Nanoparticles against Oral Pathogens. *Journal of Evolution of Medical and Dental Sciences*. 2020 Aug 17;9:2352+.
 14. Swathy S, Roy A, Rajeshkumar S. Anti-inflammatory activity of Ginger oleoresin mediated Silver nanoparticles [Internet]. Vol. 13, *Research Journal of Pharmacy and Technology*. 2020. p. 4591. Available from: <http://dx.doi.org/10.5958/0974-360x.2020.00808.2>
 15. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme Microb Technol*. 2018 Oct;117:91-5.
 16. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. *Biocatal Agric Biotechnol*. 2019 May 1;19:101138.
 17. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of *Enterococcus* sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. *Environ Sci Pollut Res*. 2020 Mar 1;27(8):8166-75.
 18. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using *Gymnema sylvestre* leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, *South African Journal of Chemical Engineering*. 2020. p. 1-4. Available from: <http://dx.doi.org/10.1016/j.sajce.2019.11.005>
 19. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. *Fuel*. 2020 Oct 1;277:118166.
 20. Anitha R, Prathoshni S, Lakshmi T. The effect of capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line [Internet]. Vol. 10, *Pharmacognosy Research*. 2018. p. 343. Available from: http://dx.doi.org/10.4103/pr.pr_46_18
 21. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. *Pesqui Bras Odontopediatria Clin Integr*. 2019 Mar 12;19(0):4668.
 22. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. *Clin Exp Pharmacol Physiol [Internet]*. 2017 Mar [cited 2021 Sep 15];44(3). Available from: <https://pubmed.ncbi.nlm.nih.gov/28004401/>
 23. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. *Case Rep Dent [Internet]*. 2019 Jan 22 [cited 2021 Sep 15];2019. Available from: <https://pubmed.ncbi.nlm.nih.gov/30805222/>
 24. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. *Oncol Rev [Internet]*. 2014 Jun 30 [cited 2021 Sep 15];8(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/25992232/>
 25. Menon S, Ks SD, R S, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. *Colloids Surf B Biointerfaces [Internet]*. 2018 Oct 1 [cited 2021 Sep 15];170. Available from: <https://pubmed.ncbi.nlm.nih.gov/29936381/>
 26. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. *J Public Health Dent [Internet]*. 2020 Jan [cited 2021 Sep 15];80(1). Available from: <https://pubmed.ncbi.nlm.nih.gov/31710096/>
 27. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. *Br J Oral Maxillofac Surg [Internet]*. 2001 Apr [cited 2021 Sep 15];39(2). Available from: <https://pubmed.ncbi.nlm.nih.gov/11286449/>
 28. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. *Int Endod J [Internet]*. 2011 Jun [cited 2021 Sep 15];44(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/21255047/>
 29. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. *Chem Biol Interact*. 2019 Aug 1;308:206-15.
 30. Kumar MS, Vamsi G, Sripriya R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. *J Periodontol*. 2006 Nov;77(11):1803-8.