Preparation of mouthwash using Ficus benghalensis assisted silver nanoparticles and a comparative analysis of its antimicrobial activity

ABSTRACT

Aim: The aim of this study is to prepare a mouthwash using Ficus benghalensis mediated silver nanoparticles (Ag NPs) and analysing its antimicrobial activity against oral pathogens.

Introduction: Nanotechnology deals with the particles which are less than 100 nm and have important roles in medicines, industries, drug gene delivery. Different parts of the F. benghalensis show medicinal properties. Leaves are used for ulcers, aerial roots are used in gonorrhoea, seeds and fruits are used for dysentery, diarrhoea and diabetes. F. benghalensis have been reported to have immunomodulatory, antibacterial activity and used to treat toothache.

Materials and method: In the present study, the mouthwash was prepared using F. benghalensis mediated Ag Nps and its antimicrobial activity against oral pathogens was analysed. Different concentrations of the synthesized F. benghalensis mediated AgNPs mouthwash were tested against Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis. and Candida albicans using agar well diffusion method. The antimicrobial effect is determined by the zone of inhibition.

Results: It has been observed that the antimicrobial effect of the prepared Ag NPs was almost similar against all the organisms used in the study with a maximum zone of inhibition against Staphylococcus aureus. It has also been observed that the antimicrobial activity of the AgNPs increased with increasing concentration.

Conclusion: As the synthesised F. benghalensis assisted silver nanoparticle mouthwash showed potent antimicrobial activity in vitro against the oral microbes: S. aureus, S. mutans, E. faecalis, C. albicans, in vivo studies are needed further to evaluate the antimicrobial effect.

Keywords: Antimicrobial activity; Ficus benghalensis; innovative technology; mouthwash; silver nanoparticles.

1. INTRODUCTION

Nanotechnology deals with particles which are less than 100 nm and have important roles in medicines, industries and drug gene delivery systems. Nanomaterials are particles having nanoscale measurement and they are estimated as small particles with upgraded synergist reactivity, warm conductivity, non-straight optical execution and compound relentlessness attributable to its enormous surface territory to its volume proportion[1,2]. Nanoparticles have begun being considered as nano antibiotics in light of their antimicrobial activities[3]. In today’s world, nanoparticles have been incorporated into different modern, wellbeing, nourishment, feed, space, compound, and beauty care products which requires a green and condition cordial way to deal with their synthesis[1]. The size of the nanoparticles is similar to most of the biological molecules and structures therefore the nanoparticles can be used for both in vitro and in vivo biomedical research and applications [4,5].

The nanoparticles were made from different materials like metal oxides and the most commonly used metals are silver and gold [6]. Silver nanoparticles (AgNPs) are commonly employed as metallic nanoparticles in health care delivery systems due to their unique physicochemical and biological properties and are used as antibacterial, antifungal, antiviral and anti-cancer agents. Since the silver is known for its antimicrobial activity, the AgNPs can be used against infection[6]. The antimicrobial activity of the synthesized silver nanoparticle is due to the silver cations released from them, pertaining to the
changes in the membrane structure of microbes which leads to the increased membrane permeability of the bacteria and finally cell death[7,8]. The AgNPs were synthesized from many plant varieties. Silver salts are well known for their antimicrobial activity since antiquity. The use of Ag NPs is increasing because of their unique properties like high electrical conductivity, chemical stability, catalytic and antimicrobial activity [9]. The size, shape and surface chemistry of AgNPs have an effect on their physical, chemical, optical and electrical properties and Small sized Ag NPs are of great use because of their potential applications in emerging biomedical, antibacterial, antifungal and antiviral areas [1,10].

These chemicals have potential toxic effects on the environment and health. Hence, green methods for synthesis of nanoparticles is preferred. Use of plants is still being explored and is considered to be a promising treatment option for various diseases. In the present study the AgNPs were synthesized from *Ficus benghalensis*. Different parts of the *F. benghalensis* how medicinal properties[8,11]. Leaves are used for ulcers, aerial roots are used in gonorrhea, seeds and fruits are used for dysentery, diarrhea and diabetes[12]. The extract of *F. benghalensis* has been reported to have more immunomodulatory, antibacterial and hair growth promoting activity [13,14]. There are many secondary metabolites present in *F. benghalensis* such as alkaloids, phenols, saponins, proteins, tannins, flavonoids. These phytochemicals can be used for many treatments such as ulcers, diarrhoea, dysentery, skin diseases, piles, gonorrhea [15].

Dental caries is a major problem mainly consisting of bacterial plaque and oral microbes, which is caused by the bacteria such as *Streptococcus mutans, Lactococcus, Staphylococcus, Enterobacter* species. Many Plants are able to produce varieties of compounds against pathogens [16,17]. Such that the roots of Ficus species can be used to treat the toothache. The herbal products used in dentistry are effective to treat dental infection. The herbal mouthwashes have lesser side effects than the conventional mouth washes. Plants products can be used as mouthwashes because they are eco-friendly, economical and effective in maintaining oral hygiene. Nano scale compound is used for the preparation materials which are used in dentistry. This method is simple and less expensive [18]. Our team has extensive knowledge and research experience that has translated into high quality publications.[19–31],[32–36] [37] [38] The aim of this study is to prepare a mouthwash using *F. benghalensis* mediated AgNPs and analysing its antimicrobial activity against oral pathogens.

2. MATERIALS AND METHODS

2.1 Preparation of plant extract

1 g of *F. benghalensis* was mixed with 100 mL of distilled water and boiled at 60-70 degree celsius in the heating mantle for 10-15 minutes. The heated solution was filtered using Whatman no.1 filter paper. 20 milli molar (0.574g) of Silver nitrate was dissolved in 60 mL of distilled water. 40 mL of filtered *F. benghalensis* extract is mixed with 60 mL of metal solution and was made into 100 mL solution.

2.2 Synthesis of nanoparticles

The solution was kept in a magnetic stirrer for nanoparticle synthesis. The colour change was observed visually and photographs were recorded in particular interval. The solution was centrifuged using lark refrigerated centrifuge. The solution was centrifuged at 8000 rpm for 10 minutes and the pellet was collected and washed with distilled water twice. The final purified pellet was collected and dried at 60°C for 24 hours. The final product was stored in an airtight eppendorf tube.

2.3 Conformation of AgNPs

The AgNPs synthesis was confirmed by using UV-vis-spectroscopy. 3mL of the solution was taken in a cuvette and scanned in a double beam UV-vis-spectrophotometer from 300 nm to 700 nm wavelength. The results were recorded for confirmation of AgNPs synthesis.

2.4 Preparation of mouthwash

The mouthwash was prepared using silver nanoparticles, ethanol, distilled water, sucrose, sodium benzoate, clove oil, Sodium dodecyl sulphate. Silver nanoparticles are the main constituent and
ethanol acts as a solvent to solubilize the ingredients. Sodium benzoate acts as a preservative and clove oil acts as a flavoring agent.

2.5 Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash against oral pathogens:
The agar well diffusion method was used to determine the antimicrobial activity of the mouthwash. Different concentrations of mouthwash were tested against *Staphylococcus aureus*, *Streptococcus mutans* (gram positive), *Enterococcus faecalis* and *Candida albicans*. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. Different concentrations of nanoparticles (25 μl, 50 μl, and 100 μl) were incorporated into the wells and the plates were incubated at 37°C for 24 hours. The antibiotics were used as positive control and zone of inhibition was recorded in each plate.

3. RESULTS AND DISCUSSION
3.1 Visual observation
It is known that Ag NPs exhibit dark brown colour depending on the intensity and size of nanoparticles. This colour arises due to the excitation of the surface plasmon resonance (SPR) of the silver nanoparticles. When *F. benghalensis* was added to the Silver nitrate solution, the colour of the solution transformed from colorless to dark brown. This colour change has been shown in figure 2. This colour change indicates the formation of AgNPs which is further confirmed by UV-vis spectrophotometry.

![Fig. 1. *F. benghalensis* plant extract](image)

![Fig. 2. Colour change exhibited by the prepared *F. benghalensis* assisted silver nanoparticles](image)

3.2 UV-vis spectroscopy
UV-vis spectroscopy is a significant technique to show the formation and stability of Ag NPs in an aqueous solution. The UV-vis spectra were recorded for the prepared *F. benghalensis* silver nitrate solution (Figure 3). It has been observed from the spectra that the AgNPs SPR peak occurs at 450 nm with high absorbance, which is specific for silver nanoparticles. The peak represents the purity of silver nanoparticles. This confirms the formation of *F. benghalensis* mediated silver nanoparticles.

![UV-vis spectrum of AgNPs](image)

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

### 3.3 Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash

The prepared *F. benghalensis* mediated AgNPs mouthwash were studied for antimicrobial activity against the following oral pathogens: *S. aureus, S. mutans, E. faecalis* and *C. albicans*. The diameter of the zone of inhibition (ZOI) in millimeters around each well in different concentrations levels of *F. benghalensis* mediated AgNPs against the bacterial species were determined (Figure 4). The results have been tabulated (Table 1) and Graph has been plotted (Figure 5).
Fig. 4. Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash against oral pathogens, *S.*mutans, *C.*albicans, *E.*faecalis, *S.*aureus

Table 1: Zone of inhibition of *F. benghalensis* mediated AgNPs mouthwash against oral pathogens, *S.*mutans, *C.*albicans, *E.*faecalis, *S.*aureus at different concentrations compared to standard antimicrobial agent.

| Oral pathogens | 25 µl | 50 µl | 100 µl | AB    |
|----------------|-------|-------|--------|-------|
| *S.* mutans    | 9 mm  | 12 mm | 21 mm  | 29 mm |
| *E.* faecalis  | 12 mm | 14 mm | 17 mm  | 33 mm |
| *S.* aureus    | 9 mm  | 14 mm | 20 mm  | 27 mm |
| *C.* albicans  | 9 mm  | 14 mm | 21 mm  | 12 mm |
Fig. 5. Antimicrobial activity of *F. benghalensis* mediated AgNPs mouthwash compared to standard antimicrobial drug (AB)

The nanoparticles obtained in the present study are prominent and possess potential antagonism against the oral pathogens. It has been observed that the mean zone of inhibition against bacterial pathogens was found to increase as the concentration of mouthwash increased, however the maximum was found for the standard antibiotic.

The zone of inhibition of the prepared AgNPs mouthwash against *Candida albicans* at 25 μl, 50 μl and 100 μl was found to be 9 mm, 14 mm and 21 mm respectively indicating excellent antifungal activity against *C. albicans* at 100 μl concentration which was better than the standard which exhibited zone of inhibition of only 12 mm. Zone of inhibition for *S. aureus* was found to be 9 mm, 14 mm and 20 mm. For *S. mutans*, the zone of inhibition were 10 mm, 11 mm and 17 mm respectively. For *E. faecalis*, it was 9 mm, 12 mm, 21 mm (Table 1). The antimicrobial activity of the synthesized AgNPs is due to the silver cations released from them, pertaining to the changes in the membrane structure of microbes, which lead to the increased membrane permeability of the bacteria and finally cell death [39].

Silver nanoparticles, due to their size, surface area and low toxicity have a significant role in nanotechnology [40,41]. The important factors in regard to the antimicrobial activity of silver are stability, adhesion and good dispersion of silver particles in organic matrix[42,43]. Silver in the form of nanoparticles has found acceptance in the field of nanotechnology, as their efficacy is primarily related to the fact that they reduce bacterial resistance. The current study was conducted to assess the antimicrobial activity of mouthwash incorporated with AgNPs against oral pathogens and showed good potential as an antimicrobial agent that maintains control of biofilm, preventing initial colonization of bacteria. They had potential antimicrobial activity of the synthesized silver nano-particles in mouthwash against gram positive bacteria, similar to various other studies[44–46] and in this current study has certain limitations, it was not tested against gram negative bacteria. Hence, further research should be conducted to gather evidence regarding [47] the potential of AgNPs and its antimicrobial activities.

4. CONCLUSION
From this study we conclude that the formation of AgNPs was observed by the colour change from colourless to dark brown and it was confirmed by UV-Vis spectroscopy. The synthesized *F. benghalensis* assisted silver nanoparticle mouthwash showed positive antimicrobial activity against the oral microbes and can be used to prevent the formation of microbial biofilm.

**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 use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

**REFERENCES**

1. Barma MD, Kannan SD, Indiran MA, Rajeshkumar S, Pradeep Kumar R. Antibacterial Activity of Mouthwash Incorporated with Silica Nanoparticles against *S. aureus*, *S. mutans*, *E. faecalis*: An in-vitro Study [Internet]. Journal of Pharmaceutical Research International. 2020. p. 25–33. Available from: http://dx.doi.org/10.9734/jpri/2020/v32i1630646

2. Ajitha P, Govindaraju L, Jenarthanan S, Subramanyam D. Antibacterial activity of various intracanal medicament against Enterococcus faecalis, Streptococcus mutans and Staphylococcus aureus: An In vitro study [Internet]. Vol. 13, Journal of Pharmacy And Bioallied Sciences. 2021. p. 157. Available from: http://dx.doi.org/10.4103/jpbs.jpbs_623_20

3. Badakar C, Gowtham A, Hugar S, Gokhale N, Davalbhakta R, Joshi R. Comparative evaluation of antibacterial efficacy of green coffee bean extract mouthwash and chlorhexidine mouthwash against Streptococcus mutans and Lactobacilli spp. – An in vitro study [Internet]. Vol. 13, Indian Journal of Health Sciences and Biomedical Research (KLEU). 2020. p. 147. Available from: http://dx.doi.org/10.4103/kleuhsj.kleuhsj_239_19

4. Pranati T, Anitha R, Rajeshkumar S, Lakshmi T. Preparation of Ag NPsusing Nutmeg oleoresin and its Antimicrobial activity against Oral pathogens [Internet]. Vol. 12, Research Journal of Pharmacy and Technology. 2019. p. 2799. Available from: http://dx.doi.org/10.5958/0974-360x.2019.00471.2

5. Shathviha PC, Ezhilarasan D, Rajeshkumar S, Selvaraj J. β-sitosterol Mediated Ag NPsInduce Cytotoxicity in Human Colon Cancer HT-29 Cells. 2020 Nov 7 [cited 2021 Aug 21];13(1). Available from: https://www.researchgate.net/publication/347575914_b-sitosterol_Mediated_Silver_Nanoparticles_Induce_Cytotoxicity_in_Human_Colon_Cancer_HT-29_Cells

6. Ganta SSL, Jeevitha M, Preetha S, Rajeshkumar S. Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles [Internet]. Journal of Pharmaceutical Research International. 2020. p. 14–9. Available from: http://dx.doi.org/10.9734/jpri/2020/v32i2830866

7. Erjaae H, Rajaian H, Nazifi S. Synthesis and characterization of novel Ag NPsusing Chamaemelum nobile extract for antibacterial application [Internet]. Vol. 8, Advances in Natural Sciences: Nanoscience and Nanotechnology. 2017. p. 025004. Available from: http://dx.doi.org/10.1088/2043-6254/aa690b
8. Mahima K, Parthiban S, Sathishkumar R. Phytochemical analysis in economically important *F. benghalensis* L. and *Ficus Krishanae* 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

9. Singh M, Jain AP. Qualitative and quantitative determination of secondary metabolites and antioxidant potential of *Nymphaea nouchali* flowers [Internet]. Vol. 8, Journal of Drug Delivery and Therapeutics. 2018. p. 111–5. Available from: http://dx.doi.org/10.22276/jddt.v8i6.s.2095

10. Analysis on Phytochemical and Antibacterial Activity for Different Medicinal Plants [Internet]. Vol. 12, International Journal of Pharmaceutical Research. 2020. Available from: http://dx.doi.org/10.31838/ijpr/2020.sp2.448

11. Chellapa LR, Shanmugam R, Indiran MA, Samuel SR. Biogenic nanoselenium synthesis, its antimicrobial, antioxidant activity and toxicity [Internet]. Vol. 9, Bioinspired, Biomimetic and Nanobiomaterials. 2020. p. 184–9. Available from: http://dx.doi.org/10.1680/jbiobn.19.00054

12. Madhumitha B, Santhakumar P, Jeevitha M, Rajeshkumar S. Green Synthesis of Selenium Nanoparticle using Capparis decidua fruit extract and its Characterization using Transmission Electron Microscopy And UV- Visible Spectroscopy. Research Journal of Pharmacy and Technology. 2021 Apr 29;14(4):2129–32.

13. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells [Internet]. Vol. 27, Environmental Science and Pollution Research. 2020. p. 8166–75. Available from: http://dx.doi.org/10.1007/s11356-019-07511-x

14. Kumar SA, Aravind Kumar S, Department of Orthodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science (SIMATS), Saveetha University, et al. Antimicrobial activity of silymarin mediated zinc oxide and hydroxyapatite nanoparticles against oral pathogens [Internet]. Vol. 16, Bioinformation. 2020. p. 863–8. Available from: http://dx.doi.org/10.6026/97320630016863

15. Ali SJ, Preetha S, Jeevitha M, Prathap L, S. R. Antifungal Activity of Selenium Nanoparticles Extracted from Capparis decidua Fruit against *Candida albicans* [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 2452–5. Available from: http://dx.doi.org/10.14260/jemds/2020/533

16. 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 Ag NPs on human colorectal HT-29 cancer cells [Internet]. Vol. 16, Bioinformation. 2020. p. 817–27. Available from: http://dx.doi.org/10.6026/97320630016817

17. S. RJ, Roy A, Shanmugam R, E. DW. Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp Lethality Assay [Internet]. Vol. 9, Journal of Evolution of Medical and Dental Sciences. 2020. p. 2894–7. Available from: http://dx.doi.org/10.14260/jemds/2020/633

18. 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

19. Ramesh A, Varghese S, Jayakumar ND, Malaippan S. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients - A case-control study. J Periodontol. 2018 Oct;89(10):1241–8.

20. 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.

21. 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.

22. Del Fabbro M, Karanxha L, Panda S, Buchi C, Nadathur Doraismamy J, Sankari M, et al. Autologous platelet concentrates for treating periodontal infrabony defects. Cochrane Database Syst Rev. 2018 Nov 26;11:CD011423.

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

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

25. Vellapally 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.

26. 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.

27. 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.

28. Venkatesan J, Singh SK, Anil S, Kim S-K, Shim MS. Preparation, Characterization and Biological Applications of Biosynthesized Ag NP with Chitosan-Fucoidan Coating. Molecules [Internet]. 2018 Jun 12;23(6). Available from: http://dx.doi.org/10.3390/molecules23061429

29. Alsubait SA, Al Ajlan R, Mitwalli H, Aburaïsi N, Mahmoud 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

30. 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.

31. 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.

32. PradeepKumar AR, Shemesh H, Nivedithitha 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 4;47(8):1196–214.

33. 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

34. 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

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

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

37. Vellappally S, Abdullah Al-Kheraif A, Anil S, Basavarajappa S, Hassanain AS. Maintaining patient oral health by using a xeno-genetic spiking neural network. J Ambient Intell Humiz Comput [Internet]. 2018 Dec 14; Available from: https://doi.org/10.1007/s12652-018-1166-8

38. Alhduwayhi 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.

39. Wu S, Rajeshkumar S, Madasamy M, Mahendran V. Green synthesis of copper nanoparticles using Cissus vitiginea and its antioxidant and antibacterial activity against urinary tract infection pathogens. Artif Cells Nanomed Biotechnol [Internet]. 2020 Dec [cited 2021 Aug 21];48(1). Available from: https://pubmed.ncbi.nlm.nih.gov/32924614/

40. Chowdhry S, Sumathi Felicita A, Shanmugam R. Antimicrobial Assay of Novel Zirconia and Silver Phytobiosynthesized Nanoparticles Biosynthesized using Ocimum Sanctum and Syzygium Aromaticum Extract-A Preliminary Study. Journal of Research in Medical and Dental Science. 2020 Aug 17;8(5):33–6.

41. Pereira WD, Rajasekar A, Rajeshkumar S. Green synthesis of selenium nanoparticles (senps) using aqueous extract of clove and cinnamon. PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY. 2020 Aug 25;85–91.

42. Kumar A, Arvina R, Rajeshkumar S. Antifungal activity of clove and cinnamon mediated selenium nanoparticles: an in vitro study. plant cell biotechnology and molecular biology. 2020 Aug 24;18–23.

43. Biochemical activities of ficus Banghalensis- A review article [Internet]. [cited 2021 Aug 21]. Available from: http://www.journalcra.com/article/bio-chemical-activities-ficus-banghalensis-review-article

44. Shovlin FE, Gillis RE. Biochemical and Antigenic Studies of Lactobacilli Isolated from Deep Dentinal
45. Antimicrobial Activity of Endangered Medicinal Plant Gloriosa Superba L [Internet]. Vol. 6, International Journal of Science and Research (IJSR). 2017. p. 1905–7. Available from: http://dx.doi.org/10.21275/art20175087

46. Jeevitha M, Rajeshkumar S. Antimicrobial Activity of Ag NPs Synthesized Using Marine Brown Seaweed Spatoglossum Asperum Against Oral Pathogens [Internet]. Vol. 10, Indian Journal of Public Health Research & Development. 2019. p. 3568. Available from: http://dx.doi.org/10.5958/0976-5506.2019.04140.8

47. Shivapriya Raje Bhonsle A, Jeevitha M, Preetha S, Rajeshkumar S. Anti-inflammatory activity of copper nanoparticles synthesized using dried ginger. Plant cell biotechnology and molecular biology. 2020 Nov 28;1–7.