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RESEARCH ARTICLE

Comparative Antimicrobial Activity of Copper Nanoparticles and Selenium Nanoparticles Synthesized Using Ficus Benghalensis Against Wound Pathogens

SOWBARANIYA S.M[1], RAJESHKUMAR.S[2*], T LAKSHMI[3]

[1]*Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India, Email: 151801094.sdc@saveetha.com*

[2]*Associate Professor , Department of Pharmacology, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India, Email : rajeshkumars.sdc@saveetha.com*

[3]*Professor, Department of pharmacology , Saveetha Dental college and Hospitals,(SIMATS), Saveetha University, Chennai, India,*

Email : lakshmi@saveetha.com

**Corresponding Author*

ABSTRACT

Introduction: The copper and selenium nanoparticles (Cu, Se Nps) were synthesized using the aqueous extract of Ficus benghalensis by the green synthesis method. This method of preparation has numerous advantages such as being nontoxic, eco accommodating, less time consuming, and easy to scale up for the synthesis of copper and selenium nanoparticles without incorporating any organic chemicals.

Aim: The present study aims at assessing the comparative antimicrobial activity of copper and selenium nanoparticles synthesised using Alampattai against wound pathogens.

Materials and Methods: The methodology includes Green synthesis of Alampattai mediated copper and selenium nanoparticles synthesis followed by test for comparative antimicrobial properties against wound pathogens

Result: It is evident that Ficus benghalensis mediated copper and selenium Nanoparticles had a significant effect against the pathogens namely S.aureus & E.coli which has the zone of inhibition of about 18 mm and 22 mm respectively which is equivalent to the standard (Amoxycillin) used.

Conclusion: From the study, it is concluded that the both copper and selenium nanoparticles had shown a proportionate increase in antimicrobial activity synthesised using Alampattai with increasing dose concentration.

KEYWORDS:
Eco-friendly, Green synthesis, Alamppatai, Copper, Silver, Antimicrobial

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INTRODUCTION

Antibiotics have an important role in the medical field and have helped us to live a healthy life. But due to the incidences of multiple resistances in human pathogenic microorganisms are on the rise and the problem has now become a global concern(1).The scientists are researching every possible aspect of antibiotic resistance to come forward with possible ways out(2).To solve this objective, new antimicrobials from various sources, where natural products still remain as one of the best sources for new compounds. The use of medicinal herbs is an age-old tradition and the recent progress in modern therapeutics has stimulated further developments(3).Herbal medicine represents traditional medicines such as a variety of efficacious plants or plant extracts, which have been known to enhance healing of various diseases for years and it has been suggested that utilization of herbal medicine may become potential treatments in the future(4).

One such potential plant is Alampattai (Ficus benghalensis) is an epiphytic plant which is commonly found in the areas of tropical and warm regions in the world(5). The bark of this plant is an area of interest which has several health benefits such as improving the immunity due to antioxidant activity(6). The paste obtained from the bark used in various skin allergies and skin problems like acne, pimples, rashes and chapalled heels,relieving arthritis(7).Numerous studies have revealed the potential of herbs as sources of drugs and have subsequently identified natural plant-based antimicrobial compounds.The activity of these plants against different bacteria, fungi and parasites might be due to the bark helps in the presence of a wide variety of active secondary metabolites such as flavonoids, phenolic acids, coumarins, terpenoids and sterols (8).

Nanoscience is among the areas of interest in research in the developing world. The materials are manipulated in such a way that it differs from the original ones in terms of shape, size and characteristics(9).Metal nanoparticles revealed special properties which include surface area as well as special surface atoms, due to their excellent physicochemical properties, which have to do with optical, magnetic, catalytic and antimicrobial properties. Synthesis of nanoparticles, especially metallic nanoparticles, is very special due to its wide application in the field of chemistry and drug development((10). Copper nanoparticles (CuNPs) have many antimicrobial properties against different strains of microorganism, causing cell lysis on E.coli, Pseudomonas, S.aureus(11).Although only a few studies has been done on the antibacterial properties of copper nanoparticles, and it has been proved they show copper nanoparticles have a significant promise as bactericidal agent (12). Copper nanoparticles can also be prepared using the methods such as thermal reduction ,chemical reduction and laser ablation and the above methods use oxygen-free atmosphere to synthesize copper nanoparticles because they are easily oxidized.Our team has extensive knowledge and research experience that has translate into high quality publications.(13-17),(18),(19),(20),(21),(22),(23),(15,24,25),(26–30) .,(31),(32)

(13–17)

In a previous arbitrary review, it has proven that Ficus bengalensis have the tendency to kill the microbes and limit the control of its growth. A large number of reports indicate the role of Selenium and copper induced nanoparticles help even in the induction of cancer cell apoptosis with minimal side effects on normal cells(33) .Thus, the pharmacological effect and toxicity is used for the various green synthesis .In this current investigation, the Alampattai is used as an antimicrobial agent to obtain copper and selenium nanoparticles through the green synthesis method .The aim of the study is to synthesis nanoparticles and subjected to the antimicrobial efficacy of Alampattai extract mediated copper and selenium nanoparticles was also tested.

MATERIALS AND METHODS

Plant extracts preparation

1g of Alampattai powder was measured and taken. The measured amount of plant powder was then mixed with 100ml 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)

Copper And Selenium nanoparticles synthesis

30 mM Copper sulphate and sodium selenite was prepared in 50ml of distilled water with 50 ml of Almapattai extract. Then the Alampattai solution was kept in the hot plate with a magnetic stirrer for nanoparticle synthesis. The spectroscopy reading based on colour change was observed (Figure 2).

Test pathogens

The antimicrobial activity of the green synthesised silver nanoparticles is tested against a variety of pathogenic microorganisms. The test pathogens were collected from a microbiology lab. For observing the antimicrobial activity, species such as gram positive aerobic Streptococcus aureus which is commonly found in the oral cavity. Gram negative species such as Pseudomonas , E.coli which is commonly found in subgingival plaque.

Antimicrobial Properties of Prepared Silver Nanoparticles

The bactericidal property of the prepared Cu & Se NP's is carried out using standard Kirby–Bauer disc diffusion assay. The test organisms are kept in nutrient broth for 24 hours and used for further experimental procedures. The agar plates are then sterilized and solidified later. Following the preparation of nutrient agar, corresponding organisms are spreaded on the petri plates using sterile glass rod in-order to obtain bacterial lawns. After this procedure, silver nanoparticles are loaded in a corresponding disc which is of required volumes such as 50 micro liter and incubated at 37 degree Celsius for 24 hours and amoxyrite used as a control. After the incubation period, the appearance of a clear zone around each disc depicts the confirmation of bactericidal property.

RESULTS

Visual observation

In the performed selenium and copper nanoparticles formulation process, sodium selenite and copper sulphate solution was mixed with prepared solution was observed. Slight colour change was observed gradually in an interval of 24 h. The UV-vis spectroscopic readings of Alampattai incorporated copper and selenium nanoparticles showed an absorbance value of 660 nm.

Fig.1: Preparation of Alampattai(F.benghalensis) Extract.

Fig.2: Preparation of Copper and Selenium Nanoparticles.

Antimicrobial activity

The zone of inhibition is referred to as the area where microbial growth is inhibited and lysis of the microbes takes place.The size of this zone depends on many factors, one being how effective the antibiotic (amoxyrite) is at stopping the growth of the bacterium. Another factor that will influence the size of a zone is the diffusion of the Amoxycillin within the agar medium and varies based on the molecular configuration of the antibiotic.The prepared extract and the commercial antibiotics are tested against the pathogens such as S.aureus (fig 3), E.coli (fig 4), Pseudomonas (fig 5).The zone of inhibition is compared to the database of existing antibiotics to determine whether the studied solution is susceptible, moderately susceptible or resistant to the Amoxycillin (Table 1)(Fig6)

Fig.3: Antimicrobial activity of synthesized nanoparticles against S.aureus

Fig.4: Antimicrobial activity of synthesised nanoparticles against E.coli

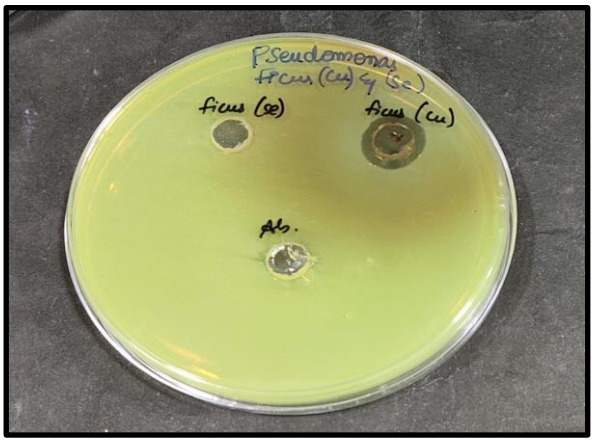

Fig.5: Antimicrobial activity of synthesised nanoparticles against Pseudomonas

Table 1: Zone Of Inhibition of the synthesized nanoparticles and the standard antibiotic(Amoxycillin).

PATHOGEN NAME	Fic. Selenium (in mm)	Fic.Copper (in mm)	Ab (in mm)
S.aureus	12	18	22
E.coli	22	18	22
Pseudomonas	9	9	14

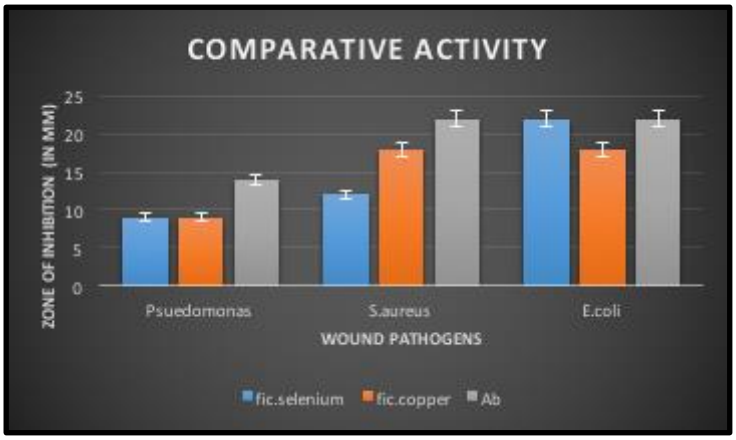

Fig.6: This graph represents comparative antimicrobial activity of synthesized nanoparticles and the Amoxycillin. X-axis represents wound pathogens and the Y-axis represents the zone of inhibition (in mm). Blue colour represents fic.selenium , orange colour represents fic.copper and grey colour represents standard (Amoxycillin).

From the prepared green synthesis, Ficus benghalensis mediated copper nanoparticles had a zone of inhibition of about 18 mm against the wound pathogen S.aureus where the prepared nanoparticles activity is approximating the standard

drug Amoxicillin . The activity of Ficus benghalensis mediated selenium nanoparticles had a zone of inhibition of about 22 mm which is equal to the standard (Amoxycillin) used. So , Ficus benghalensis mediated copper and silver nanoparticles had more or less equivalent activity to the standard used i.e.,Amoxycillin.

DISCUSSION

The development of new drugs is due to microorganisms having become resistant to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines(34). So, it is necessary to find out new antimicrobial agents to fight against diseases. Nanotechnology is an emerging technology and has led to a new revolution in every field of science.Among the various inorganic Nanoparticles available, Copper and selenium nanoparticles have easy processing methods, are inexpensive, have a wide range of applications in dentistry and are a safe material. Due to these properties, copper and selenium pulls a particular interest among researchers (35).

Green synthesis offers numerous benefits that are easily available, long term uses, biomedical applications, where toxic chemicals are not used for the synthesis protocol.The active ingredients of plants against microorganisms are mostly some of the secondary metabolites that are present in abundance in herbs that make it more effective(36). Previous article by Ahamed et Al ;aqueous extracts of the bark F. bengalensis and exhibited significant antibacterial activity against tested bacterial strains. Presence of tannins and saponins in higher concentration than the other phytochemicals suggests that these phytochemicals could likely be responsible for the antibacterial activity(37). However further studies are needed to establish that these plant extracts could form effective antimicrobial therapy against common bacterial diseases(38).

In this study potential increase in the antimicrobial property of Ficus benghalensis mediated copper and selenium nanoparticles in increasing concentration when compared to the standard shows that this formulation can be of good therapeutic benefit in case of bacterial diseases. There has been a considerable increase in the use of nanoparticle based therapeutics for the treatment of any kind of bacterial and microbial disease.

Future Aspects

The synthesized NPs selected from alampattai extract study elicit protective effects against various wound pathogens and mechanisms involved can be studied in vitro and in vivo. Our study will pave the way for the development of a drug that can treat, prevent, or cure various wound pathogens .

CONCLUSION

This study proves that there are anti-microbial mechanisms adopted by the selenium nanoparticles and copper nanoparticles (39-48).The F.benghalensis copper and selenium nanoparticle formation is known to adopt cellular mechanisms to counteract and inhibit microbial growth .In future these nanoparticles can be used for new drug designing and targeting and offer treatment for various bacterial disorders with minimal side effects. Also, these green synthesis and nanoparticle formulations were demonstrated to have biocompatibility, as well as strong potential for application in the fields of medicine and food.

CONFLICT OF INTEREST

There is no conflict of Interest

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REFERENCES

1. Baran MF. Synthesis, Characterization And Investigation Of Antimicrobial Activity Of Silver Nanoparticles From Cydonia Oblonga Leaf [Internet]. Vol. 17, Applied Ecology and Environmental Research. 2019. p. 2583–92. Available from: http://dx.doi.org/10.15666/aeer/1702_25832592
2. Timi D, Gopalakrishnan S, Maino M. Antimicrobial Application and Assessment of Green Synthesized Silver Nanoparticles Using Aqueous Leaf Extract of Ficus Copiosa [Internet]. Vol. 6, Journal of Biomedical Engineering and Medical Imaging. 2019. p. 06–15. Available from: http://dx.doi.org/10.14738/jbemi.63.7641
3. Rajeshkumar S, Veena P, Santhiyaa RV. Synthesis and Characterization of Selenium Nanoparticles Using Natural Resources and Its Applications [Internet]. Exploring the Realms of Nature for Nanosynthesis. 2018. p. 63–79. Available from: http://dx.doi.org/10.1007/978-3-319-99570-0_4
4. Yasmin A, Ramesh K, Rajeshkumar S. Optimization and stabilization of gold nanoparticles by using herbal plant extract with microwave heating. Nano Converg [Internet]. 2014 Apr 11;1(1):12. Available from: http://dx.doi.org/10.1186/s40580-014-0012-8
5. Riaz N. a-Glucosidase Inhibitory Constituents from Ficus bengalensis [Internet]. Vol. 5, Pakistan Journal of Chemistry. 2015. p. 42–8. Available from: http://dx.doi.org/10.15228/2015.v05.i01.p07201506081206
6. Mahima K, Parthiban S, Sathishkumar R. Phytochemical analysis in economically important Ficus Benghalensis L. and Ficus Krishnae C.DC. using GC-MS [Internet]. Vol. 10, International Journal of pharma and Bio Sciences. 2019. Available from: http://dx.doi.org/10.22376/ijpbs.2019.10.4.p5-13
7. Patel P, Patel M, Patel M. Effects of ethanol extract of ficus bengalensis (bark) on inflammatory bowel disease [Internet]. Vol. 42, Indian Journal of Pharmacology. 2010. p. 214. Available from: http://dx.doi.org/10.4103/0253-7613.68420
8. Baheti JR, Navale SD. Investigation of anti-ulcer activity of Ficus bengalensis Linn bark in laboratory animals [Internet]. Vol. 79, Planta Medica. 2013. Available from: http://dx.doi.org/10.1055/s-0033-1351950
9. Francis T, Rajeshkumar S, Roy A, Lakshmi T. Anti-inflammatory

and Cytotoxic Effect of Arrow Root Mediated Selenium Nanoparticles [Internet]. Vol. 12, Pharmacognosy Journal. 2020. p. 1363–7. Available from: http://dx.doi.org/10.5530/pj.2020.12.188

10. Website [Internet]. [cited 2021 May 21]. Available from: Menon, Soumya, Shrudhi Devi KS, R. Santhiya, S. Rajeshkumar, and Venkat Kumar. "Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism." Colloids and Surfaces B: Biointerfaces 170 (2018): 280-292.https://doi.org/10.1016/j.colsurfb.2018.06.006.
11. Rajeshkumar S, Menon S, Venkat Kumar S, Tambuwala MM, Bakshi HA, Mehta M, et al. Antibacterial and antioxidant potential of biosynthesized copper nanoparticles mediated through Cissus arnotiana plant extract [Internet]. Vol. 197, Journal of Photochemistry and Photobiology B: Biology. 2019. p. 111531. Available from: http://dx.doi.org/10.1016/j.jphotobiol.2019.111531
12. Rajeshkumar S, Rinitha G. Nanostructural characterization of antimicrobial and antioxidant copper nanoparticles synthesized using novel Persea americana seeds [Internet]. Vol. 3, OpenNano. 2018. p. 18–27. Available from: http://dx.doi.org/10.1016/j.onano.2018.03.001
13. Rajeshkumar S, Kumar SV, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles usingMangifera indica leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. Enzyme Microb Technol [Internet]. 2018 Oct;117:91–5. Available from: http://dx.doi.org/10.1016/j.enzmictec.2018.06.009
14. Nandhini NT, Rajeshkumar S, Mythili S. The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation. Biocatal Agric Biotechnol [Internet]. 2019 May 1;19:101138. Available from: https://www.sciencedirect.com/science/article/pii/S1878818118308235
15. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res [Internet]. 2020 Mar 1;27(8):8166–75. Available from: https://doi.org/10.1007/s11356-019-07511-x
16. Gomathi M, Prakasam A, Rajkumar PV, Rajeshkumar S, Chandrasekaran R, Anbarasan PM. Green synthesis of silver nanoparticles using Gymnema sylvestre leaf extract and evaluation of its antibacterial activity [Internet]. Vol. 32, South African Journal of Chemical Engineering. 2020. p. 1–4. Available from: http://dx.doi.org/10.1016/j.sajce.2019.11.005
17. Rajasekaran S, Damodharan D, Gopal K, Rajesh Kumar B, De Poures MV. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. Fuel [Internet]. 2020 Oct 1;277:118166. Available from: https://www.sciencedirect.com/science/article/pii/S0016236120311625
18. Santhoshkumar J, Sowmya B, Venkat Kumar S, Rajeshkumar S. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of Passiflora caerulea. S Afr J Chem Eng [Internet]. 2019 Jul;29:17–23. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1026918519300253
19. Raj R K, D E, S R. ß-Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. J Biomed Mater Res A [Internet]. 2020 Sep;108(9):1899–908. Available from: http://dx.doi.org/10.1002/jbm.a.36953
20. Saravanan M, Arokiyaraj S, Lakshmi T, Pugazhendhi A. Synthesis of silver nanoparticles from Phenerochaete chrysosporium (MTCC-787) and their antibacterial activity against human pathogenic bacteria. Microb Pathog [Internet]. 2018 Apr;117:68–72. Available from: http://dx.doi.org/10.1016/j.micpath.2018.02.008
21. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species–mediated cytotoxicity in HepG2 cells. Hum Exp Toxicol [Internet]. 2019 Jun 1;38(6):694–702. Available from: https://doi.org/10.1177/0960327119839173
22. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. Hepatobiliary Pancreat Dis Int [Internet]. 2018 Jun;17(3):192–7. Available from: http://dx.doi.org/10.1016/j.hbpd.2018.04.003
23. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. Arab J Gastroenterol [Internet]. 2018 Jun;19(2):56–64. Available from: http://dx.doi.org/10.1016/j.ajg.2018.03.002
24. Gomathi AC, Xavier Rajarathinam SR, Mohammed Sadiq A, Rajeshkumar S. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of Tamarindus indica on MCF-7 human breast cancer cell line. J Drug Deliv Sci Technol [Internet]. 2020 Feb 1;55:101376. Available from: https://www.sciencedirect.com/science/article/pii/S1773224719313693
25. Dua K, Wadhwa R, Singhvi G, Rapalli V, Shukla SD, Shastri MD, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res [Internet]. 2019 Sep;80(6):714–30. Available from: http://dx.doi.org/10.1002/ddr.21571
26. 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
27. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. Arch Oral Biol [Internet]. 2021 Feb;122:105030. Available from: http://dx.doi.org/10.1016/j.archoralbio.2020.105030
28. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry? Oral Surg Oral Med Oral Pathol Oral Radiol [Internet]. 2021 Jul;132(1):118–9. Available from: http://dx.doi.org/10.1016/j.oooo.2021.01.025
29. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. J Oral Pathol Med [Internet]. 2019 Feb;48(2):115–21. Available from: http://dx.doi.org/10.1111/jop.12806
30. Duraisamy R, Krishnan CS, Ramasubramanian H, Sampathkumar J, Mariappan S, Navarasampatti Sivaprakasam A. Compatibility of Nonoriginal Abutments With Implants: Evaluation of Microgap at the Implant-Abutment Interface, With Original and Nonoriginal Abutments. Implant Dent [Internet]. 2019 Jun;28(3):289–95. Available from: http://dx.doi.org/10.1097/ID.0000000000000885
31. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. Aquaculture [Internet]. 2019 May 30;507:1–6. Available

from: https://www.sciencedirect.com/science/article/pii/S004484861 8328072

32. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther [Internet]. 2021 Mar 18;12(1):192. Available from: http://dx.doi.org/10.1186/s13287-021-02265-1
33. Website [Internet]. [cited 2021 May 21]. Available from: Rajeshkumar S, Ganesh L, Santhoshkumar J. Selenium Nanoparticles as Therapeutic Agents in Neurodegenerative Diseases [Internet]. Nanobiotechnology in Neurodegenerative Diseases. 2019. p. 209–24. Available from: http://dx.doi.org/10.1007/978-3-030-30930-5_8
34. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artif Cells Nanomed Biotechnol [Internet]. 2020 Dec;48(1):1153–8. Available from: http://dx.doi.org/10.1080/21691401.2020.1817053
35. B S, Sadhvi B, Rajeshkumar S, Roy A, Lakshmi T. Copper oxide nanoparticles synthesis and characterization using UV-vis spectrophotometer and TEM [Internet]. Vol. 10, International Journal of Research in Pharmaceutical Sciences. 2019. p. 2845–8. Available from: http://dx.doi.org/10.26452/ijrps.v10i4.1562
36. Rajeshkumar S, Tharani M, Jeevitha M, Santhoshkumar J. Anticariogenic Activity of Fresh Aloe Vera Gel Mediated Copper Oxide Nanoparticles [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3664. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04158.5
37. Ahmad S. Phytochemical composition and pharmacological prospectus of Ficus bengalensis Linn. (Moraceae)- A review [Internet]. Vol. 5, Journal of Medicinal Plants Research. 2011. Available from: http://dx.doi.org/10.5897/jmpr11.455
38. Menon S, Agarwal H, Venkat Kumar S, Rajeshkumar S. Biomemetic synthesis of selenium nanoparticles and its biomedical applications [Internet]. Green Synthesis, Characterization and Applications of Nanoparticles. 2019. p. 165–97. Available from: http://dx.doi.org/10.1016/b978-0-08-102579-6.00008-3
39. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative Evaluation of Remineralizing Potential of a Paste Containing Bioactive Glass and a Topical Cream Containing Casein Phosphopeptide-Amorphous Calcium Phosphate: An in Vitro Study. Pesqui Bras Odontopediatria Clin Integr [Internet]. 2019 Mar 12 [cited 2021 Sep 15];19(0):4668. Available from: http://revista.uepb.edu.br/index.php/pboci/article/view/4668
40. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol [Internet]. 2017 Mar [cited 2021 Sep 15];44(3). Available from: https://pubmed.ncbi.nlm.nih.gov/28004401/
41. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated Growth Factors as an Ingenious Biomaterial in Regeneration of Bony Defects after Periapical Surgery: A Report of Two Cases. Case Rep Dent [Internet]. 2019 Jan 22 [cited 2021 Sep 15];2019. Available from: https://pubmed.ncbi.nlm.nih.gov/30805222/
42. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev [Internet]. 2014 Jun 30 [cited 2021 Sep 15];8(1). Available from: https://pubmed.ncbi.nlm.nih.gov/25992232/
43. Menon S, Ks SD, R S, S R, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces [Internet]. 2018 Oct 1 [cited 2021 Sep 15];170. Available from: https://pubmed.ncbi.nlm.nih.gov/29936381/
44. Samuel SR, Acharya S, Rao JC. School Interventions-based Prevention of Early-Childhood Caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent [Internet]. 2020 Jan [cited 2021 Sep 15];80(1). Available from: https://pubmed.ncbi.nlm.nih.gov/31710096/
45. Praveen K, Narayanan V, Muthusekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. Br J Oral Maxillofac Surg [Internet]. 2001 Apr [cited 2021 Sep 15];39(2). Available from: https://pubmed.ncbi.nlm.nih.gov/11286449/
46. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J [Internet]. 2011 Jun [cited 2021 Sep 15];44(6). Available from: https://pubmed.ncbi.nlm.nih.gov/21255047/
47. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact [Internet]. 2019 Aug 1 [cited 2021 Sep 15];308:206–15. Available from: http://dx.doi.org/10.1016/j.cbi.2019.05.028
48. Kumar MS, Vamsi G, Sripriya R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J Periodontol [Internet]. 2006 Nov;77(11):1803–8. Available from: http://dx.doi.org/10.1902/jop.2006.050293