

Controlling oral pathogens using *Ficus benghalensis* mediated silver nanoparticles

ABSTRACT

Aim: To find antimicrobial property of *Ficus benghalensis* mediated silver nanoparticles (AgNPs)

Introduction: Nanoparticles have been studied in recent years because of certain properties like physical, chemical, electronic, thermal, magnetic, optical, dielectric and biological. This study was conducted in order to evaluate antimicrobial properties of *F. benghalensis* mediated AgNPs.

Materials and methods: In this study, extract of *F. benghalensis* was used to synthesize silver nanoparticles characterized using UV- visible spectrophotometer, Fresh *F. benghalensis* mediated AgNPs show excellent antimicrobial activity against oral pathogens, *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*.

Results: *F. benghalensis* mediated AgNPs showed antimicrobial activity against gram positive *S. aureus* with a zone of inhibition of 16.22 ± 0.31 mm at the concentration of 100 μ l. The zone of inhibition against *S. mutans* was 12.14 ± 0.21 mm followed by zone of inhibition against *E. faecalis* was 12.12 ± 0.2 mm and antimicrobial activity against *C. albicans* showed a zone of inhibition of 14.02 ± 0.24 mm at the concentration of 100 μ l. The zone of inhibition of the nanoparticles was shown to be increased with increase in concentration against all the pathogens and the maximum inhibition was shown against *S. aureus*.

Conclusion: Overall, antimicrobial activity was seen to be increased with increase in concentration. Thus there was potent antimicrobial activity in *F. benghalensis* mediated AgNPs which could be beneficial when applied in treatment of infectious oral diseases in future.

Keywords: Antimicrobial activity; *Ficus benghalensis*; innovative technology; nanoparticles; oral pathogens

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1.INTRODUCTION

Nanoparticles size range from 10- 1000 nm so they are known as particulate dispersion or solid particles. The drugs are now dissolved, trapped and encapsulated or in other words bound to a matrix of nanoparticles. The medicinal plant from centuries used as an alternative remedy for treating human disease. This is due to various active constituents of therapeutic value. Microbial resistance development against antibiotics made the research to investigate various alternative sources to treat the resistance strain. 80% of the population of the world realized that medicine derived from the plant was severe as a first line of defense in maintenance of health and combating many diseases (1). *F. benghalensis* is commonly known as Banyan tree or vats or vada tree in Ayurveda. Silver has been used for centuries as an antimicrobial. In order to fight infections, disease and stop the spoilage, it is known that silver-based ion compounds are more toxic to both Gram-negative and Gram-positive microorganisms (2). The Barks, stems, leaves, flowers and fruits of plants, various animal tissues from which natural antimicrobials are derived. Optimum levels of total phenolic and flavonoid compounds in *F. benghalensis* aerial root are found to be present in 70 mg of extract (3). Several metal nanoparticles, for example magnesium, gold, Iron, copper, silver, zinc have evolved. silver nanoparticles (AgNPs) have been established to be simplest because of the need sensible antimicrobial activity against various microorganisms which is in the form of nanoparticles (NPs) this can be used as more effective bactericidal materials because of their enhanced reactivity, that result from their high surface/volume ratio (4). Particularly, AgNPs are known to show strong biocidal effects on various bacterial species that include even multidrug resistant bacteria (5). Plant based synthesis of AgNPs found to be very simple, rapid, dependable,

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eco-friendly and non-toxic. The synthesis of metal nanoparticles using plant extracts produces an advantage over other types of biological synthesis methods which have difficulties maintaining microbial cultures. After the synthesis of AgNPs, characterization of the AgNPs is important for investigating their characteristic features like size, shape, surface area, morphology, solubility and aggregation etc. (6). The physical and chemical properties of nanoparticles may need a considerable influence on their biological properties (7). Characterization of AgNPs is important before evaluating their toxicity(8). Different analytical techniques had been employed for the characterization of the nanoparticles, like Transmission electron microscope (TEM), Scanning electron microscopy (SEM), Ultraviolet visible spectroscopy (UV-vis), X-ray diffractometry (XRD), Atomic force microscopy (AFM), Energy Dispersive Analysis (EDAX) and Fourier transform infrared spectroscopy (FT-IR) etc (9). The antibacterial effects of AgNPs against bacterial cells are always complicated (10). The direct morphological analysis by TEM or SEM gives structural modification of the bacterial cell (11). This would give us useful information and good understanding on the bactericidal activity of AgNPs against bacterial cells. And the proper antibacterial mechanism of the AgNPs is still in a mysterious situation. Therefore, the antibacterial activities and its mechanisms of AgNPs against several bacteria were reported in the past. The present study is based on various plant based methods for AgNPs synthesis, characterizations, and predicted antibacterial activity against various bacteria. Before we have done various studies on biological synthesized different nanoparticles. (12–24),(25–29) (30) (31). The present study is to evaluate the antimicrobial activity of *F. benghalensis* mediated AgNPs against oral pathogens.

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2. MATERIALS AND METHODS

2.1 Preparation of plant extract

1 mg of *F. benghalensis* was collected from the local field and was dried in the shade region. After getting dried they were crushed & powdered and mixed with 100 mL of distilled water in a conical flask. The solution was labelled and boiled in 60-70 degree Celsius in the heating mantle for 10-15 minutes. The heated solution was taken out where there was an appearance of small bubbles. After the heating process, the solution was filtered using Whatman no.1 filter paper (Figure 1).

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Fig. 1. Synthesis of AgNPs from *F. benghalensis* extract

2.2 Synthesis of nanoparticles

20 milli molar (0.574g) of Ag was dissolved in 100 mL of distilled water. 40 mL of filtered *F. benghalensis* extract was mixed with 60 mL of Silver nitrate. The flask containing the mixture was incubated in an orbital shaker and observed for colour change at various periods of incubation time. The synthesized nanoparticles were optically measured using a double beam UV-vis spectrometer. AgNPs synthesized from *F. benghalensis* was tested for antimicrobial activity by agar well diffusion method against *S.aureus*, *S.mutans*, *E.faecalis*, *C. albicans*. 1 millimolar of silver mixed with double distilled water. The plant extract of *F. benghalensis* was

added with the metal solution and was made into a 100 mL solution. The colour change has been observed visually and photographs were recorded (Figure 2). The solution is kept in a magnetic stirrer/orbital shaker for nanoparticle synthesis.

2.3 Antimicrobial activity

The agar well diffusion method was used to determine the antimicrobial activity of silver nanoparticles. Different concentrations of silver were tested against *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis*, and *Candida albicans*. The freshly prepared bacterial suspension was made to disperse on the Muller Hinton agar plates. Different concentrations of nanoparticles like 25 μ l, 50 μ l, 100 μ l and standard (Ab) were incorporated into each of the well and plates were incubated at 37°C for 24 h. The Zone of inhibition for different concentrations of *F. benghalensis* mediated AgNPs were measured.

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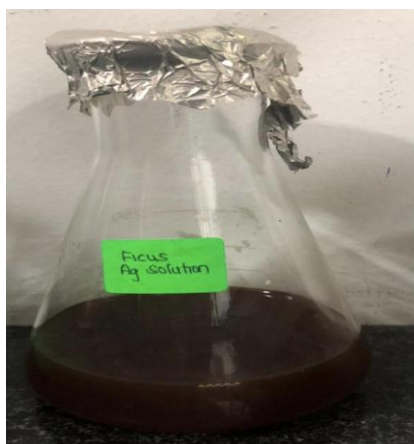


Fig. 2. Synthesis of AgNps visually identified by color change

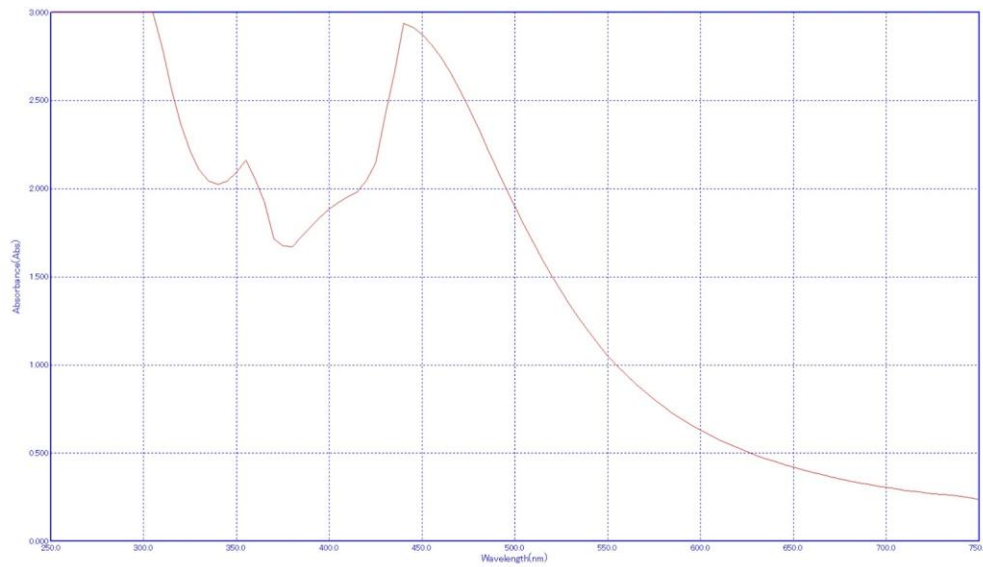


Fig. 3. Spectroscopic analyses of *F. benghalensis* mediated AgNPs mouthwash recorded as function of time

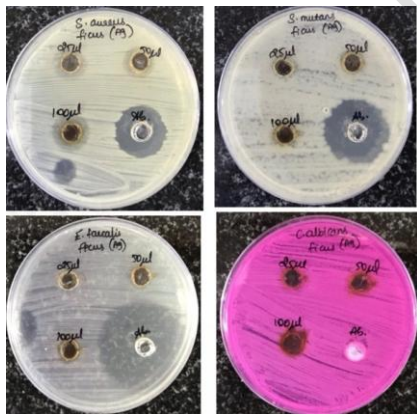


Fig. 4. Antimicrobial activity of *F. benghalensis* mediated AgNPs against oral pathogens

3. RESULTS & DISCUSSION

As observed visually at various periods of incubation, the colour of the solution transformed from colorless to dark brown which indicated formation of AgNPs (Figure 2). UV- vis spectrophotometer revealed surface

plasmon resonance peak positioned at 450 nm (Figure 3). The antimicrobial activity of *F. benghalensis* mediated AgNPs was assayed by well diffusion method (Figure 4). Table 1 shows the inhibition of bacterial growth in various concentrations of *F. benghalensis* mediated AgNPs against *S. aureus*, *S. mutans*, *E. faecalis*, *C. albicans*. As the concentration increased gradually the antimicrobial activity of *F. benghalensis* assisted AgNPs also increased (Figure 5). The obtained results were comparable with that of the standard antimicrobial agents. The *F. benghalensis* mediated AgNPs showed antimicrobial activity against gram positive *S. aureus* with maximum zone of inhibition of 16.22 ± 0.31 mm at the concentration of 100 μ l. The zone of inhibition against *S. mutans* was 12.14 ± 0.21 mm followed by zone of inhibition against *E. faecalis* was 12.12 ± 0.2 mm and antimicrobial activity against *C. albicans* showed a zone of inhibition of 14.02 ± 0.24 mm at the concentration of 100 μ l. The zone of inhibition of the nanoparticles was shown to be increased with increase in concentration against all the pathogens and the maximum inhibition was shown against *S. aureus*.

The results obtained were comparable to similar biosynthesized nanoparticles. From the dried ginger copper nanoparticles were synthesised and these were characterised by UV-vis spectroscopy and exhibited potent antimicrobial activity against common oral pathogens (32). In their study, the approach on green synthesis of iron nanoparticles by using dried ginger can provide pharmacological evidence of antioxidant activity (33). Previous studies have shown that adding metal nanoparticles to various materials will improve the antimicrobial activity (11,34). Initially the iron nanoparticles were identified by stable dark brown colour and the surface plasmon resonance was at the peak positioned at 370 nm. Their study supported that dried ginger Zingiber are important sources for potent biologic activities and thus these plant-based nanoparticles may be essential in the treatment of various pathologic conditions (35, 36). The morphology of copper nanoparticles synthesized from the extract of *Eclipta prostrata* leaves as analysed by HRTEM shows spherical and agglomerated particles ranging from 28 to 45 nm (37). Silver nanoparticles has shown to have higher antifungal activity against *C. albicans* and *Candida tropicalis* which will represent an alternative for fungal infection treatment (38). Further research on antimicrobial studies in vivo assigns possible applications in the dental field which will be efficient to treat oral infectious diseases.

Table1: Antimicrobial activity of AgNPs against oral pathogens

Pathogens	25 μ l	50 μ l	100 μ l	Ab
<i>S. aureus</i>	13.02 ± 0.34	15.41 ± 0.33	16.22 ± 0.31	21.02 ± 0.23
<i>S. mutans</i>	9.24 ± 0.12	9.62 ± 0.24	12.14 ± 0.21	27.14 ± 0.31
<i>E. faecalis</i>	9.42 ± 0.22	9.02 ± 0.22	12.12 ± 0.21	14.02 ± 0.24
<i>C. albicans</i>	9.06 ± 0.14	9.04 ± 0.42	14.02 ± 0.24	12.04 ± 0.21

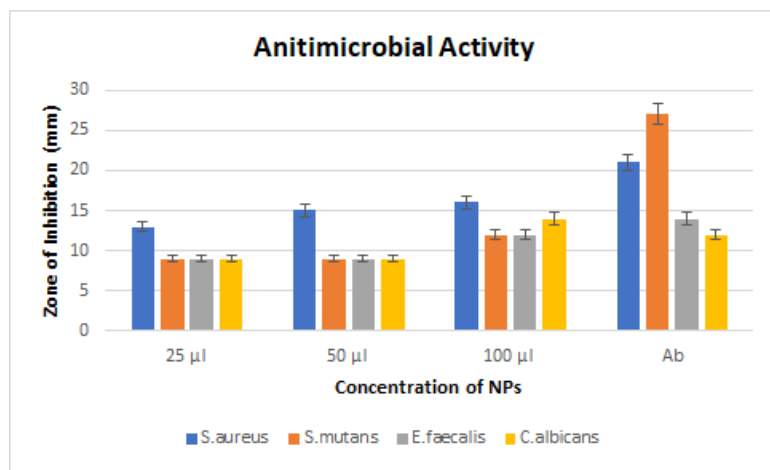


Fig. 5. Analysis of Antimicrobial activity of *F. benghalensis* mediated AgNPs against oral pathogens

4. CONCLUSION

The present study reported that silver nanoparticles can be synthesized in a simple and easy method using *F. benghalensis* extract. *F. benghalensis* mediated AgNPs showed excellent antimicrobial activity against oral pathogens. This study showed an increase in the zone of inhibition was seen while increasing the dosage. The *F. benghalensis* mediated AgNPs are potential candidates in biomedical applications with various benefits such as cost-effectiveness, less side effects and large scale commercial production.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used 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. Mohanraj VJ, Chen Y. Nanoparticles - A review [Internet]. Vol. 5, Tropical Journal of Pharmaceutical Research. 2007. Available from: <http://dx.doi.org/10.4314/tjpr.v5i1.14634>

2. Nadkarni KM. [Indian materia medica] ; Dr. K. M. Nadkarni's Indian materia medica : with Ayurvedic, Unani-Tibbi, Siddha, allopathic, homeopathic, naturopathic & home remedies, appendices & indexes. 1. Popular Prakashan; 1996. 1319 p.
3. Mondal A. Indian Mottled Freshwater Eel, *Anguilla bengalensis Bengalensis* (Gray, 1831), A Threatened Species of Indian Subcontinent- A Review [Internet]. Vol. 3, International Journal of Oceanography & Aquaculture. 2019. Available from: <http://dx.doi.org/10.23880/ijoac-16000162>
4. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles – A review on biomolecules involved, characterisation and antibacterial activity [Internet]. Vol. 273, Chemico-Biological Interactions. 2017. p. 219–27. Available from: <http://dx.doi.org/10.1016/j.cbi.2017.06.019>
5. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria [Internet]. Vol. 275, Journal of Colloid and Interface Science. 2004. p. 177–82. Available from: <http://dx.doi.org/10.1016/j.jcis.2004.02.012>
6. Jyoti K, Baunthiyal M, Singh A. Characterization of silver nanoparticles synthesized using *Urtica dioica* Linn. leaves and their synergistic effects with antibiotics [Internet]. Vol. 9, Journal of Radiation Research and Applied Sciences. 2016. p. 217–27. Available from: <http://dx.doi.org/10.1016/j.jrras.2015.10.002>
7. Tran QH, Nguyen VQ, Le A-T. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives [Internet]. Vol. 4, Advances in Natural Sciences: Nanoscience and Nanotechnology. 2013. p. 033001. Available from: <http://dx.doi.org/10.1088/2043-6262/4/3/033001>
8. Vogel U, Savolainen K, Wu Q, van Tongeren M, Brouwer D, Berges M. Handbook of Nanosafety: Measurement, Exposure and Toxicology. Elsevier; 2013. 376 p.
9. Sapsford KE, Tyner KM, Dair BJ, Deschamps JR, Medintz IL. Analyzing Nanomaterial Bioconjugates: A Review of Current and Emerging Purification and Characterization Techniques [Internet]. Vol. 83, Analytical Chemistry. 2011. p. 4453–88. Available from: <http://dx.doi.org/10.1021/ac200853a>
10. Manivasagan P, Venkatesan J, Senthilkumar K, Sivakumar K, Kim S-K. Biosynthesis, Antimicrobial and Cytotoxic Effect of Silver Nanoparticles Using a Novel *Nocardia* sp. MBRC-1 [Internet]. Vol. 2013, BioMed Research International. 2013. p. 1–9. Available from: <http://dx.doi.org/10.1155/2013/287638>
11. Hwang ET, Lee JH, Chae YJ, Kim YS, Kim BC, Sang B-I, et al. Analysis of the Toxic Mode of Action of Silver Nanoparticles Using Stress-Specific Bioluminescent Bacteria [Internet]. Vol. 4, Small. 2008. p. 746–50. Available from: <http://dx.doi.org/10.1002/sml.200700954>
12. 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.
13. 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.
14. 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.
15. 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.
16. Paramasivam A, Vijayashree Priyadharsini J. MitomiRs: new emerging microRNAs in mitochondrial

dysfunction and cardiovascular disease. *Hypertens Res.* 2020 Aug;43(8):851–3.

17. Jayaseelan VP, Arumugam P. Dissecting the theranostic potential of exosomes in autoimmune disorders. *Cell Mol Immunol.* 2019 Dec;16(12):935–6.
18. 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.
19. 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.
20. 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.
21. 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>
22. 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>
23. 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.
24. 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.
25. 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.
26. 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>
27. 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>
28. Sarode SC, Gondivkar S, Sarode GS, Gadbaile A, Yuwanati M. Hybrid oral potentially malignant disorder: A neglected fact in oral submucous fibrosis. *Oral Oncol.* 2021 Jun 16;105390.
29. Kavarthapu A, Gurumoorthy K. Linking chronic periodontitis and oral cancer: A review. *Oral Oncol.* 2021 Jun 14;105375.
30. 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>
31. 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.

32. Shankar S, Rhim J-W. Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles [Internet]. Vol. 132, Materials Letters. 2014. p. 307–11. Available from: <http://dx.doi.org/10.1016/j.matlet.2014.06.014>
33. Jackson K, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Nadu T, et al. Cytotoxic potentials of silibinin assisted silver nanoparticles on human colorectal HT-29 cancer cells [Internet]. Vol. 16, Bioinformation. 2020. p. 817–27. Available from: <http://dx.doi.org/10.6026/97320630016817>
34. Guerreiro-Tanomaru JM, Trindade-Junior A, Costa BC, da Silva GF, Drullis Cifali L, Basso Bernardi MI, et al. Effect of zirconium oxide and zinc oxide nanoparticles on physicochemical properties and antibiofilm activity of a calcium silicate-based material. ScientificWorldJournal. 2014 Nov 6;2014:975213.
35. Devi RS, Shruthi Devi R, Jeevitha M, Preetha S, Rajeshkumar S. Free Radical Scavenging Activity of Copper Nanoparticles Synthesized from Dried Ginger [Internet]. Journal of Pharmaceutical Research International. 2020. p. 1–7. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i1930703>
36. Begum A, Jeevitha M, Preetha S, Rajeshkumar S. Cytotoxicity of Iron Nanoparticles Synthesized Using Dried Ginger [Internet]. Journal of Pharmaceutical Research International. 2020. p. 112–8. Available from: <http://dx.doi.org/10.9734/jpri/2020/v32i2530829>
37. Chung I, Rahuman AA, Marimuthu S, Kirthi AV, Anbarasan K, Padmini P, et al. Green synthesis of copper nanoparticles using Eclipta prostrata leaves extract and their antioxidant and cytotoxic activities [Internet]. Experimental and Therapeutic Medicine. 2017. Available from: <http://dx.doi.org/10.3892/etm.2017.4466>
38. Zhou L, Zhao X, Li M, Lu Y, Ai C, Jiang C, et al. Antifungal activity of silver nanoparticles synthesized by iturin against *Candida albicans* in vitro and in vivo. Appl Microbiol Biotechnol. 2021 May;105(9):3759–70.