Cytotoxic Effect of Red Sandal Mediated Silver Nanoparticles Mouthwash Using Brine Shrimp Lethality Assay: An In-vitro Study

B. Amritha a, S. Balaji Ganesh b* and S. Rajesh Kumar c

a Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.
b Department of Periodontics, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.
c Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India.

ABSTRACT

Background: Silver Nanoparticles (AgNP) plays a crucial role in nanoscience, nanotechnology and nanomedicine. AgNP has antibacterial, antifungal, antiviral, anti-inflammatory, anti-antigenic, anticancer and cytotoxic properties. Cytotoxic effects of mouthwashes on the gingival tissue have always been a priority. Pterocarpus santalinus, with the common name of red sandalwood. The aim of the study is to evaluate the cytotoxic effect of red sandal mediated silver nanoparticles mouthwash using brine shrimp lethality assay.

Materials and Methods: The mouthwash was prepared using red sandal mediated silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, sodium dodecyl phosphate with 0.1% in concentration. The methodology includes green synthesis of Red sanders mediated Silver Nanoparticles synthesis followed by tests for Cytotoxic effect using mouthwash.

Results: Red sanders mediated silver nanoparticles have high potent cytotoxic potential, it has
been proved with the help of brine shrimps. LD50 concentration was obtained to be 80µL, with half the population of nauplii in the respective well surviving post incubation. **Conclusion:** From the observed result, it has been concluded that Pterocarpus santalinus/red sandal mediated silver nanoparticles mouthwash has a considerably moderate cytotoxic effect. It is a rich source of various potential phytochemicals and is a simple, rapid, stable and cost effective method for nano based medical applications.

**Keywords:** Green synthesis; cytotoxic effect; mouthwash; Pterocarpus santalinus; silver nanoparticles.

1. **INTRODUCTION**

Various medicinal plants have been used for hundreds of years and are found to be effective in the treatment of many diseases [1]. Natural products of plants possess several biological activities including cytotoxic effects. The cytotoxic level of medicinal plants must even be evaluated against host cells. The safety of plants as potential therapeutically agents must be ascertained and the therefore side effects should be acceptable to the host [2]. Bioactive compounds with no or less toxic effect to the host are good for formulation of medicine. Many researches are done to understand the cytotoxic effect constituents of medicinal plants for the treatment of the disease [3].

Nanosized inorganic particles, of either simple or composite nature, display physical and chemical properties and represent an increasingly important material development of novel nanodevices which may be utilised in numerous physical, biological, biomedical, and pharmaceutical applications [4]. Silver Nanoparticles (AgNP) plays a crucial role in nanoscience, nanotechnology and nanomedicine. AgNP has antibacterial, antifungal, antiviral, anti-inflammatory, anti-antigenic, anticancer and cytotoxic properties [5]. AgNP has engrossing properties and its low cost and simply available in natural sources. AgNP has an impractical potential in comparison to gold nanoparticles [6]. AgNPs are increasingly utilized in various fields, including medical, food, health care, cosmetics, and industrial purposes, due to their unique physical and chemical properties [7]. Silver nanoparticles may be a popular additive in many health products as listed above due to its unique ability to fight infectious diseases, slow the growth of bacteria, mold and germs [8]. Biologically prepared AgNPs show high yield, solubility, and high stability [9]. Several synthetic methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches which will produce well-defined size and morphology under optimized conditions for translational research [10].

Cytotoxic effects of mouthwashes on the gingival tissue have always been a priority. Chlorhexidine gluconate mouthwash had no cytotoxic effects on human gingival fibroblasts. Mouthwashes are composed of strontium chloride, potassium nitrate, sodium citrate and sodium fluoride as desensitizing agents [11]. Pterocarpus santalinus, with the common name of red sandalwood. The wood is traditionally considered not aromatic. A wide array of biological activities and potential health benefits of P. santalinus have been reported including antioxidative, antidiabetic, antimicrobial, anticancer, anti-inflammatory cytotoxic properties as well as protective effects on the liver, gastric mucosa and nervous systems [12]. All these protective effects were attributed to bioactive compounds present in P. santalinus. The effect of this herb against most of the oral pathogens is essentially unexplored [13]. Our team has extensive knowledge and research experience that has translated into high quality publications [14–26],[27–31],[32],[33]. The aim of the study is to evaluate the cytotoxic effect of red sandal mediated silver nanoparticles mouthwash using brine shrimp lethality assay.

2. **MATERIALS AND METHODS**

2.1 **Preparation of Silver Nanoparticles**

The dried powdered plant material 0.5gm of red sandal was diluted with 100ml of distilled water, and heated for 9 mins at 60-80°C under vacuum. Then it was filtered using Whatman’s filter paper and plant extract was prepared. 80ml of distilled water was taken in a conical flask and 20ml of plant extract was added to it. 1mM of Silver particles were added and then kept in a shaker. Preliminary reading was taken every 2 hours for about 72hrs. After 72hrs fill the AgNP plant extract into the 6 centrifuge tube, 12ml each and centrifuged for about 10 mins.
2.2 Preparation of Mouthwash

The mouthwash was prepared using red sandal mediated silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, sodium dodecyl phosphate with 0.1% in concentration. Silver nanoparticles are the main constituent, ethanol acts as a solvent to solubilise the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavouring agent.

2.3 Cytotoxic Effect

Brine shrimp eggs were obtained from a new aqua laboratory. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. After 2 days, when the shrimp were ready, 5 different nanoparticles solution was added to each test tube, 10 shrimps were introduced in each tube. After 24 hrs, the number of surviving shrimps were counted and recorded. LC50 of less than 100 ppm was considered as potent.

3. RESULTS

The test for cytotoxic properties was assessed using brine shrimps. Descriptive statistics were used in this study. Ten nauplii were placed in each of six wells with one standard and the remaining with nanoparticle concentrations 5 µL, 10 µL, 20 µL, 40 µL and 80 µL. LD50 concentration was obtained to be 80µL, with half the population of nauplii in the respective well surviving post incubation. 10 numbers of nauplii are alive on day1 in different concentrations. On day 2, 9 nauplii are alive on 5 µL concentration, 8 nauplii are alive on 10 µL concentration, 9 nauplii are alive on 20 µL concentration, 8 nauplii are alive on 40 µL concentration and 7 nauplii are alive on 80 µL concentration. All nauplii are alive in control value (Table 1, Fig. 1).

Table 1. depicts the cytotoxic activity of red sandal silver nanoparticles mouthwash

| Concentration(µL) | Day 1 | Day 2 |
|-------------------|-------|-------|
| 5µL               | 10    | 9     |
| 10µL              | 10    | 9     |
| 20µL              | 10    | 9     |
| 40µL              | 10    | 8     |
| 80µL              | 10    | 8     |
| Control           | 10    | 10    |

Fig. 1. Graph showing cytotoxic effect of silver nanoparticles based mouthwash of red sandal. Blue represents day 1 and orange represents day 2. X axis denotes number of nauplii alive and Y axis denotes concentration. All nauplii are alive on day 1. On day 2, 9 nauplii are alive on 5µL, 8 nauplii are alive in 10µL, 9 nauplii are alive in 20µL, 8 nauplii are alive in 40µL and 7 nauplii are alive in 80µL. In comparison between day 1 and day 2, as concentration increases, the number nauplii decreases.
4. DISCUSSION

Medicinal plants have unlimited capacity to synthesize bioactive compounds that are effective and have fewer side effects compared to synthetic drugs. Bioactive compounds from plants have shown over the years to have various biological activities [34]. Scientists have developed a greater interest in using these compounds in formulation of new and novel drugs, because of their biological activities and reliability [35]. *P. santalinus* could be used to treat various medical complications such as hemorrhage, dysentery, eye diseases, and mental aberrations, and to act as an aphrodisiac and diaphoretic.

*P. santalinus* is highly valued for its heavy, dark claret-red sanders, which yields 16% of the red coloring matter santalin, used as a coloring agent in pharmaceutical preparations and foodstuff [36]. The presence of various types of plant secondary metabolites such as anthocyanins, flavonoids, glycerides, isoflavone glucoside, phenols, pterocarpol, pterocarpatriol, pterocarpodiolones with β-eudesmol, saponins, steroids, tannins and triterpenoids in the *P. santalinus* L. has been reported [37].

Cytotoxicity of dental materials and oral hygiene products has always been a concern for dental clinicians. These products should be biocompatible; otherwise, they would cause inflammatory reactions. Nanotechnology favorably changed the properties of many dental materials. Mouthwashes are composed of strontium chloride, potassium nitrate, sodium citrate and sodium fluoride as desensitizing agents. Clinical and human intervention studies are very limited; therefore the biological and physiological effects of the isolated compounds of the heartwood are also worth investigation. The key advances in the tissue culture-based biotechnology of economically important red sandals [38]. The development of treatment methods has evoked great expectations in the future. This plant has been exploited to treat a wide variety of ailments, with reported antimicrobial and antioxidant properties, as well as cytotoxic effects against some human cancer cell lines. In vitro cytotoxicity effects of leaf, stem and bark of *Pterocarpus santalinus* Linn.F. was evaluated in a study where they found that *P. santalinus* showed a cytotoxic effect against cancer cell lines [39]. The limitation of this study is that we did not do clinical trials in patients.

5. CONCLUSION

From the observed result, it has been concluded that *Pterocarpus santalinus/red sandal* mediated silver nanoparticles mouthwash has a considerably moderate cytotoxic effect [40-53]. It is a rich source of various potential phytochemicals and is a simple, rapid, stable and cost effective method for nano based medical applications.

DISCLAIMER

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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

I would like to thank the Department of periodontics, Saveetha Dental College, Chennai for their valuable inputs in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Das K, Gowthami V, Dang R. Comparative proximate analysis, phytochemical screening and antioxidant study of leaf and root extracts of Decalepis hamiltonii Wight & Arn [Internet]. Annals of Phytomedicine: An International Journal, 2017;6. Available: http://dx.doi.org/10.21276/ap.2017.6.2.12
2. Gurunathan S, Park JH, Han JW, Kim J-H. Comparative assessment of the apoptotic potential of silver nanoparticles synthesized by Bacillus tequilensis and
Calocybe indica in MDA-MB-231 human breast cancer cells: targeting p53 for anticancer therapy. Int J Nanomedicine [Internet]. 2015;10:4203–22. Available: http://dx.doi.org/10.2147/IJN.S83953

3. Breuer U. Plastics from Bacteria - Natural Functions and Applications. By Guo-Qiang Chen (Editor), Alexander Steinbüchel (Series Editor) [Internet]. Biotechnology Journal. 2010;5:1351–1351. Available: http://dx.doi.org/10.1002/biot.201009064

4. Paul RP, Roy A, Maajida AM, Shanmugam R. Antibacterial Activity of White Pepper Oleoresin Mediated Silver Nanoparticles against Oral Pathogens [Internet]. Journal of Evolution of Medical and Dental Sciences. 2020;9:2352–5. Available: http://dx.doi.org/10.14260/jemds/2020/510

5. Lal HM. Polymer Nanocomposites Based on Silver Nanoparticles: Synthesis, Characterization and Applications [Internet]. Springer Nature. Available: https://books.google.com/books/about/Polymer_Nanocomposites_Based_on_Silver_N.html?hl=en&id=G00dEAAQBAJ

6. Chernousova S, Epple M. Silver as Antibacterial Agent: Ion, Nanoparticle, and Metal [Internet]. Angewandte Chemie International Edition. 2013;52:1636–53. Available: http://dx.doi.org/10.1002/anie.201205923

7. Wang H, Li L-L. In Vivo Self-Assembly Nanotechnology for Biomedical Applications [Internet]. Springer. 2018:201. Available: https://books.google.com/books/act

8. Lofrano G, Libralato G, Brown J. Nanotechnologies for Environmental Remediation: Applications and Implications [Internet]. Springer; 2017:325 . Available: https://books.google.com/books/act

9. Li L-S, Hu J, Yang W, Paul Alivisatos A. Band Gap Variation of Size- and Shape-Controlled Colloidal CdSe Quantum Rods [Internet]. Nano Letters. 2001;1:349–51. Available: http://dx.doi.org/10.1021/nl010559r

10. Karthik L, Vishnu Kirthi A, Ranjan S, Mohana Srinivasan V. Biological Synthesis of Nanoparticles and Their Applications [Internet]. CRC Press. 2019:284. Available: https://books.google.com/books/act

11. Coelho AS, Laranjo M, Gonçalves AC, Paula A, Paulo S, Abrantes AM, et al. Cytotoxic effects of a chlorhexidine mouthwash and of an enzymatic mouthwash on human gingival fibroblasts. Odontology [Internet]. 2020;108(2):260–70. Available: http://dx.doi.org/10.1007/s10266-019-00465-z

12. Manjunatha BK. Hepatoprotective activity of Pterocarpus santalinus L.f., an endangered medicinal plant [Internet]. Vol. 38, Indian Journal of Pharmacology. 2006:25. Available: http://dx.doi.org/10.4103/0253-7613.19848

13. Bindhu MR, Umadevi M. Antibacterial activities of green synthesized gold nanoparticles [Internet]. Vol. 120, Materials Letters. 2014:122–5. Available: http://dx.doi.org/10.1016/j.matlet.2014.01.108

14. 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;89(10):1241–8. Available: http://dx.doi.org/10.1002/jper.2017-0445

15. Paramasivam A, Priyadharshini JV, Raghunandhakumar S, Elumalai P. A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res [Internet]. 2020;43(7):729–30. Available: http://dx.doi.org/10.1038/s41440-020-0461-x

16. SG, TG, KV, Faleh AA, Sukumaran A, PNS. Development of 3D scaffolds using nanochitosan/silk-fibroin/hyaluronic acid biomaterials for tissue engineering applications. Int J Biol Macromol [Internet]. 2018;120(Pt A):876–85. Available: http://dx.doi.org/10.1016/j.ijbiomac.2018.08.149

17. 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;11:CD011423. Available: http://dx.doi.org/10.1002/14651858.CD011423.pub2

18. Paramasivam A, Vijayashree Priyadharshini J. MitomiRs: new emerging microRNAs in mitochondrial dysfunction and...
cardiovascular disease. Hypertens Res [Internet]. 2020;43(8):851–3. Available: http://dx.doi.org/10.1038/s41440-020-0423-3

19. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. Cell Mol Immunol [Internet]. 2019;16(12):935–6. Available: http://dx.doi.org/10.1038/s41423-019-0310-5

20. 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;148:176–84. Available: https://www.sciencedirect.com/science/article/pii/S0140366419307017

21. 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 Mmectic Multivariable Logistic Regression Analysis Approach (MLRA2). J Med Syst [Internet]. 2018;42(10):183. Available: http://dx.doi.org/10.1007/s10916-018-1037-z

22. 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;83(4):445–50. Available: http://dx.doi.org/10.21815/JDE.019.054

23. 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: http://dx.doi.org/10.3390/molecules23061429

24. Alsubait SA, Al Ajan 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;8(3). Available: http://dx.doi.org/10.3390/biom8030068

25. Venkatesan J, Rekha PD, Anil S, Bhatnagar I, Sudha PN, Dechasakulwatana C, et al. Hydroxyapatite from Cuttlefish Bone: Isolation, Characterizations, and Applications. Biotechnol Bioprocess Eng [Internet]. 2018;23(4):383–93. Available: https://doi.org/10.1007/s12257-018-0169-9

26. 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;135:672–7. Available: https://www.sciencedirect.com/science/article/pii/S0263224118311333

27. PradeepKumar AR, Shemes H, Nivedhitha MS, Hashir MM, 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;47(8):1198–214. Available: http://dx.doi.org/10.1016/j.joen.2021.04.022

28. 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: http://dx.doi.org/10.1111/odi.13937

29. 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: http://dx.doi.org/10.1111/odi.13998

30. 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;105390. Available: http://dx.doi.org/10.1016/j.oraloncology.2021.105390

31. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. Oral Oncol [Internet]. 2021 Jun 14;105375. Available: http://dx.doi.org/10.1016/j.oraloncology.2021.105375

32. 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.
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;14:2851–61. Available: https://doi.org/10.2147/RMHP.S306880

34. Cruz D, Falé PL, Mourato A, Vaz PD, Luisa Serralheiro M, Lino ARL. Preparation and physicochemical characterization of Ag nanoparticles biosynthesized by Lippia citriodora (Lemon Verbena) [Internet]. Vol. 81, Colloids and Surfaces B: Biointerfaces. 2010:67–73. Available: http://dx.doi.org/10.1016/j.colsurfb.2010.06.025

35. Dash SS, Bag BG. Synthesis of gold nanoparticles using renewable Punica granatum juice and study of its catalytic activity [Internet]. Vol. 4, Applied Nanoscience. 2014:55–9. Available: http://dx.doi.org/10.1007/s13204-012-0179-4

36. Krishnaveni KS, Srinivasa Rao JV. A New Acylated Isoflavone Glucoside from Pterocarpus santalinus [Internet]. Vol. 48, Chemical and Pharmaceutical Bulletin. 2000:1373–4. Available: http://dx.doi.org/10.1248/cpb.48.1373

37. Balaraju K, Agastian P, Ignacimuthu S, Park K. A rapid in vitro propagation of red sanders (Pterocarpus santalinus L.) using shoot tip explants [Internet]. Vol. 33, Acta Physiologiae Plantarum. 2011:2501–10. Available: http://dx.doi.org/10.11738-011-0795-8

38. Arunkumar AN, Joshi G. Pterocarpus santalinus (Red Sanders) an Endemic, Endangered Tree of India: Current Status, Improvement and the Future [Internet]. Vol. 4, Journal of Tropical Forestry and Environment. 2014. Available from: http://dx.doi.org/10.31357/jtf.e.437

39. Donga S, Moteriya P, Chanda S. Evaluation of antimicrobial and synergistic antimicrobial properties of pterocarpus santalinus [Internet]. Asian Journal of Pharmaceutical and Clinical Research. 2017;10:204. Available: http://dx.doi.org/10.22159/ajpcr.2017.v10i11.20939

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;68(7):1565–7. Available: 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]. Physica B: Condensed Matter. 2010;405:20–4. Available: 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;4(2):78–83. Available: 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: 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]. Journal of Organometallic Chemistry. 2014;753:72–80. Available: 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]. Energy Conversion and Management. 2016;117:466–74. Available: 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
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: 225–9. Available: 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]. Powder Technology. 2014;253:129–37. Available: 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]. Journal of Toxicology and Environmental Health, Part A. 2014;77:177–91. Available: http://dx.doi.org/10.1080/15287394.2013.853224

50. Neelakantan P, Cheng CQ, Mohanraj R, Sriman P, Subbarao C, Sharma S. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser in vitro [Internet]. International Endodontic Journal. 2015;48:602–10. Available: 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]. Journal of Molecular Structure. 2014;1056-1057: 307–13. Available: http://dx.doi.org/10.1016/j.molstruc.2013.10.014

52. Gopalakannan S, Senthivelan 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: 685–90. Available: http://dx.doi.org/10.1016/j.proeng.2012.06.086

53. 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 [Internet]. 2016;134(Pt 2):433–9. Available: http://dx.doi.org/10.1016/j.ecoenv.2015.11.005