

Anticariogenic Activity of Mucuna Pruriens Mediated Titanium Dioxide Nanoparticles.

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ABSTRACT

Introduction: Dental caries which is the most common disease of the oral cavity are caused due to demineralization of the inorganic substance and the subsequent destruction of the organic substance of the tooth. They start with the adherence of bacteria to the structures of the tooth, which then will gradually lead to the formation of plaque, culminating in the demineralization of enamel caused by the fermentation of dietary carbohydrates. Mucuna pruriens has long been used as Ayurvedic medicine, as a means to treat Parkinsonism primarily. It is also known to have a variety of other activities which include antiepileptic, antidiabetic, anticariogenic, anti-inflammatory, antioxidative, and also cytotoxic effects.

Materials and Methods: 1g Mucuna pruriens was dissolved in distilled water and was boiled for 5 minutes, Titanium dioxide extract was prepared by dissolving the extract in 90ml of distilled water. The mixture was then placed on an orbital shaker for an hour.

Result: Maximum activity against all the three oral pathogens were observed at 100 µL.

Conclusion: From this study, we can conclude that the high potential of Mucuna pruriens mediated Titanium dioxide nanoparticles is evident on S. mutans and E. faecalis

KEYWORDS:

Mucuna pruriens, anticariogenic activity, Staphylococcus aureus; Staphylococcus mutans, Enterococcus faecalis.

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INTRODUCTION

Dental caries, which is the most common disease of the oral cavity are caused due to demineralization of the inorganic substance and the subsequent destruction of the organic substance of the tooth(1)(2,3). There is a formation of a plaque biofilm that protects the pathogenic microorganisms from the host's defense mechanism(4). They start with the adherence of bacteria to the structures of the tooth, which then will gradually lead to the formation of plaque, culminating in the demineralization of enamel caused by the fermentation of dietary carbohydrates(5)(6). There is the formation of initial caries that manifest as a white spot lesion and occurs in a

subsurface beneath an area that relatively seems to be intact and is caused by both Staphylococcus mutans and Lactobacillus acidophilus(7).

Mucuna pruriens has long been used as traditional Indian medicine, which is better known as Ayurveda, as a means to treat Parkinsonism primarily. It is also known to have a variety of other activities which include antiepileptic, antidiabetic, anticariogenic, anti-inflammatory, antioxidative, and also cytotoxic effects. Ever since the dawn of time, we have been reliant on plants as a source of food and medicine. Yet we still are not in a state where the true potential of plants has not been explored and hence the knowledge about which still

largely remains unknown. With countries like India having a high demand predominantly for herbal medicines and the increase seen in the demand for the same in the global market(8), we need to explore the potential that these unexplored plants have. There have been studies where antimicrobial activity has been reported against both Gram-Negative and Gram positive bacteria (9). It has shown to have efficacy against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *B. subtilis*(10).

We live in an ever-improving and ever-progressing world, hence we need more effective means for every activity that we are performing. This is applicable for means of drug delivery and nanoparticles(11)(12)(13). Nanoparticles are defined as materials, manufactured or naturally occurring, that are in the size range of 1-100nm. They have incredible potential to serve as anticariogenic agents because of their enhanced physicochemical properties, large surface area to mass ratio, and increased chemical reactivity(14)(15-17). Our team has extensive knowledge and research experience that has translated into high quality publications(18-22),(23)-(24),(20,25,26),(27-31),(32)-(33)(34) (35) (36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) (47)

The aim of this study is to find the anticariogenic activity of *Mucuna pruriens* mediated Titanium dioxide nanoparticles.

MATERIALS AND METHODS

Green synthesis of Nanoparticle

1g *Mucuna pruriens* was dissolved in distilled water and was boiled for 5 minutes. Titanium dioxide extract was prepared by dissolving the extract in 90ml of distilled water. The mixture was then placed on an orbital shaker for an hour.

Anticariogenic activity

Anticariogenic activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Enterococcus faecalis* and *S. mutans*. 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 sterilised plates were allowed to solidify. 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.

RESULT & DISCUSSION

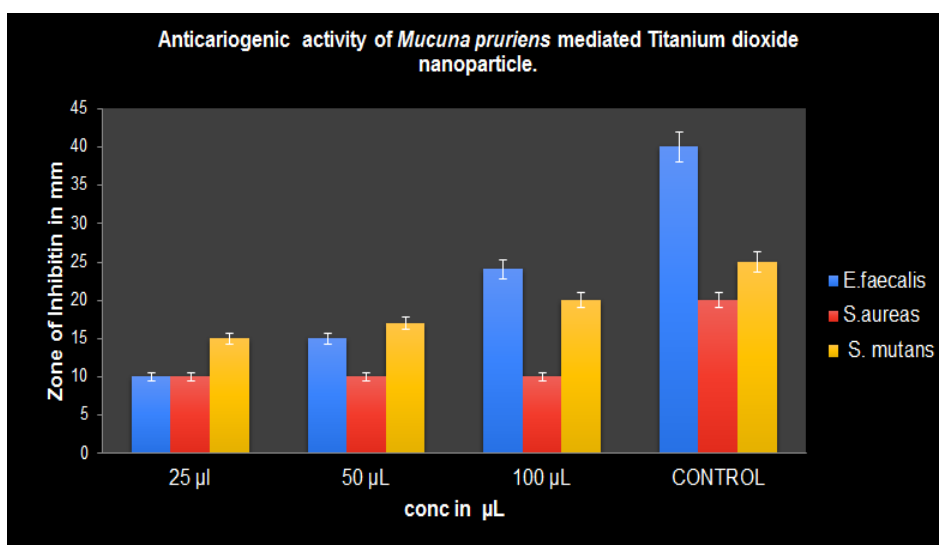


Fig.1: Graph representing Anticariogenic activity of. The bar graph depicts the Anticariogenic activity of *Mucuna pruriens* mediated Titanium dioxide nanoparticles. x axis represents the concentration and y axis represents the zone of inhibition in mm, data implies as mean±SEM

In the present study, a test for antimicrobial activity was carried out using the Agar well diffusion method. Three agar plates for identifying the inhibitory effect over *Staphylococcus mutans*, *Staphylococcus aureus* and *Enterococcus faecalis* respectively, were used. Each plate had four wells each with different nanoparticle concentrations being 25µL, 50 µL, 100 µL while the fourth was a standard. Against *Enterococcus faecalis*, the diameter of the zone of inhibition of the nanoparticles at 25 µL, 50 µL, 100 µL is observed to be 10mm, 15 mm and 24mm respectively. With *Staphylococcus mutans*, the diameter of the zone of inhibition of the nanoparticles at 25 µL, 50 µL, 100 µL was obtained as 15mm, 17mm and 20 mm respectively. For *Staphylococcus aureus*, the diameter of the

zones of inhibition found at 25 µL, 50 µL, 100 µL was obtained at 10mm, 10mm and 10mm. Thus, maximum activity for all the three was observed at 100 µL. The control group against *E. faecalis*, had a diameter of 40mm, against *S. aureus* it was found to be at 20 and for *S. mutans*, was found to be at 20mm. While it is evident that at 100µL, the activity was found to be maximum for both *Enterococcus faecalis* and *Staphylococcus mutans*, *Staphylococcus aureus* did not have a differing diameter of the zones of inhibition around it.

This was also observed in a study by(48),(49)and (50,51), they compared the action of *Mucuna pruriens* against various cariogenic pathogens. They also found that the zone of inhibition found around *S. mutans* was measured the same

diameter around them. When seen in the study by (52) and (53), we see that with the help of nanoparticles the method of delivery is far more efficient as the zone of inhibition found is larger in diameter.

This study also adds to the already existing knowledge regarding the effectiveness of nanoparticles against a variety of diseases. We already had preexisting knowledge about its effectiveness against cancer and its cytotoxic activity to add to that we can also mention its effectiveness against bacteria and microbes (11,54-57).

CONCLUSION

From this study, we can conclude that the high potential of *Mucuna pruriens* mediated Titanium dioxide nanoparticles is evident on *S. mutans* and *E. faecalis* (58-67). With this study we can find its use in making oral hygiene products such as toothpastes, mouthwashes which can help reduce the incidence of caries and be highly effective.

CONFLICT OF INTEREST

There was no conflict of interest

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REFERENCES

1. Vikneshan M, Saravanakumar R, Mangaiyarkarasi R, Rajeshkumar S, Samuel SR, Suganya M, et al. Algal biomass as a source for novel oral nano-antimicrobial agent [Internet]. Vol. 27, Saudi Journal of Biological Sciences. 2020. p. 3753-8. Available from: <http://dx.doi.org/10.1016/j.sjbs.2020.08.022>
2. Sivaraj R, Pattanathu K S, Rajiv P, Narendhran S, Venckatesh R. Biosynthesis and characterization of *Acalypha indica* mediated copper oxide nanoparticles and evaluation of its antimicrobial and anticancer activity [Internet]. Vol. 129, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2014. p. 255-8. Available from: <http://dx.doi.org/10.1016/j.saa.2014.03.027>
3. Shankar SB, Barani Shankar S, Arivarasu L, Rajeshkumar S. Biosynthesis of Hydroxy Citric Acid Mediated Zinc Nanoparticles and Its Antioxidant and Cytotoxic Activity [Internet]. Journal of Pharmaceutical Research International. 2020. p. 108-12. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2630845>
4. Fernandes G, Delbem A, do Amaral J, Gorup L, Fernandes R, de Souza Neto F, et al. Nanosynthesis of Silver-Calcium Glycerophosphate: Promising Association against Oral Pathogens [Internet]. Vol. 7, Antibiotics. 2018. p. 52. Available from: <http://dx.doi.org/10.3390/antibiotics7030052>
5. Barma MD. Synthesis of Triphala Incorporated Zinc Oxide Nanoparticles and Assessment of its Antimicrobial Activity Against Oral Pathogens : An In-Vitro Study [Internet]. Vol. 13, Bioscience Biotechnology Research Communications. 2020. p. 74-8. Available from: <http://dx.doi.org/10.21786/bbrc/13.7/14>
6. Snehal* P, Shivakumar KM, Hathiwala S, Srinivasan SR, Khatri S. Prevalence of untreated dental caries among the preschool children of Western Maharashtra. JDOH. 2015 Nov 30;7(11):175-8.
7. Loesche W. Dental caries and periodontitis: contrasting two infections that have medical implications. Infect Dis Clin North Am. 2007 Jun;21(2):471-502, vii.
8. Fung SY, Sim SM, Kandiah, Jeyaseelan, Armugam A, Aguiyi JC, et al. Prophylactic effect of *Mucuna pruriens* Linn (velvet bean) seed extract against experimental *Naja sputatrix* envenomation: gene expression studies. Indian J Exp Biol. 2014 Sep;52(9):849-59.
9. Gupta M, Upal Kanti Mazumder. In Vitro Lipid Peroxidation and Antimicrobial Activity of *Mucuna pruriens* Seeds. 2005 Jan 1 [cited 2021 Mar 13]; Available from: <http://dx.doi.org/>
10. Salau AO, Odeleye OM. Antimicrobial activity of *Mucuna pruriens* on selected bacteria. Afr J Biotechnol [Internet]. 2007 [cited 2021 Mar 13];6(18). Available from: <https://www.ajol.info/index.php/ajb/article/view/57964>
11. Barma MD, Kannan SD, Indiran MA, Rajeshkumar S, Pradeep Kumar R. Antibacterial Activity of Mouthwash Incorporated with Silica Nanoparticles against *S. aureus*, *S. mutans*, *E. faecalis*: An in-vitro Study. Journal of Pharmaceutical Research International. 2020 Aug 24;25-33.
12. Kisin ER, Murray AR, Keane MJ, Shi X-C, Schwegler-Berry D, Gorelik O, et al. Single-walled Carbon Nanotubes: Geno- and Cytotoxic Effects in Lung Fibroblast V79 Cells [Internet]. Vol. 70, Journal of Toxicology and Environmental Health, Part A. 2007. p. 2071-9. Available from: <http://dx.doi.org/10.1080/15287390701601251>
13. Robertson TA, Sanchez WY, Roberts MS. Are Commercially Available Nanoparticles Safe When Applied to the Skin? J Biomed Nanotechnol. 2010 Oct 1;6(5):452-68.
14. Saafan A, Zaazou MH, Sallam MK, Mosallam O, El Danaf HA. Assessment of Photodynamic Therapy and Nanoparticles Effects on Caries Models [Internet]. Vol. 6, Open Access Macedonian Journal of Medical Sciences. 2018. p. 1289-95. Available from: <http://dx.doi.org/10.3889/oamjms.2018.241>
15. Karthik V, Arivarasu L, Rajeshkumar S. Hyaluronic Acid Mediated Zinc Nanoparticles against Oral Pathogens and Its Cytotoxic Potential [Internet]. Journal of Pharmaceutical Research International. 2020. p. 113-7. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930716>
16. Shree MK, Kavya Shree M, Arivarasu L, Rajeshkumar S. Cytotoxicity and Antimicrobial Activity of Chromium Picolinate Mediated Zinc Oxide Nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020. p. 28-32. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2030726>
17. Jaisankar AI, Arivarasu L. Free Radical Scavenging and Anti-Inflammatory Activity of Chlorogenic Acid Mediated Silver Nanoparticle [Internet]. Journal of Pharmaceutical Research International. 2020. p. 106-12. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930715>
18. 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.
19. 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.
 20. 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.
 21. 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>
 22. 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.
 23. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea*. *S Afr J Chem Eng.* 2019 Jul;29:17-23.
 24. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. *Arab J Gastroenterol.* 2018 Jun;19(2):56-64.
 25. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of *Tamarindus indica* on MCF-7 human breast cancer cell line. *J Drug Deliv Sci Technol.* 2020 Feb 1;55:101376.
 26. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. *Drug Dev Res.* 2019 Sep;80(6):714-30.
 27. Ramesh A, Varghese S, Jayakumar ND, Malaiappan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. *J Periodontol.* 2018 Oct;89(10):1241-8.
 28. 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.
 29. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2021 Jul;132(1):118-9.
 30. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. *Syzygium cumini* extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med.* 2019 Feb;48(2):115-21.
 31. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprakasam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments. *Implant Dent.* 2019 Jun;28(3):289-95.
 32. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. *Aquaculture.* 2019 May 30;507:1-6.
 33. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. *Stem Cell Res Ther.* 2021 Mar 18;12(1):192.
 34. Pushpaanjali G, Geetha RV, Lakshmi T. Knowledge and Awareness about Antibiotic Usage and Emerging Drug Resistance Bacteria among Dental Students. *Journal of Pharmaceutical Research International.* 2020 Aug 24;34-42.
 35. Aathira CM, Geetha RV, Lakshmi T. Knowledge and Awareness about the Mode of Transmission of Vector Borne Diseases among General Public. *Journal of Pharmaceutical Research International.* 2020 Aug 24;87-96.
 36. Baskar K, Lakshmi T. Knowledge, Attitude and Practices Regarding HPV Vaccination among Undergraduate and Postgraduate Dental Students in Chennai. *Journal of Pharmaceutical Research International.* 2020 Aug 25;95-100.
 37. Manya Suresh LT. Wound Healing Properties of *Aloe Barbadensis* Miller-In Vitro Assay. *Journal of Complementary Medicine Research.* 2020;11(5):30-4.
 38. First Report on Marine Actinobacterial Diversity around Madras Atomic Power Station (MAPS), India [Internet]. [cited 2021 Aug 31]. Available from: <http://alinteridergisi.com/article/first-report-on-marine-actinobacterial-diversity-around-madras-atomic-power-station-maps-india/>
 39. Physicochemical Profile of *Acacia Catechu* Bark Extract - An in Vitro Stud - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://eijppr.com/article/physicochemical-profile-of-acacia-catechu-bark-extract-an-in-vitro-stud>
 40. Lakshmi T. Antifungal Activity of *Ficus racemosa* Ethanolic Extract against Dermatophytes-An in vitro Study. *Journal of Research in Medical and Dental Science.* 2021;9(2):191-3.
 41. Awareness of Drug Abuse among Teenagers - *International Journal of Pharmaceutical and Phytopharmacological Research* [Internet]. [cited 2021 Aug 31]. Available from: <https://eijppr.com/article/awareness-of-drug-abuse-among-teenagers>
 42. Mangal CSK, Anitha R, Lakshmi T. Inhibition of Nitric oxide Production and Nitric oxide Synthase Gene Expression in LPS Activated RAW 264 .7 Macrophages by Thyme oleoresin from *Thymus vulgaris*. *J Young Pharm.* 2018;10(4):481.
 43. COX2 Inhibitory Activity of *Abutilon Indicum* - *Pharmaceutical Research and Allied Sciences* [Internet]. [cited 2021 Aug 31]. Available from: <https://ijpras.com/article/cox2-inhibitory-activity-of-abutilon-indicum>
 44. Jibu RM, Geetha RV, Lakshmi T. Isolation, Detection and Molecular Characterization of *Staphylococcus aureus* from Postoperative Infections. *Journal of Pharmaceutical Research International.* 2020 Aug 24;63-7.
 45. Sindhu PK, Thangavelu L, Geetha RV, Rajeshkumar S, Raghunandhakumar S, Roy A. Anorectic drugs: an experimental and clinical perspective ♦A Review. *Journal of Complementary Medicine Research.* 2020;11(5):106-12.
 46. Nivethitha R, Thangavelu L, Geetha RV, Anitha R, RajeshKumar S, Raghunandhakumar S. In Vitro Anticancer Effect of *Sesamum Indicum* Extract -. *Journal of Complementary Medicine Research.* 2020;11(5):99-105.
 47. Mariona P, Roy A, Lakshmi T. Survey on lifestyle and food habits of patients with PCOS and obesity. *Journal of Complementary Medicine Research.* 2020;11(5):93-8.
 48. Anticariogenic Activity of Some Indian Traditional Medicinal Plants [Internet]. [cited 2021 Mar 13]. Available from:

- <https://scialert.net/fulltext/?doi=ejdm.2017.1.8>
49. Mastan SA, Janaki Ramayya P, Mutyala Naidu L, Mallikarjuna K. Antimicrobial Activity of Various Extracts of *Mucuna Pruriens* Leaves. *Biomedical and Pharmacology Journal*. 2015 Feb 15;2(1):55-60.
 50. Melvin SS. In Vitro evaluation of the antibacterial activity of *Mucuna pruriens* leaf and callus extracts [Internet]. Vol. 7, *African Journal of Microbiology Research*. 2013. p. 3101-11. Available from: <http://dx.doi.org/10.5897/ajmr12.2213>
 51. Agarwal H, Menon S, Shanmugam VK. Functionalization of zinc oxide nanoparticles using *Mucuna pruriens* and its antibacterial activity [Internet]. Vol. 19, *Surfaces and Interfaces*. 2020. p. 100521. Available from: <http://dx.doi.org/10.1016/j.surfin.2020.100521>
 52. G S, Saurabh G, Komal S. Comparative Characterization for Antimicrobial Activity and Bioactive Compounds Present in Leaf Extract of *Ocimum sanctum* [Internet]. Vol. 03, *Journal of Food & Industrial Microbiology*. 2018. Available from: <http://dx.doi.org/10.4172/2572-4134.1000121>
 53. S SK, Satheesha KS. In-Vitro Antibacterial Activity of Black Tea (*Camellia sinensis*) Mediated Zinc Oxide Nanoparticles Against Oral Pathogens [Internet]. Vol. 13, *Bioscience Biotechnology Research Communications*. 2020. p. 2077-80. Available from: <http://dx.doi.org/10.21786/bbrc/13.4/66>
 54. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Employing sulphated polysaccharide (fucoïdan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines [Internet]. Vol. 26, *Materials Today Communications*. 2021. p. 101975. Available from: <http://dx.doi.org/10.1016/j.mtcomm.2020.101975>
 55. Shunmugam R, Balusamy SR, Kumar V, Menon S, Lakshmi T, Perumalsamy H. Biosynthesis of gold nanoparticles using marine microbe (*Vibrio alginolyticus*) and its anticancer and antioxidant analysis [Internet]. Vol. 33, *Journal of King Saud University - Science*. 2021. p. 101260. Available from: <http://dx.doi.org/10.1016/j.jksus.2020.101260>
 56. Nasim I, Kamath K, Rajeshkumar S. Evaluation of the remineralization capacity of a gold nanoparticle-based dental varnish: An in vitro study [Internet]. Vol. 23, *Journal of Conservative Dentistry*. 2020. p. 390. Available from: http://dx.doi.org/10.4103/jcd.jcd_315_20
 57. Aathira CM, Arivarasu L, Rajeshkumar S. Antioxidant and Anti-Inflammatory Potential of Chromium Picolinate Mediated Zinc Oxide Nanoparticle [Internet]. *Journal of Pharmaceutical Research International*. 2020. p. 118-21. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930717>
 58. 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.
 59. 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/>
 60. 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/>
 61. 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/>
 62. 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/>
 63. 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/>
 64. 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/>
 65. 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/>
 66. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. *Chem Biol Interact*. 2019 Aug 1;308:206-15.
 67. 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.