

GREEN SYNTHESIS OF COPPER NANOPARTICLES USING STEM EXTRACT OF MUSA SAPIENTUM AND ITS CHARACTERISATION - AN IN VITRO STUDY

ABSTRACT:

Introduction: Metallic nanoparticles (NPs) have attracted great interest because of their unique physical and chemical properties and among which copper nanoparticles are used for its low cost, better efficacy and environmentally friendly. *Musa sapientum* has been considered as one of the best medicinal value plants and can be used for the treatment of various periodontal diseases.

The aim of the study is to synthesize and characterize *Musa sapientum* mediated copper nanoparticles.

Materials and Methods: The Copper nanoparticles (CuNPs) were synthesized using *Musa sapientum* extract and characterization was done by UV-Visible Spectroscopy and Transmission Electron Microscope (TEM).

Results: The results confirmed that the synthesized *Musa sapientum* mediated nanoparticle was eco-friendly and non-toxic. The peak value has been centered at 75 nm, which has been mainly associated with absorbance of CuNPs. Absorbance rate increases with increase in wavelength. The formation of CuNPs as well as their morphological dimensions in the TEM study demonstrate that the average size is from 5.67 – 9.10 nm. The shapes of the CuNPs are proved to be spherical. TEM analysis reveals that the copper nanoparticles are predominantly spherical. The overall morphology of the copper nanoparticles produced by reduction of Cu^{2+} ions with 2Mm CuSO_4 is composed of almost uniform nanoparticles.

Conclusion: In this study, a simple, biological and low-cost approach was done for the preparation of copper nanoparticles using stem extract of *Musa sapientum*. Thus, *Musa sapientum* mediated copper nanoparticles can be subjected to the various other biological activities such as antibacterial, antifungal, cytotoxic evaluation to know the efficiency of these nanoparticles.

Keywords: *Musa sapientum*, copper, nanoparticles, green synthesis.

INTRODUCTION:

Metallic nanoparticles have multifunctionality nature, and they have been extensively used in a variety of sectors of industries and medicine including drug delivery, cancer treatment, wastewater treatment, and DNA analysis. Recently metallic nanoparticles (NPs) have attracted great interest because of their unique physical and chemical properties(1). The green synthesis of metallic nanoparticles has been proposed as a cost-effective and environmentally friendly alternative to chemical and physical methods. In recent years, copper nanoparticles (CuNPs) have attracted much attention from researchers due to its various applications in industries, medicine and various other fields(2). This study supports the use of copper nanoparticles as a biocidal agent because of its great antibacterial property which is known for a long time (3). ↔

Many plant parts or whole plants have been used for the green synthesis of Cu NPs due to the presence of a large number of bioactive compounds in plants. The extracts of plants have been efficiently applied for this purpose (4). Synthesis of Cu NPs has been successful with extracts of various parts of plant species that include stem extract of *Musa sapientum*. Bananas (Genus *Musa*) have been grown for a long time throughout the world (5). Pharmacological investigations shows that all parts of banana have nutritional and traditional medicinal uses and in many vitro studies, animal model studies and clinical studies suggest that various parts of banana can be used for the treatment of various diseases like diabetes, hypertension, cancer, ulcers, diarrhoea, and Alzheimer's disease (6). Other medicinal uses are in surgical dressing, pain relief, food and pharmaceuticals, nano medicine, pollution control, apoptosis and cell cycle.

Our team has extensive knowledge and research experience that has translated into high quality publications-(7-19)_(20-24)_(25)_(26). For the first time, CuNPs were successfully synthesized using *Musa sapientum* stem extract in the current investigation. *Musa sapientum*, commonly known as banana, is an herbaceous plant of the Musaceae family. Different parts of the banana plant contain carotenoids, phenolic compounds, and biogenic amines such as dopamine, serotonin, noradrenaline, tryptophan, and tyrosine (27). Traditionally it is used in the treatment of various diseases like dysentery, intestinal lesions and various other abdominal infections (28).

Aqueous extract of banana stem has more antimicrobial activity which could be useful against the gram negative bacteria such as staphylococcus and the bio actives such as flavonoids, tannins are also useful against pathogenic bacteria causing periodontitis-(29). Currently, a biological method has been followed for the green chemistry synthesis of metal nanoparticles due to it being free from hazardous chemicals (30). The metal nanoparticle synthesis method from plant extracts has more advantages over the microbial synthesis method because the microbial process is highly expensive due to the cost of microorganism isolation and their culture maintenance (31). Cu nanoparticles have been synthesised using biological sources and have been reported in potential applications such as for antimicrobials and photocatalytic activity (32). In recent times, plant-mediated synthesis of nanoparticles has garnered wide interest owing to its inherent features such as rapidity, simplicity, eco-friendliness, and cheaper costs. The analytical studies revealed that the synthesized copper sulphate nanoparticles from these two different methods have almost identical size and morphology (33). The aim of the study is to synthesize and characterize *Musa sapientum* mediated copper nanoparticles.

MATERIALS AND METHODS:

Plant extracts preparation:

1 g of *Musa sapientum* powder was measured and taken. The measured amount of plant powder was then mixed with 100 mL of distilled water and boiled for 5-10 mins. The contents were filtered using a filter paper, funnel and measuring cylinder. A viscous filtrate was obtained (Figure 1).

Synthesis of *Musa sapientum* mediated CuNps

0.01 mg of CuSO_4 was weighed and mixed with distilled water of 8 mL and mixed with filtered extract. The extract is permitted to stand in the stirrer for a duration of 1 h and kept in the shaker for intermixing of the particles to obtain green synthesis (Figure 2). The reduction of CuSO_4 to CuNps was periodically monitored by an ultraviolet (UV) spectrometer.

UV-Vis spectra analysis:

UV-Vis spectroscopy was used for monitoring the signature of CuNps. This is a powerful tool for the characterization of colloidal particles. Metal particles are ideal candidates for study with UV-Vis spectroscopy since they exhibit strong surface plasmon resonance (SPR) absorption in

the visible region and are highly sensitive to the surface modification. The presence of CuNP was confirmed by SPR property



Figure 1: Preparation Of *Musa sapientum* stem extract.

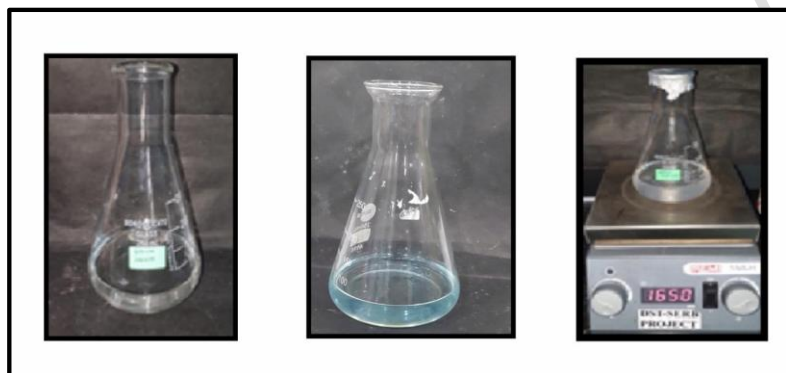


Figure 2 : Preparation of *Musa Sapientum* copper nanoparticles.



Figure 3: Colour change from light green colour to brown colour.

RESULTS:

UV-Vis spectroscopy analysis as the sample composition extract was mixed in the CuSO_4 solution, the color started to change from light green to brown due to the reduction of copper ions which indicated the formation of CuNps (Figure 3). Table 1 shows that as the wavelength increases with a rate of 0.25 AU absorbance rate is also increased and the maximum absorbance is found at the wavelength of 800 nm. Wavelength and the absorbance is directly proportional as the wavelength is increased absorbance is also increased at the rate of 0.25 AU. More color changes and Cu settled in the synthesized exhibited at 750 nm (Graph 1). The average SPR phenomenon is absorbed at 780-840 nm. Nano-sized particles exhibit unique optical properties having an exponential-decay profile. UV-Visible absorbance spectroscopy has proved to be a very useful technique for studying metal nanoparticles because the peak positions and shapes are sensitive to particle size. The effect of ascorbic concentration in the extract on the UV-Visible absorbance spectroscopy of the synthesized CuNp showed a single peak at around 750 nm. The surface Plasmon peak of CuNp has been reported to appear at around 570 nm. However, when the particle size is less than 4 nm, the distinctive Plasmon peak is known to be broadened and replaced by a featureless absorbance, which increases monotonically towards higher energies. In our work, the resulting Cu dispersion did not show a plasmon peak at around 750 nm, but displayed a broadened peak with a higher wavelength, indicating the presence of more separated CuNp.

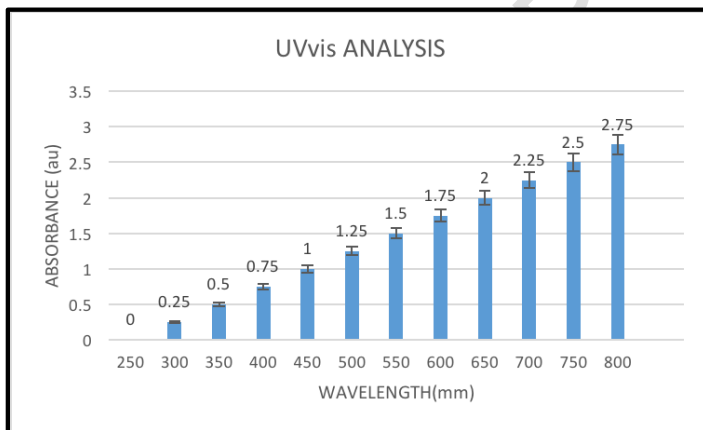
TEM technique was employed to visualize the size and shape of copper nanoparticles. The TEM image of the produced CuNPs is shown in Figure 4. The formation of CuNPs as well as their morphological dimensions in the TEM study demonstrates that the average size is from 5.67 – 9.10 nm. ~~The shapes of the CuNPs are proved to be spherical.~~ TEM analysis reveals that the copper nanoparticles are predominantly spherical. The overall morphology of the copper nanoparticles produced by reduction of Cu^{2+} ions, with 2Mm CuSO_4 is composed of almost uniform nanoparticles

Formatted: Superscript

Formatted: Subscript

Table 1: This table represents the UV- vis spectroscopic analysis of copper nanoparticles. As the absorbance rate increases the wavelength also increases at the rate of 50 nm. Absorbance and the wavelength is directly proportional and the maximum absorbance is seen at the wavelength of 800 nm.

Absorbance (AU)	0.25	0.5	0.75	1.00	1.25	1.50	1.75	2	2.25	2.5	2.75
wavelength (nm)	300	350	400	450	500	550	600	650	700	750	800



Graph 1: This graph represents UV-vis spectroscopic analysis of copper nanoparticles. X axis represents various wavelengths of the green synthesized copper nanoparticles (in nm), while the Y axis represents the Absorbance rate at which the copper nanoparticles were absorbed (in Astronomical Unit). Absorbance rate increases with increase in wavelength.

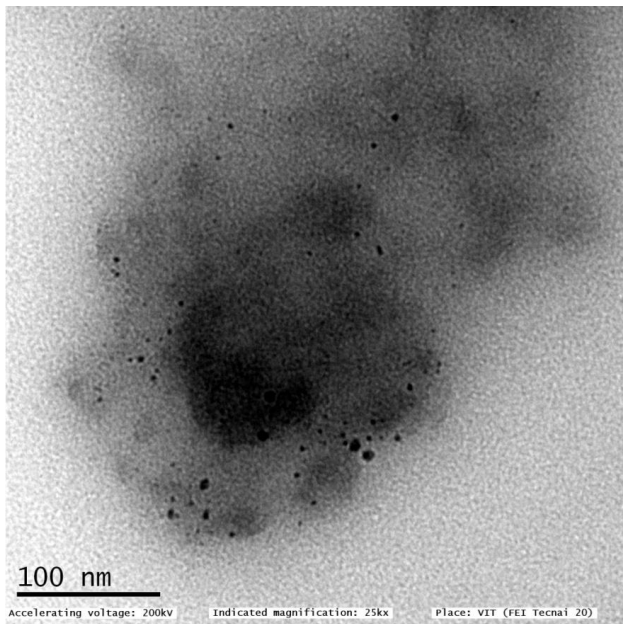


Figure 4: Transmission electron microscopic analysis of nanoparticles

DISCUSSION:

According to the previous article suggested-, the UV-Visible absorption spectrum recorded for copper nano- particles, shown exhibits maximum absorption at 580 nm which was in a varied range (34). Another study suggested that the nanosized copper particles typically exhibit a surface plasmon peak at around 556 to 580 nm and the surface plasmon band at 580 nm which indicates the existence of copper nanoparticles (35). The previous article on the phytochemical content and free radical scavenging activity of banana peel indicates that peel extracts from these varieties may be useful to combat free radical mediated diseases (36)-. In recent days more use of herbs as the natural phytochemicals helps in suppressing the alveolar bone loss, which is the striking feature in periodontitis and have shown to possess a wide array of biological properties such as antimicrobial, antioxidant, and anti-inflammatory effects.

Recently, copper (Cu) nanoparticles have been synthesised, characterised and applied in various fields by different researchers. Previous articles on the oxidation of different Cu NPs have reported that those studies are incomplete, and learning about the initial oxidation and oxidation progress through time in different environmental conditions is needed (37). The value of this knowledge is to learn the oxidation degree of the Cu NPs after storage and to know the role of the coating in the chemical stability.

CONCLUSION:

Significant biological activity of *Musa sapientum* synthesized CuNP, established biological method is highly preferred in comparison to other methods as it is eco-friendly and requires less number of downstream processing(40)(Danda 2010) (41) (42) (43) (44) (45) (46) (47) (48) (49) (50) ((50,51) (52) (53). The SPR property of synthesized NP was studied by UV-Vis spectroscopy, and the peak of the spectra was found to be at 750 nm, which is characteristic property of CuNP.

REFERENCES:

1. Ramyadevi J, Jeyasubramanian K, Marikani A, Rajakumar G, Rahuman AA. Synthesis and antimicrobial activity of copper nanoparticles [Internet]. Vol. 71, Materials Letters. 2012. p. 114–6. Available from: <http://dx.doi.org/10.1016/j.matlet.2011.12.055>
2. Pohanka M. Copper and copper nanoparticles toxicity and their impact on basic functions in the body. Bratisl Lek Listy [Internet]. 2019 [cited 2021 May 24];120(6). Available from: <https://pubmed.ncbi.nlm.nih.gov/31223019/>
3. Kruk T, Szczepanowicz K, Stefańska J, Socha RP, Warszński P. Synthesis and antimicrobial activity of monodisperse copper nanoparticles [Internet]. Vol. 128, Colloids and Surfaces B: Biointerfaces. 2015. p. 17–22. Available from: <http://dx.doi.org/10.1016/j.colsurfb.2015.02.009>
4. Murthy HCA, Ananda Murthy HC, Desalegn T, Kassa M, Abebe B, Assefa T. Synthesis of Green Copper Nanoparticles Using Medicinal Plant *Hagenia abyssinica* (Brace) JF. Gmel. Leaf Extract: Antimicrobial Properties [Internet]. Vol. 2020, Journal of Nanomaterials. 2020. p. 1–12. Available from: <http://dx.doi.org/10.1155/2020/3924081>

5. Kibria AA, Kamrunnessa, Rahman MM, Kar A. Extraction and Evaluation of Phytochemicals from Banana Peels (*Musa sapientum*) and Banana Plants (*Musa paradisiaca*) [Internet]. Vol. 2, Malaysian Journal of Halal Research. 2019. p. 22–6. Available from: <http://dx.doi.org/10.2478/mjhr-2019-0005>
6. Reddy AJ, Dubey AK, Handu SS, Sharma P, Mediratta PK, Ahmed QM, et al. Anticonvulsant and Antioxidant Effects of Stem Extract on Acute and Chronic Experimental Models of Epilepsy. *Pharmacognosy Res* [Internet]. 2018 Jan;10(1):49–54. Available from: http://dx.doi.org/10.4103/pr.pr_31_17
7. 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>
8. 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>
9. 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>
10. 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>
11. 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>
12. 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>
13. 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>
14. 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>

15. 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>
16. 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>
17. 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>
18. 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>
19. 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>
20. 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>
21. 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>
22. 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>
23. 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>
24. 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>

25. 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>
26. 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>
27. Apriasari ML, Iskandar, Suhartono E. Bioactive Compound and Antioxidant Activity of Methanol Extract Mauli Bananas (*Musa sp*) Stem [Internet]. Vol. 4, *International Journal of Bioscience, Biochemistry and Bioinformatics*. 2014. p. 110–5. Available from: <http://dx.doi.org/10.7763/ijbbb.2014.v4.321>
28. Dikshit P, Shukla K, Tyagi MK, Garg P, Gambhir JK, Shukla R. Antidiabetic and antihyperlipidemic effects of the stem of *Musa sapientum* Linn. in streptozotocin-induced diabetic rats. *J Diabetes* [Internet]. 2012 Dec;4(4):378–85. Available from: <http://dx.doi.org/10.1111/j.1753-0407.2012.00198.x>
29. Bährle-Rapp M. *Musa Sapientum* Extract [Internet]. Springer Lexikon Kosmetik und Körperpflege. 2007. p. 361–361. Available from: http://dx.doi.org/10.1007/978-3-540-71095-0_6723
30. Mali SC, Dhaka A, Githala CK, Trivedi R. Green synthesis of copper nanoparticles using *Celastrus paniculatus* Willd. leaf extract and their photocatalytic and antifungal properties [Internet]. Vol. 27, *Biotechnology Reports*. 2020. p. e00518. Available from: <http://dx.doi.org/10.1016/j.btre.2020.e00518>
31. Kothai S, Umamaheswari R. Green Synthesis, Characterization of Copper Nanoparticles Derived from *Ocimum Sanctum* Leaf Extract and their Antimicrobial Activities [Internet]. Vol. 8, *Journal of Chemistry and Chemical Sciences*. 2018. p. 984–92. Available from: <http://dx.doi.org/10.29055/jccs/670>
32. Malathi S, Balasubramanian S. Synthesis Of Copper Nanoparticles And Their Biomedical Applications: Green Synthesis Of Copper Nanoparticles [Internet]. LAP Lambert Academic Publishing; 2012. 52 p. Available from: https://books.google.com/books/about/Synthesis_Of_Copper_Nanoparticles_And_Th.html?hl=&id=fhp5MAEACAAJ
33. Mohan Jain S. *Banana Improvement: Cellular, Molecular Biology, and Induced Mutations* [Internet]. Science Pub Incorporated; 2004. 382 p. Available from: https://books.google.com/books/about/Banana_Improvement.html?hl=&id=hUgjAQAAMA AJ
34. Kumar B, Smita K, Debut A, Cumbal L. Andean Sacha Inchi (*L.*) Leaf-Mediated Synthesis of CuO Nanoparticles: A Low-Cost Approach. *Bioengineering (Basel)* [Internet]. 2020 Jun 6;7(2). Available from: <http://dx.doi.org/10.3390/bioengineering7020054>

35. Amin F, Fozia, Khattak B, Alotaibi A, Qasim M, Ahmad I, et al. Green Synthesis of Copper Oxide Nanoparticles Using Leaf Extract and Their Characterization and Investigation of Antimicrobial Potential and Cytotoxic Activities. *Evid Based Complement Alternat Med* [Internet]. 2021 Jun 18;2021:5589703. Available from: <http://dx.doi.org/10.1155/2021/5589703>
36. Dmochowska A, Czajkowska J, Jędrzejewski R, Stawiński W, Migdał P, Fiedot-Toboła M. Pectin based banana peel extract as a stabilizing agent in zinc oxide nanoparticles synthesis. *Int J Biol Macromol* [Internet]. 2020 Dec 15;165(Pt A):1581–92. Available from: <http://dx.doi.org/10.1016/j.ijbiomac.2020.10.042>
37. Medvedeva XV, Li F, Maokhamphiou A, Medvedev JJ, Ahmed A, Klinkova A. Shape control in seed-mediated synthesis of non-elongated Cu nanoparticles and their optical properties. *Nanoscale* [Internet]. 2021 Jul 6; Available from: <http://dx.doi.org/10.1039/d1nr01358k>
38. Patil SP. assisted green synthesis of metal nanoparticles: A mini review. *Biotechnol Rep (Amst)* [Internet]. 2020 Dec;28:e00569. Available from: <http://dx.doi.org/10.1016/j.btre.2020.e00569>
39. Rajagopal G, Nivetha A, Sundar M, Panneerselvam T, Murugesan S, Parasuraman P, et al. Mixed phytochemicals mediated synthesis of copper nanoparticles for anticancer and larvicidal applications. *Heliyon* [Internet]. 2021 Jun;7(6):e07360. Available from: <http://dx.doi.org/10.1016/j.heliyon.2021.e07360>
40. 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>
41. 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>
42. 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>
43. 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>
44. 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>

45. Dhinesh B, Isaac Joshua Ramesh 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>
46. Pradeep Kumar 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>
47. 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>
48. 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>
49. 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>
50. 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>
51. 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>
52. 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>
53. 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>

UNDER PEER REVIEW

