

## PREPARATION OF MOUTHWASH USING BANANA STEM EXTRACT MEDIATED COPPER NANOPARTICLES AND ITS CYTOTOXIC EFFECT - AN IN VITRO STUDY

### ABSTRACT:

#### Background:

Copper nanoparticles have recently attracted researchers due to their low cost, availability and other novel properties. Various parts of *Musa sapientum* which belong to the family Musaceae have been used for various medicinal purposes. The aim of the study is to assess the cytotoxic effect of mouth wash prepared using banana stem mediated copper nanoparticles.

**Materials and method:** 100 ml of distilled water is mixed with 1g of banana stem extract and distilled. The filtrate is mixed with 0.477g of copper sulphate mixed in 60 ml of distilled water. There were a total of 10 shrimps per dilution. The artificial seawater upto 10ml per test tube is controlled. The test tube was left uncovered under a lamp. The number of surviving shrimps were counted and recorded after 24hrs. The percentage of motility was calculated by dividing the total number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death of nauplii is attributed to the compounds present in nanoparticles.

**Results:** Percentage of lethality for the banana stem extract mediated copper nanoparticles mouthwash at concentrations of 5 $\mu$ l, 10 $\mu$ l, 20 $\mu$ l, 40 $\mu$ l, 80 $\mu$ l were 60%, 70%, 100%, 100%, 100% respectively.

**Conclusion:** Thus with an increase in concentration of the banana stem mediated copper nanoparticles mouthwash, the cytotoxic activity increases. Thus from the study, it is evident that natural mouthwash can be prepared with banana stem mediated copper nanoparticles with less side effects.

**Keywords :** Banana stem extract, Copper nanoparticle, Cytotoxicity, Mouthwash, Green synthesis

**Running title:** Banana stem extract mediated copper nanoparticles mouthwash and its cytotoxic effect

## INTRODUCTION

Nanobiotechnology deals with nano sized materials in different fields of biological sciences. Biosynthesis of metallic nanoparticles using plant extract is eco friendly (1). Metal nanoparticles have gained more attention in recent years due to its use in various fields. Copper nanoparticles have recently attracted researchers due to their low cost, availability and other novel properties. These properties are different from their bulk metals (2,3). Copper nanoparticles are used in various aspects. They are used in wound dressings. Copper nanoparticles have potential industrial use such as gas sensors, catalytic processes, high temperature superconductors, solar cells and so on. In addition to the anti-inflammatory and antioxidant properties, copper nanoparticles also have the characteristics of cytotoxicity(4).

*Musa sapientum* which is also known as banana, commonly grown in South India for its fruits, stem, flowers, roots and leaves. Various parts of *Musa sapientum* which belong to the family Musaceae have been used for various medicinal purposes. The area of interest here is banana stem extract since it is cost efficient and is widely distributed in the region where research is carried out. The major uses of banana pseudo-stem fiber are in making specialized and high-quality sanitary products such as baby pampers, textiles, and papers such as banknotes(5,6). The banana pseudo-stem fiber can also be used for ropes such as marine rope since this fiber has good resistance to sea water and has buoyancy properties. Other uses of this fiber are for making coffee and tea bags, filter cloths, as reinforcement fibers for plaster, disposable fabrics, and light-density woven fabrics (5–7). Banana stem is used to treat disorders such as dysentery diarrhea, inflammation, pain and snake bite. The antimicrobial compound present in the banana stem extract is tannic acid which plays a huge role in the study since it helps in the cytotoxic effect of banana stem extract(8). Banana stem is also a source of polyphenols, gentisic acid which plays an important role in its antioxidant activity (9,10).

Oral rinses, also called mouthwashes, are most often used to reduce the microbial load in the oral cavity or to control or reduce bad breath, depending on the ingredients in the particular oral rinse(11). Oral rinses also contain chemicals, phenols glycerin as a humectant, sodium benzoate as a buffer, flavors, coloring, and emulsifiers that serve as stabilizers. These mouth wash provides strength to gums thereby preventing gingival recession and other periodontal disease (12) . Mouth wash also acts in healing wounds in the oral cavity to a certain extent. Cytotoxicity is an important characteristic of mouthwash since it eliminates micro organisms which serve the purpose of mouthwash. Similar research has been carried out by fellow researchers(13) (14) (15) (16). The aim of the study is to assess the cytotoxic effect of mouth wash prepared using banana stem mediated copper nanoparticles.

## **MATERIALS AND METHOD**

### **Preparation of plant extract:**

The preparation of plant extract involves the selection of banana stem extract in powder form (Figure 1). Each glassware was thoroughly washed with distilled water and dried in a hot air oven prior to use. The plant powder that weighs approximately 1g was taken and mixed in a conical flask with 100 ml of distilled water. The solution is then boiled and filtered through filter paper for 5 to 10 minutes. The extract thus obtained will be purified and then used for further experiments (Figure 2).

### **Preparation of banana stem mediated Copper Nanoparticles:**

0.477 g of copper sulphate ( $0.179 * 3$ ) is weighed and taken for the synthesis of Copper nanoparticles. The  $\text{CuSO}_4$  weight is combined with 50 ml distilled water. Now, it is blended with the 50 ml solution for plant extract. Finally, 100 ml of a mixture of the solution CuNP and banana stem extract is collected .

### **Green Synthesis of banana stem mediated Copper nanoparticles:**

The mixture was put for two days in a laboratory shaker until the changes in color were detected. The reading was taken every hour in the UV-vis spectrophotometer (Beckman DU-20 spectrophotometer) and color variations were also noted. In addition, the reaction mixture was subjected to 10 minutes of centrifugation and the resulting pellet was collected in one tube.

### **Cytotoxic activity of banana stem mediated Copper Nanoparticles:**

There were a total of 10 shrimps per dilution. The artificial sea water up to 10mL per test tube is controlled. The test tube was left uncovered under a lamp. The concentrations taken were 5 $\mu$ l, 10 $\mu$ l, 20 $\mu$ l, 40 $\mu$ l, 80 $\mu$ l and a control group. The number of surviving shrimps were counted and recorded after 24hrs. The percentage of motility was calculated by dividing the total number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death of nauplii is attributed to the compounds present in nanoparticles.

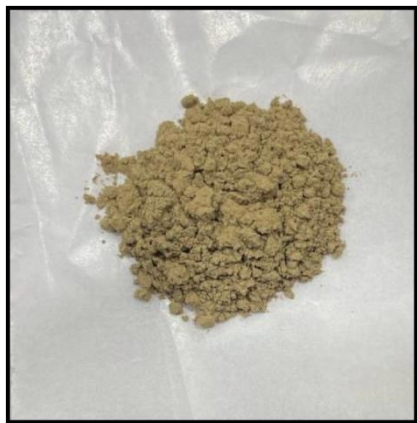


Figure 2 represents the preparation of plant extract.

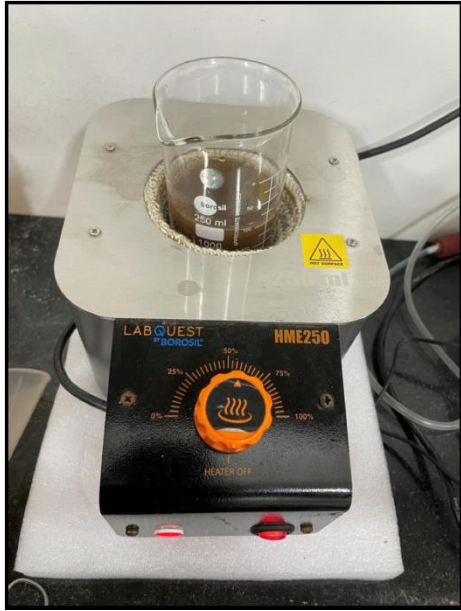


Figure 2 depicts the heating of the solution of banana stem extract.

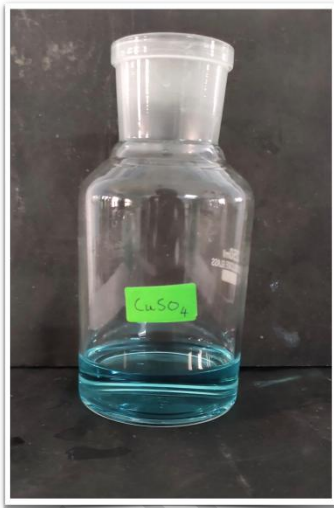


Figure 3 represents Copper sulphate solution.



Figure 4 represents the filtrate prepared.

## RESULTS

The figures 1, 2, 3 and 4 represent the methodology in preparation of mouthwash through green synthesis using banana stem extract mediated copper nanoparticles. Descriptive statistics was used. The concentration of the lethality dosage (LD50) was measured at 95% confidence intervals using Probit analysis. LD50 below 100 ppm was considered (active) to be effective. As Meyer and others have stated, the LD50 value of less than 1000  $\mu\text{g} / \text{mL}$  is toxic while the LD50 value of more than 1000  $\mu\text{g} / \text{mL}$  is non-toxic. The mortality percentage (%M) was also calculated by dividing the number of dead nauplii by the total number and multiplying by 100%. This is to ensure nauplii mortality is due to the substances in the nanoparticles. The cytotoxic effect of banana stem mediated CuNP shows that as the concentration increases there is a significant increase in the percentage of lethality. The result shows that a significant cytotoxic effect is achieved at 5  $\mu\text{l}$  reaching 100% of lethality at 20 $\mu\text{l}$ , 40 $\mu\text{l}$ , 80 $\mu\text{l}$ . (Figure 5) With reference to the below table it can be seen that the % of lethality for the concentrations 5 $\mu\text{l}$ , 10 $\mu\text{l}$ , 20 $\mu\text{l}$ , 40 $\mu\text{l}$ , 80 $\mu\text{l}$  were 60%, 70%, 100%, 100%, 100% respectively. (Table 1).

**Table 1:** represents the data of concentration of the mouthwash and its respective cytotoxic effect.

Concentration in $\mu\text{l}$	% of lethality
Control	0%
5 $\mu\text{l}$	60%
10 $\mu\text{l}$	70%
20 $\mu\text{l}$	100%
40 $\mu\text{l}$	100%
80 $\mu\text{l}$	100%

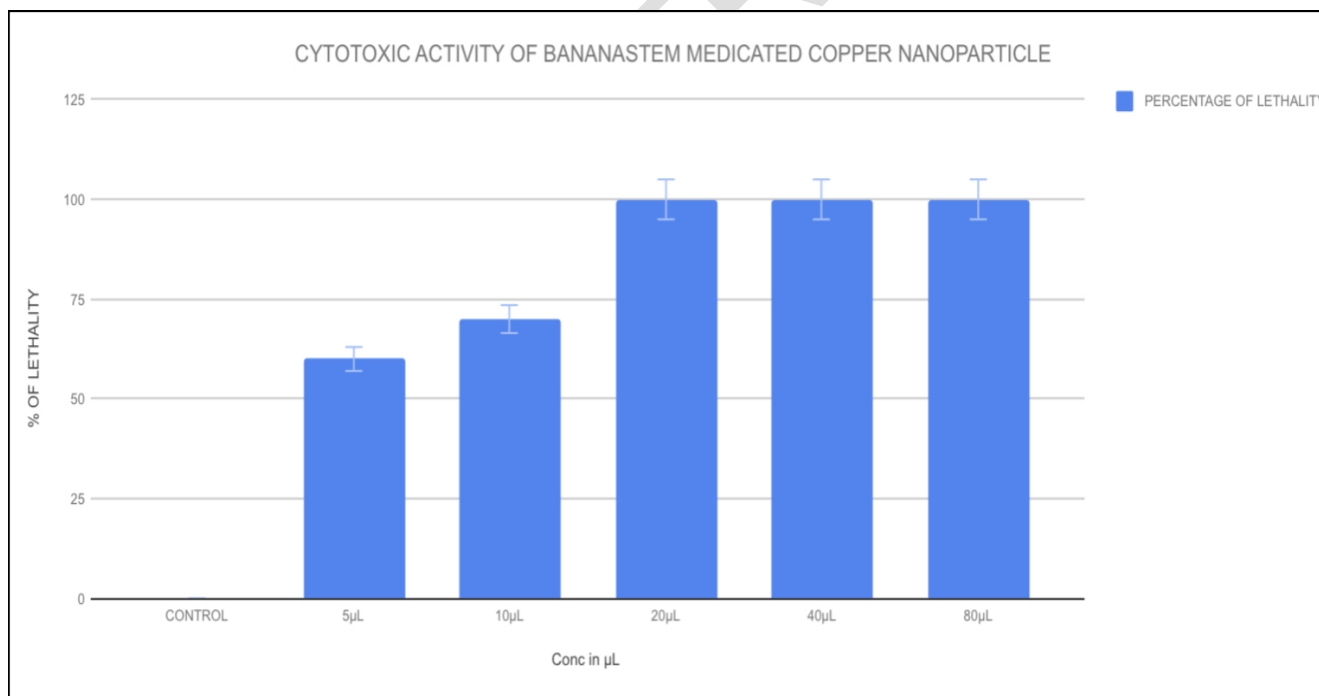


Figure 5: Graph showing the cytotoxic activity of banana stem mediated copper nanoparticles. X

axis represents conc.in  $\mu\text{l}$  and Y axis represents % of lethality. The graph shows that with increase in concentration, the lethality of the mouthwash increases.

## DISCUSSION

Our team has extensive knowledge and research experience that has translated into high quality publications (17–29),(30–34),(35),(36). From the results, it is seen that the cytotoxic effects increase with concentration reaching 100% lethality at 80  $\mu\text{l}$ . This shows that banana stem mediated copper nanoparticles has a significant cytotoxic effect and plays an important role in the characteristics of the mouth wash. This attribute helps in leading to reduced microbial count in the oral cavity at a reduced time.

In a study Avinash et al has described the cytotoxic effect of copper nanoparticles also explaining about the bioactivity mechanism of action(37). Reza et al, has also examined the cytotoxicity of copper nanoparticles, in which the aim of their experiment was the green synthesis of copper nanoparticles using *Allium saralicum* and assessment of their cytotoxicity, antioxidant, antibacterial, antifungal, and cutaneous wound healing effects under *in vitro* and *in vivo conditions*. These nanoparticles were characterized by Fourier transformed infrared spectroscopy (FT-IR), UV–visible spectroscopy, field emission scanning electron microscopy (FE-SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM) (38).

Oral cavity is a mirror of an individual's healthy state. Maintaining the oral cavity is essential for everyone to prevent themselves from many gingival and periodontal diseases that include aggressive and chronic periodontitis which further leads to tooth loss. Thus, each individual has to be aware of their dental status and maintain their oral hygiene by using the mouthwashes as mentioned to minimise the effect of microbial growth within the oral cavity. Since this research is underway, further modifications have to be done like adding some flavouring agents and a better mode of preparing the mouth wash to reduce the loss of the flavonoids present in the mouthwash. Clinical trials are to be done. This study will be helpful in preparation of eco friendly mouthwash which is completely devoid of chemical substances and has low adverse effects in the future. The study also helps in providing a better mouthwash which can provide good results in a shorter time.

## CONCLUSION

Thus with an increase in concentration of the banana stem mediated copper nanoparticles mouthwash, the cytotoxic activity increases (39) (40) (41) (42) (43) (44) (45) (46) (47) (48) (49) ((49,50) (51) (52). Thus from the study, it is evident that natural mouthwash can be prepared with banana stem mediated copper nanoparticles with less side effects.

## REFERENCES

1. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity [Internet]. Vol. 273, *Chemico-Biological Interactions*. 2017. p. 219–27. Available from: <http://dx.doi.org/10.1016/j.cbi.2017.06.019>
2. Agarwal H, Venkat Kumar S, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles – An eco-friendly approach [Internet]. Vol. 3, *Resource-Efficient Technologies*. 2017. p. 406–13. Available from: <http://dx.doi.org/10.1016/j.reffit.2017.03.002>
3. Shukla AK, Iravani S. *Green Synthesis, Characterization and Applications of Nanoparticles* [Internet]. Elsevier; 2018. 548 p. Available from: <https://play.google.com/store/books/details?id=LzV8DwAAQBAJ>
4. Fathima T, Rajeshkumar S, Nagalingam M. Green synthesis of silver nanoparticles using *Symplocos racemosa* and its antifungal activity against *Candida albicans* [Internet]. Vol. 14, *RESEARCH JOURNAL OF PHARMACY AND TECHNOLOGY*. 2021. p. 775–8. Available from: <http://dx.doi.org/10.5958/0974-360x.2021.00135.9>
5. Fawcett W, West India Committee. The banana, its cultivation, distribution and commercial uses [Internet]. 1921. Available from: <http://dx.doi.org/10.5962/bhl.title.31195>
6. Murrill WA. The Banana and its Uses [Internet]. Vol. 125, *Scientific American*. 1921. p. 118–9. Available from: <http://dx.doi.org/10.1038/scientificamerican1221-118>
7. Jirukkakul N. Physical Properties of Banana Stem and Leaf Papers Laminated with Banana Film [Internet]. Vol. 16, *Walailak Journal of Science and Technology (WJST)*. 2018. p. 753–63. Available from: <http://dx.doi.org/10.48048/wjst.2019.3471>
8. Arunkumar P, Vedagiri H, Premkumar K. Rapid bioreduction of trivalent aurum using banana stem powder and its cytotoxicity against MCF-7 and HEK-293 cell lines [Internet]. Vol. 15, *Journal of Nanoparticle Research*. 2013. Available from: <http://dx.doi.org/10.1007/s11051-013-1481-0>

9. Hölscher D, Buerkert A, Schneider B. Phenylphenalenones Accumulate in Plant Tissues of Two Banana Cultivars in Response to Herbivory by the Banana Weevil and Banana Stem Weevil [Internet]. Vol. 5, Plants. 2016. p. 34. Available from: <http://dx.doi.org/10.3390/plants5030034>
10. Vazhacharickal PJ, Santhosh ES, Sajeshkumar NK, Mathew JJ, Sreejith PE, Sabu M. Green synthesis of copper and zinc nanoparticles using different varieties of banana starch, latex and sap and evaluation of their antifungal activity against *Fusarium oxysporum cubense* [Internet]. Amazon Publishers, USA; Available from: <https://play.google.com/store/books/details?id=hCnxDwAAQBAJ>
11. Ramamurthy J, Mg V. COMPARISON OF EFFECT OF HIORA MOUTHWASH VERSUS CHLORHEXIDINE MOUTHWASH IN GINGIVITIS PATIENTS: A CLINICAL TRIAL [Internet]. Vol. 11, Asian Journal of Pharmaceutical and Clinical Research. 2018. p. 84. Available from: <http://dx.doi.org/10.22159/ajpcr.2018.v11i7.24783>
12. Thamaraiselvan M, Elavarasu S, Thangakumaran S, Gadagi JS, Arthie T. Comparative clinical evaluation of coronally advanced flap with or without platelet rich fibrin membrane in the treatment of isolated gingival recession. *J Indian Soc Periodontol* [Internet]. 2015 Jan;19(1):66–71. Available from: <http://dx.doi.org/10.4103/0972-124X.145790>
13. An experimental analysis on the influence of fuel borne additives on the single cylinder diesel engine powered by *Cymbopogon flexuosus* biofuel. *J Energy Inst* [Internet]. 2017 Aug 1 [cited 2021 Sep 16];90(4):634–45. Available from: <http://dx.doi.org/10.1016/j.joei.2016.04.010>
14. Campeau PM, Kasperaviciute D, Lu JT, Burrage LC, Kim C, Hori M, et al. The genetic basis of DOORS syndrome: an exome-sequencing study. *Lancet Neurol* [Internet]. 2014 Jan;13(1):44–58. Available from: [http://dx.doi.org/10.1016/S1474-4422\(13\)70265-5](http://dx.doi.org/10.1016/S1474-4422(13)70265-5)
15. Sathish T, Karthick S. Wear behaviour analysis on aluminium alloy 7050 with reinforced SiC through taguchi approach [Internet]. Vol. 9, Journal of Materials Research and Technology. 2020. p. 3481–7. Available from: <http://dx.doi.org/10.1016/j.jmrt.2020.01.085>
16. Krishnaswamy H, Muthukrishnan S, Thanikodi S, Arockiaraj G, Venkatraman V. Investigation of air conditioning temperature variation by modifying the structure of passenger car using computational fluid dynamics [Internet]. Vol. 24, Thermal Science. 2020. p. 495–8. Available from: <http://dx.doi.org/10.2298/tsci190409397k>
17. 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* [Internet]. 2018 Oct;89(10):1241–8. Available from: <http://dx.doi.org/10.1002/JPER.17-0445>
18. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19

and its effects on cardiovascular disease. *Hypertens Res* [Internet]. 2020 Jul;43(7):729–30. Available from: <http://dx.doi.org/10.1038/s41440-020-0461-x>

19. S G, T G, K V, Faleh A A, Sukumaran A, P N S. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. *Int J Biol Macromol* [Internet]. 2018 Dec;120(Pt A):876–85. Available from: <http://dx.doi.org/10.1016/j.ijbiomac.2018.08.149>
20. Del Fabbro M, Karanxha L, Panda S, Bucchi C, Nadathur Doraiswamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. *Cochrane Database Syst Rev* [Internet]. 2018 Nov 26;11:CD011423. Available from: <http://dx.doi.org/10.1002/14651858.CD011423.pub2>
21. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. *Hypertens Res* [Internet]. 2020 Aug;43(8):851–3. Available from: <http://dx.doi.org/10.1038/s41440-020-0423-3>
22. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. *Cell Mol Immunol* [Internet]. 2019 Dec;16(12):935–6. Available from: <http://dx.doi.org/10.1038/s41423-019-0310-5>
23. Vellappally S, Al Kheraif AA, Divakar DD, Basavarajappa S, Anil S, Fouad H. Tooth implant prosthesis using ultra low power and low cost crystalline carbon bio-tooth sensor with hybridized data acquisition algorithm. *Comput Commun* [Internet]. 2019 Dec 15;148:176–84. Available from: <https://www.sciencedirect.com/science/article/pii/S0140366419307017>
24. Vellappally S, Al Kheraif AA, Anil S, Assery MK, Kumar KA, Divakar DD. Analyzing Relationship between Patient and Doctor in Public Dental Health using Particle Memetic Multivariable Logistic Regression Analysis Approach (MLRA2). *J Med Syst* [Internet]. 2018 Aug 29;42(10):183. Available from: <http://dx.doi.org/10.1007/s10916-018-1037-z>
25. 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* [Internet]. 2019 Apr;83(4):445–50. Available from: <http://dx.doi.org/10.21815/JDE.019.054>
26. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, Characterization and Biological Applications of Biosynthesized Silver Nanoparticles with Chitosan-Fucoidan Coating. *Molecules* [Internet]. 2018 Jun 12;23(6). Available from: <http://dx.doi.org/10.3390/molecules23061429>
27. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaisi N, Mahmood A, Muthurangan M, et al. Cytotoxicity of Different Concentrations of Three Root Canal Sealers on Human Mesenchymal Stem Cells. *Biomolecules* [Internet]. 2018 Aug 1;8(3). Available from: <http://dx.doi.org/10.3390/biom8030068>

28. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechsakulwatana C, et al. Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. *Biotechnol Bioprocess Eng* [Internet]. 2018 Aug 1;23(4):383–93. Available from: <https://doi.org/10.1007/s12257-018-0169-9>
29. Vellappally S, Al Kheraif AA, Anil S, Wahba AA. IoT medical tooth mounted sensor for monitoring teeth and food level using bacterial optimization along with adaptive deep learning neural network. *Measurement* [Internet]. 2019 Mar 1;135:672–7. Available from: <https://www.sciencedirect.com/science/article/pii/S0263224118311333>
30. PradeepKumar AR, Shemesh H, Nivedhitha MS, Hashir MMJ, Arockiam S, Uma Maheswari TN, et al. Diagnosis of Vertical Root Fractures by Cone-beam Computed Tomography in Root-filled Teeth with Confirmation by Direct Visualization: A Systematic Review and Meta-Analysis. *J Endod* [Internet]. 2021 Aug;47(8):1198–214. Available from: <http://dx.doi.org/10.1016/j.joen.2021.04.022>
31. R H, Ramani P, Tilakaratne WM, Sukumaran G, Ramasubramanian A, Krishnan RP. Critical appraisal of different triggering pathways for the pathobiology of pemphigus vulgaris-A review. *Oral Dis* [Internet]. 2021 Jun 21; Available from: <http://dx.doi.org/10.1111/odi.13937>
32. Ezhilarasan D, Lakshmi T, Subha M, Deepak Nallasamy V, Raghunandhakumar S. The ambiguous role of sirtuins in head and neck squamous cell carcinoma. *Oral Dis* [Internet]. 2021 Feb 11; Available from: <http://dx.doi.org/10.1111/odi.13798>
33. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncol* [Internet]. 2021 Jun 16;105390. Available from: <http://dx.doi.org/10.1016/j.oraloncology.2021.105390>
34. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. *Oral Oncol* [Internet]. 2021 Jun 14;105375. Available from: <http://dx.doi.org/10.1016/j.oraloncology.2021.105375>
35. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanein AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. *J Ambient Intell Humaniz Comput* [Internet]. 2018 Dec 14; Available from: <https://doi.org/10.1007/s12652-018-1166-8>
36. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, et al. Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. *Risk Manag Healthc Policy* [Internet]. 2021 Jul 7;14:2851–61. Available from: <http://dx.doi.org/10.2147/RMHP.S306880>
37. avinash. G, IJAR. EFFECT OF COPPER ON LIPID PEROXIDATION AND ENZYMATIC ANTIOXIDANTS IN SORGHUM BICOLOR [Internet]. Vol. 5, *International Journal of Advanced Research*. 2017. p. 424–30. Available from: <http://dx.doi.org/10.21474/ijar01/5346>

38. Tahvilian R, Zangeneh MM, Falahi H, Sadrjavadi K, Jalalvand AR, Zangeneh A. Green synthesis and chemical characterization of copper nanoparticles using *Allium saralicum* leaves and assessment of their cytotoxicity, antioxidant, antimicrobial, and cutaneous wound healing properties [Internet]. Vol. 33, *Applied Organometallic Chemistry*. 2019. Available from: <http://dx.doi.org/10.1002/aoc.5234>
39. Danda AK. Comparison of a single noncompression miniplate versus 2 noncompression miniplates in the treatment of mandibular angle fractures: a prospective, randomized clinical trial. *J Oral Maxillofac Surg* [Internet]. 2010 Jul;68(7):1565–7. Available from: <http://dx.doi.org/10.1016/j.joms.2010.01.011>
40. Robert R, Justin Raj C, Krishnan S, Jerome Das S. Growth, theoretical and optical studies on potassium dihydrogen phosphate (KDP) single crystals by modified Sankaranarayanan–Ramasamy (mSR) method [Internet]. Vol. 405, *Physica B: Condensed Matter*. 2010. p. 20–4. Available from: <http://dx.doi.org/10.1016/j.physb.2009.08.015>
41. Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. *J Adv Pharm Technol Res* [Internet]. 2013 Apr;4(2):78–83. Available from: <http://dx.doi.org/10.4103/2231-4040.111523>
42. Soh CL, Narayanan V. Quality of life assessment in patients with dentofacial deformity undergoing orthognathic surgery—A systematic review [Internet]. Vol. 42, *International Journal of Oral and Maxillofacial Surgery*. 2013. p. 974–80. Available from: <http://dx.doi.org/10.1016/j.ijom.2013.03.023>
43. Lekha L, Kanmani Raja K, Rajagopal G, Easwaramoorthy D. Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines [Internet]. Vol. 753, *Journal of Organometallic Chemistry*. 2014. p. 72–80. Available from: <http://dx.doi.org/10.1016/j.jorganchem.2013.12.014>
44. Dhinesh B, Isaac JoshuaRamesh Lalvani J, Parthasarathy M, Annamalai K. An assessment on performance, emission and combustion characteristics of single cylinder diesel engine powered by *Cymbopogon flexuosus* biofuel [Internet]. Vol. 117, *Energy Conversion and Management*. 2016. p. 466–74. Available from: <http://dx.doi.org/10.1016/j.enconman.2016.03.049>
45. PradeepKumar AR, Shemesh H, Jothilatha S, Vijayabharathi R, Jayalakshmi S, Kishen A. Diagnosis of Vertical Root Fractures in Restored Endodontically Treated Teeth: A Time-dependent Retrospective Cohort Study. *J Endod* [Internet]. 2016 Aug;42(8):1175–80. Available from: <http://dx.doi.org/10.1016/j.joen.2016.04.012>
46. Vijayakumar GNS, Nixon Samuel Vijayakumar G, Devashankar S, Rathnakumari M, Sureshkumar P. Synthesis of electrospun ZnO/CuO nanocomposite fibers and their dielectric and non-linear optic studies [Internet]. Vol. 507, *Journal of Alloys and Compounds*. 2010. p. 225–9. Available from: <http://dx.doi.org/10.1016/j.jallcom.2010.07.161>

47. Kavitha M, Subramanian R, Narayanan R, Udhayabanu V. Solution combustion synthesis and characterization of strontium substituted hydroxyapatite nanocrystals [Internet]. Vol. 253, Powder Technology. 2014. p. 129–37. Available from: <http://dx.doi.org/10.1016/j.powtec.2013.10.045>
48. Sahu D, Kannan GM, Vijayaraghavan R. Size-Dependent Effect of Zinc Oxide on Toxicity and Inflammatory Potential of Human Monocytes [Internet]. Vol. 77, Journal of Toxicology and Environmental Health, Part A. 2014. p. 177–91. Available from: <http://dx.doi.org/10.1080/15287394.2013.853224>
49. Neelakantan P, Cheng CQ, Mohanraj R, Sriraman P, Subbarao C, Sharma S. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser in vitro [Internet]. Vol. 48, International Endodontic Journal. 2015. p. 602–10. Available from: <http://dx.doi.org/10.1111/iej.12354>
50. Lekha L, Kanmani Raja K, Rajagopal G, Easwaramoorthy D. Synthesis, spectroscopic characterization and antibacterial studies of lanthanide(III) Schiff base complexes containing N, O donor atoms [Internet]. Vols. 1056-1057, Journal of Molecular Structure. 2014. p. 307–13. Available from: <http://dx.doi.org/10.1016/j.molstruc.2013.10.014>
51. Gopalakannan S, Senthilvelan T, Ranganathan S. Modeling and Optimization of EDM Process Parameters on Machining of Al 7075-B4C MMC Using RSM [Internet]. Vol. 38, Procedia Engineering. 2012. p. 685–90. Available from: <http://dx.doi.org/10.1016/j.proeng.2012.06.086>
52. Parthasarathy M, Isaac Joshua Ramesh Lalvani J, Dhinesh B, Annamalai K. Effect of hydrogen on ethanol-biodiesel blend on performance and emission characteristics of a direct injection diesel engine. Ecotoxicol Environ Saf [Internet]. 2016 Dec;134(Pt 2):433–9. Available from: <http://dx.doi.org/10.1016/j.ecoenv.2015.11.005>