RED TEA MEDIATED SYNTHESIS AND CHARACTERISATION OF COPPER NANOPARTICLES

Running Title: Synthesis and characterisation of copper nanoparticles using red tea

ABSTRACT:

Background: The synthesis of metallic nanoparticles is a lively area of education and, more importantly, "application research" in nanotechnology. A wide range of chemical and physical procedures might be used for synthesis of metallic nanoparticles. However, these methods are swarming with many problems including use of toxic solvents, generation of hazardous by-products, and high energy consumption. Accordingly, there's a vital need to develop environmentally benign procedures for synthesis of metallic nanoparticles. A promising approach to realize this objective is to take advantage of the wide array of biological resources in nature. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for production of low-cost, energy-efficient, and nontoxic metallic nanoparticles.

Aim: The aim of the current study was to synthesise and characterize the copper Nanoparticles mediated with red tea.

Materials and Methods: The green synthesis of the copper Nanoparticles was done using red tea. Morphological characters like the shape and size of the obtained copper nanoparticles were done by transmission electron microscopy (TEM).

Results: The results confirmed that the synthesised red tea mediated nanoparticles are eco-friendly, good and non toxic. TEM images showed that the copper nanoparticles were well dispersed, crystalline in nature. Copper nanoparticles are spherical in nature. The particles size was ranging from 40 to 90 nm, The TEM image shows that nanoparticles are not combined but are separated by equal interspace between the particles, which was confirmed by microscopy visualising under the higher resolution.

Conclusion: In this study, a simple, biological and low-cost approach was done for the preparation of copper nanoparticles using red tea extract. Thus the green synthesized copper nanoparticles can be subjected to the various other biological activities to test their biological efficiency and can be a pioneering step towards the shift to eco friendly medicine.

Keywords: Copper nanoparticles; Green synthesis; Innovative; Nanotechnology; Red Tea

INTRODUCTION:

Over the past few years, considerable interest has been focused on metal nanoparticles because of their potential applica- tions in diverse fields including catalysis, magnetic recording media, or microelectronics. Various methods are now known which enable one to organize these nanoparticles with controlled size and shape, these include metal vapour deposition, electro(1–3)chemical reduction, radiolytic reduction, thermal decomposition, mechanical attrition and chemical reduction. Among these methods, the solution method is found to be simple and most versatile for metal nanoparticles.

Nanoparticles are of great scientific interest as they bridge the gap between bulk materials and atomic or molecular structures. A bulk material has constant physical properties despite its size, but at the nanoscale this is often not the case.(4) Several well-characterised bulk materials are found to possess the most interesting properties when studied within the nanoscale. There are many reasons for this including the very fact that nanoparticles possess a really high aspect ratio. Metallic nanoparticles have possible applications in diverse areas like biotechnology, cosmetics, coatings, electronics and packaging. For instance, nanoparticles are often induced to merge into a solid at relatively lower temperatures, often without melting, resulting in improved and easy-to-create coatings for electronics applications (eg: capacitors).(5,6) Nanoparticles possess a wavelength below the critical wavelength of light. This renders them transparent, a property that creates them very useful for applications in packing, cosmetics and coatings. Metallic nanoparticles are often attached to single strands of DNA nondestructively. This exposes avenues for medical diagnostic applications. Nanoparticles can traverse through the vasculature and localize any organ. This potentially can cause novel therapeutic, imaging, and biomedical applications. (7–9)(10–13)

There are two alternative approaches for synthesis of metallic nanoparticles: the "bottom-up" approach and therefore the "top-down" approach. Bottom-up, or self assembly, refers to the development of a structure atom-by-atom, molecule-by-molecule, or cluster- by-cluster. In this approach, initially the nanostructured building blocks are formed and, subsequently, assembled into the ultimate material using chemical or biological procedures for synthesis.(14–18) A definite advantage of the bottom-up approach is the enhanced possibility of obtaining metallic nanoparticles with comparatively lesser defects and more homogeneous chemical compositions. In the top-down approach, an appropriate starting material is reduced in size using mechanical or chemical means. An important drawback of the top-down approach is the imperfection of the surface structure. Such defects within the surface structure can have a big impact on physical properties and surface chemistry of the metallic nanoparticles because of its high aspect ratio. (19–23)

Copper is one among the most widely used materials in the world. It has an excellent significance in all industries, particularly within the electrical sector because of it's low cost. Copper nanoparticles are synthesized and characterized by different methods.(18) Stability and reactivity are the two most important factors that impede the utilization and development of the metal cluster during a new generation of nanodevices. The copper nanoparticles have been promising in the field of medicine and dentistry due to their properties such as their interaction with pathogens, their large active surface area, and their high biological and chemical reactivity.(24–26)

Our team has extensive knowledge and research experience that has translated into high quality publications.(27–39),(40–44) (45) (46) In this context, this study was done to synthesise and characterise the copper nanoparticles using red tea extract.

MATERIALS AND METHODS:

Preparation of Red Tea extract:

A sample of Red Tea powder is taken and measured accurately to 1g to which 100mL of distilled water is added and boiled for 15-20 minutes at 60-70 degrees and the obtained extract is cooled for sometime, then the solution is filtered by using whatman no.10 filter paper. The filtered extract was collected and stored in the refrigerator for further use.

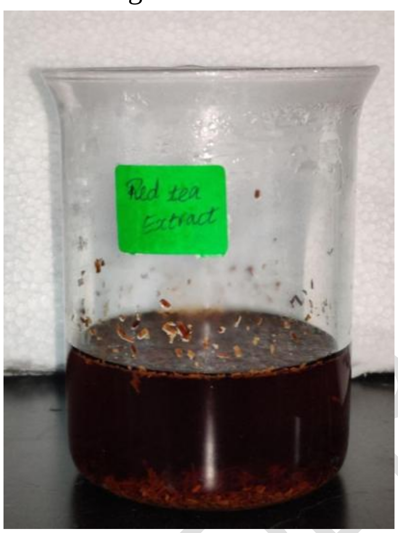

Figure 1: Mixture of Red Tea extract in distilled water

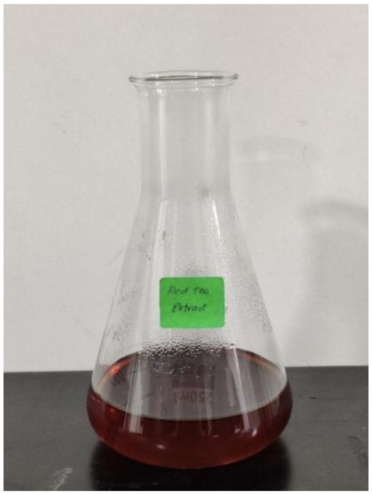

Figure 2: Concentrated extract of Red Tea extract

Synthesis of Copper Nanoparticles:

Synthesis of Copper Nanoparticles is done biologically using red tea. 20mM of copper sulphate is added to the obtained extract. The colour change was observed visually and photographed. The solution is kept in a magnetic stirrer for nanoparticle synthesis. The reaction mixture of copper sulphate and red tea was centrifuged at 8,000xg for 10 minutes. The resulting pellet was washed three times with distilled water and filtered and the supernatant so formed was collected.

Figure 3: Mixture of Copper sulphate and Red tea

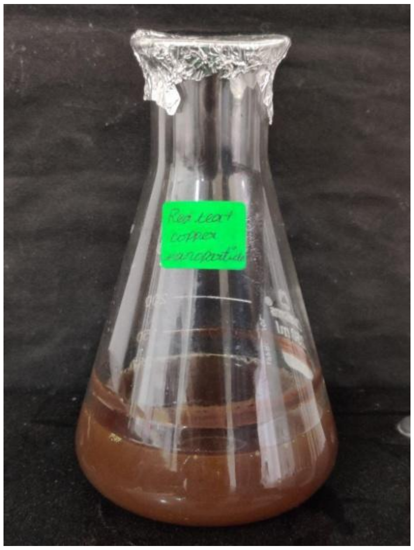

Figure 4: The final reaction with red tea mediated CUSO4 Nanoparticles

UV spectrometric analysis of synthesized nanoparticle:

Spectrometric analysis was evaluated by UV-visible spectroscopy. The biologically reduced solution mixture was scanned by Shimazdu, Lambda UV mini-1240 instrument operated at a resolution of 1 nm. The UV-visible analysis was performed in the absorption wavelength of 200 to 700 nm periodically for one hour to observe rapid reduction of copper nanoparticles and the results were recorded for the graphical analysis.

Characterisation of prepared Copper Nanoparticles:

The synthesized Cu NPs were characterised using TEM (Transmission Electron Microscope). The morphological analysis of the particle was done with TEM. A sample of Cu NPs was loaded on a carbon-coated copper grid, followed by solvent evaporation at room temperature for an hour. The TEM micrograph images were recorded on Zeiss- EM10C instrument on carbon coated copper grids with an accelerating voltage of 80 KV. The clear microscopic views were observed and documented in different ranges of magnifications.

RESULTS:

Nanoparticle synthesis was predicted through visual observation of solution color change from light brown to brownish black and also nanoparticle synthesis were later confirmed using UV–Visible spectroscopy.

Copper nanoparticles were successfully synthesized using red tea extract after being subjected to continuous heat and stirring. The reddish brown reaction mixture slowly changed to a thick brown suspension after several minutes of reaction. The development of intense brown colour owing to the surface plasmon resonance confirmed the synthesis of the copper nanoparticles. Colour changes of the reaction mixture 240 minutes after the bioreduction process, which were recorded by UV-visible spectrophotometer. UV–Visible readings were recorded in the wavelength range of 200 - 600nm. The absorption formed in the reaction media has an absorbance peak at 350 nm. The surface plasmon resonance absorbance was very sensitive to size and shape of the particles. It was observed that the SPR bands are located at the range 357 nm which is the characteristic absorption peak for copper nanoparticles in this study. (Figure 5)

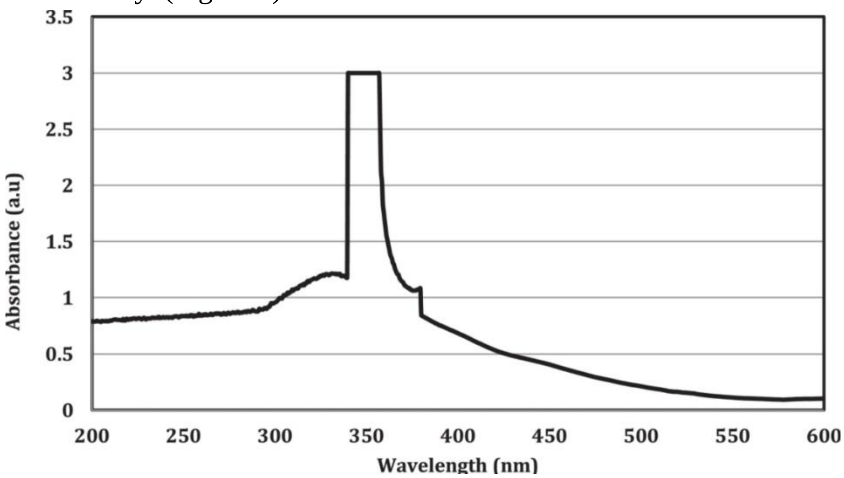

Figure 5: UV-Vis spectroscopy of copper nanoparticles synthesised using red tea extract. The X-axis shows the wavelength (nm) and the Y axis shows the absorbance(Abs). UV-visible spectra of the copper nanoparticles showed the peak at 350 nm

The centrifuged substrate was then subjected to the TEM analysis for the characterisation so as to determine the size, shape and distribution of nanoparticles. TEM images show that particles are well dispersed, crystalline in nature is shown in the figure below, Copper nanoparticles were spherical in nature. The particles size was ranging from 40 to 90 nm, The TEM image showed that nanoparticles are not combined but are separated by equal interspace between the particles, which was confirmed by microscopy visualizing under the higher resolution. This image explains that the copper nanoparticles are bounded with the phytochemicals of the plant extract. (Figure 6)

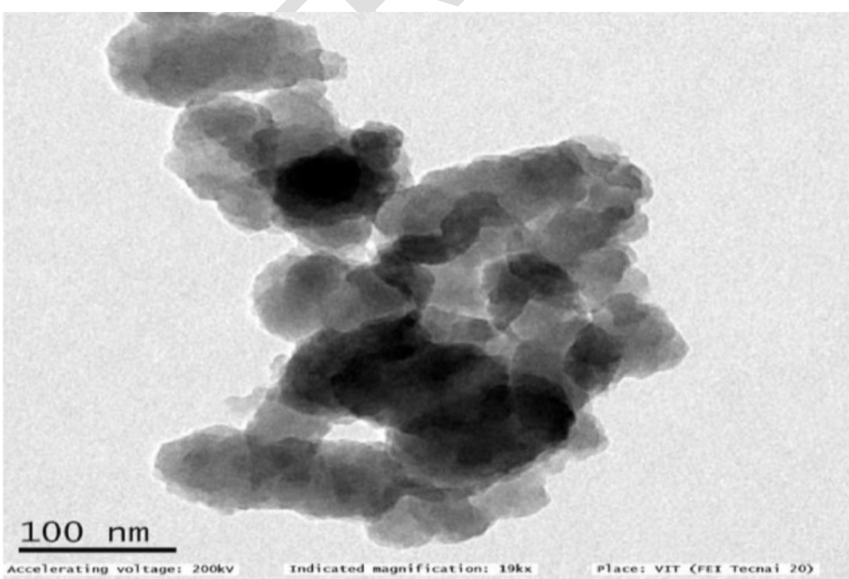

Figure 6: Image showing the Copper Nanoparticles under TEM. The copper nanoparticles under TEM are spherical and are clustered.

DISCUSSION:

The current study was undertaken to synthesize and characterise copper nanoparticles mediated through red tea extract. So as to analyse its biologic properties to be used as adjunct in medical and dental fields.

Nandhini JT et al in 2021 synthesised copper nanoparticles using sea weed and their morphological features were characterised. The UV-visible analysis was performed in the absorption wavelength of 200-700 nm periodically for one hour to observe rapid reduction of zinc oxide nanoparticles by action of grape seed extracts after which the nanoparticles were subjected to TEM analysis. The TEM analysis in this study confirmed the spherical shape of the zinc oxide nanoparticles with their size ranging between 20 to 30 microns and were seen as clusters in the transmission electron microscope. (12)

Also in another study, titanium nanoparticles were synthesized using grape seed extract and their morphological characteristics were evaluated using transmission electron microscope. The TEM analysis revealed the spherical shape of titanium nanoparticles of size 15 to 30 nm synthesised using grape seed extract. (47)

Similarly, Periera et al., synthesised selenium nanoparticles using aqueous extract of clove and cinnamon and characterised the morphological features with transmission electron microscopy. In this study, the absorbance peak was noted at 355 nm. The TEM analysis confirmed the spherical and square shape of the titanium nanoparticles with the particle size ranging between 40 to 90 nm. (47,48)

The current study was conducted similar to the previous studies discussed and their characterisation done using transmission electron microscope revealed similar results to the articles mentioned above. However, extensive research needs to be conducted to study the antibacterial, antifungal, antioxidant and cytotoxic activities of the synthesized nanoparticles for therapeutic purposes.

CONCLUSION:

In this study, a simple, biological and low-cost approach was used for the preparation of copper Nanoparticles using Red Tea(49) (50) (51) (52) (53) (54) (55) (56) (57) (58) (59) ((59,60) (61) (62). The green synthesized copper Nanoparticles can be subjected to the various other biological activities such as antibacterial, antifungal, cytotoxic evaluation to know the efficiency of these nanoparticles do that they can be used as a substitute for conventional chemical products thereby reducing the cytotoxicity.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no

conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES:

1. Li Y, Duan X, Qian Y, Yang L, Liao H. Nanocrystalline Silver Particles: Synthesis, Agglomeration, and Sputtering Induced by Electron Beam [Internet]. Vol. 209, Journal of Colloid and Interface Science. 1999. p. 347–9. Available from: http://dx.doi.org/10.1006/jcis.1998.5879

2. Chen JC, Lin ZH, Ma XX. Evidence of the production of silver nanoparticles via pretreatment of Phoma sp.3.2883 with silver nitrate. Lett Appl Microbiol. 2003;37(2):105–8.

3. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles [Internet]. Vol. 3, Nanomedicine: Nanotechnology, Biology and Medicine. 2007. p. 95–101. Available from: http://dx.doi.org/10.1016/j.nano.2006.12.001

4. Khanna PK, Kale TS, Shaikh M, Koteswar Rao N, Satyanarayana CVV. Synthesis of oleic acid capped copper nano-particles via reduction of copper salt by SFS [Internet]. Vol. 110, Materials Chemistry and Physics. 2008. p. 21–5. Available from: http://dx.doi.org/10.1016/j.matchemphys.2008.01.013

5. Surmawar NV, Thakare SR, Khaty NT. One-Pot, Single Step Green Synthesis of Copper Nanoparticles: SPR Nanoparticles [Internet]. Vol. 3, International Journal of Green Nanotechnology. 2011. p. 302–8. Available from: http://dx.doi.org/10.1080/19430892.2011.633478

6. Grouchko M, Kamyshny A, Ben-Ami K, Magdassi S. Synthesis of copper nanoparticles catalyzed by pre-formed silver nanoparticles [Internet]. Vol. 11, Journal of Nanoparticle Research. 2009. p. 713–6. Available from: http://dx.doi.org/10.1007/s11051-007-9324-5

7. Khanna PK, Gaikwad S, Adhyapak PV, Singh N, Marimuthu R. Synthesis and characterization of copper nanoparticles [Internet]. Vol. 61, Materials Letters. 2007. p. 4711–4. Available from: http://dx.doi.org/10.1016/j.matlet.2007.03.014

8. J A, Aparna J, Rajeshkumar S. Cytotoxic and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesised Using Maranta Arundinacea Root Extract [Internet]. Vol. 11, International Journal of Research in Pharmaceutical Sciences. 2020. p. 4372–7. Available from: http://dx.doi.org/10.26452/ijrps.v11i3.2655

9. ANTI-INFLAMMATORY ACTIVITY OF TITANIUM DIOXIDE NANOPARTICLES SYNTHESISED USING GRAPE SEED EXTRACT: AN in vitro STUDY [Internet]. [cited 2021 Aug 16]. Available from: https://paperpile.com/app/p/2fe87431-ef18-02f1-a4d4-c018f73e5b66

10. EFFICACY OF Aloe vera GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING IN THE MANAGEMENT OF CHRONIC PERIODONTITIS [Internet]. [cited 2021 Aug 16]. Available from: https://paperpile.com/app/p/74fa086a-c658-0095-afbd-baccfb582a75

11. Kishen A, Rajeshkumar S, Preejitha VB. Cynodon dactylon Mediated Synthesis of Selenium Nanoparticles and Its Antimicrobial Activity Against Oral Pathogens [Internet]. Vol. 11, International Journal of Research in Pharmaceutical Sciences. 2020. p. 4152–6. Available from: http://dx.doi.org/10.26452/ijrps.v11i3.2621

12. Rajeshkumar S, Nandhini NT, Manjunath K, Sivaperumal P, Krishna Prasad G, Alotaibi SS, et al.

Environment friendly synthesis copper oxide nanoparticles and its antioxidant, antibacterial activities using Seaweed (Sargassum longifolium) extract [Internet]. Vol. 1242, Journal of Molecular Structure. 2021. p. 130724. Available from: http://dx.doi.org/10.1016/j.molstruc.2021.130724

13. ANTIFUNGAL ACTIVITY OF GRAPE SEED EXTRACT MEDIATED ZINC OXIDE NANOPARTICLES - AN In vitro STUDY [Internet]. [cited 2021 Aug 16]. Available from: https://paperpile.com/app/p/d2434c40-e3a0-0fed-9ebe-2a13d846baf9

14. Chen H, Lee J-H, Kim Y-H, Shin D-W, Park S-C, Meng X, et al. Metallic copper nanostructures synthesized by a facile hydrothermal method. J Nanosci Nanotechnol. 2010 Jan;10(1):629–36.

15. Kowshik M, Ashtaputre S, Kharrazi S, Vogel W, Urban J, Kulkarni SK, et al. Extracellular synthesis of silver nanoparticles by a silver-tolerant yeast strain MKY3 [Internet]. Vol. 14, Nanotechnology. 2003. p. 95–100. Available from: http://dx.doi.org/10.1088/0957-4484/14/1/321

16. Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier; 2018. 548 p.

17. Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, et al. Fungus-Mediated Synthesis of Silver Nanoparticles and Their Immobilization in the Mycelial Matrix: A Novel Biological Approach to Nanoparticle Synthesis [Internet]. Vol. 1, Nano Letters. 2001. p. 515–9. Available from: http://dx.doi.org/10.1021/nl0155274

18. Khanna PK, More P, Jawalkar J, Patil Y, Koteswar Rao N. Synthesis of hydrophilic copper nanoparticles: effect of reaction temperature [Internet]. Vol. 11, Journal of Nanoparticle Research. 2009. p. 793–9. Available from: http://dx.doi.org/10.1007/s11051-008-9441-9

19. Shenton W, Douglas T, Young M, Stubbs G, Mann S. Inorganic-Organic Nanotube Composites from Template Mineralization of Tobacco Mosaic Virus [Internet]. Vol. 11, Advanced Materials. 1999. p. 253–6. Available from: http://dx.doi.org/10.1002/(sici)1521-4095(199903)11:3<253::aid-adma253>3.0.co;2-7

20. Shahverdi AR, Fakhimi A, Shahverdi HR, Minaian S. Synthesis and effect of silver nanoparticles on the antibacterial activity of different antibiotics against Staphylococcus aureus and Escherichia coli. Nanomedicine. 2007 Jun;3(2):168–71.

21. Husseiny MI, Abd El-Aziz M, Badr Y, Mahmoud MA. Biosynthesis of gold nanoparticles using Pseudomonas aeruginosa [Internet]. Vol. 67, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2007. p. 1003–6. Available from: http://dx.doi.org/10.1016/j.saa.2006.09.028

22. Marshall MJ, Beliaev AS, Dohnalkova AC, Kennedy DW, Shi L, Wang Z, et al. c-Type Cytochrome-Dependent Formation of U(IV) Nanoparticles by Shewanella oneidensis [Internet]. Vol. 4, PLoS Biology. 2006. p. e268. Available from: http://dx.doi.org/10.1371/journal.pbio.0040268

23. Dameron CT, Reese RN, Mehra RK, Kortan AR, Carroll PJ, Steigerwald ML, et al. Biosynthesis of cadmium sulphide quantum semiconductor crystallites [Internet]. Vol. 338, Nature. 1989. p. 596–7. Available from: http://dx.doi.org/10.1038/338596a0

24. Website [Internet]. Available from: Menon, S., Rajeshkumar, S., & Kumar, V. (2017). A review on biogenic synthesis of gold nanoparticles, characterization, and its applications. Resource-Efficient Technologies, 3 (4), 516-527. https://doi.org/10.1016/j.reffit.2017.08.002.

25. Website [Internet]. Available from: Rajeshkumar, S., and Poonam Naik. “Synthesis and biomedical applications of cerium oxide nanoparticles–a review.” Biotechnology Reports 17 (2018): 1-5. https://doi.org/10.1016/j.btre.2017.11.008.

26. Agarwal H, Menon S, Venkat Kumar S, Rajeshkumar S. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route [Internet]. Vol. 286, Chemico-Biological Interactions. 2018. p. 60–70. Available from: http://dx.doi.org/10.1016/j.cbi.2018.03.008

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. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res. 2020 Jul;43(7):729–30.

29. 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. 2018 Dec;120(Pt A):876–85.

30. 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. 2018 Nov 26;11:CD011423.

31. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and cardiovascular disease. Hypertens Res. 2020 Aug;43(8):851–3.

32. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol. 2019 Dec;16(12):935–6.

33. 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. 2019 Dec 15;148:176–84.

34. 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. 2018 Aug 29;42(10):183.

35. 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.

36. 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

37. 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

38. 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.

2018 Aug 1;23(4):383–93.

39. 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. 2019 Mar 1;135:672–7.

40. 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. 2021 Aug;47(8):1198–214.

41. 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

42. 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

43. Sarode SC, Gondivkar S, Sarode GS, Gadbail A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. Oral Oncol. 2021 Jun 16;105390.

44. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol. 2021 Jun 14;105375.

45. 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

46. 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. 2021 Jul 7;14:2851–61.

47. SYNTHESIS AND CHARACTERIZATION OF GRAPE SEED MEDIATED TITANIUM DIOXIDE NANOPARTICLES: AN in vitro STUDY [Internet]. [cited 2021 Aug 16]. Available from: https://paperpile.com/app/p/3479aa0f-9f72-0224-9cf3-85f5b6492e82

48. GREEN SYNTHESIS OF SELENIUM NANOPARTICLES (SeNPs) USING AQUEOUS EXTRACT OF CLOVE AND CINNAMON [Internet]. [cited 2021 Aug 16]. Available from: https://paperpile.com/app/p/8deb9cd6-c0cc-04e5-9d5b-a1a14726faac

49. 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. 2010 Jul;68(7):1565–7.

50. 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

51. Krishnan V, Lakshmi T. Bioglass: A novel biocompatible innovation. J Adv Pharm Technol Res. 2013 Apr;4(2):78–83.

52. 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

53. 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

54. 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

55. 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. 2016 Aug;42(8):1175–80.

56. 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

57. 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

58. 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

59. 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 laserin vitro [Internet]. Vol. 48, International Endodontic Journal. 2015. p. 602–10. Available from: http://dx.doi.org/10.1111/iej.12354

60. 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

61. 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

62. Parthasarathy M, Isaac JoshuaRamesh 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. 2016 Dec;134(Pt 2):433–9.