

Original Research Article

PREPARATION OF MOUTH WASH USING *Musa sapientum* MEDIATED SILVER NANOPARTICLES AND ITS ANTIMICROBIAL ACTIVITY

Running title: Antimicrobial activity of silver nanoparticles synthesized using *Musa sapientum*.

ABSTRACT:

INTRODUCTION: *Musa sapientum* are primarily grown in tropical and subtropical countries, and are commonly used around the world for their nutritional values. Peels were examined for mineral, nutritional content. The product of the mineral content is that the concentration of potassium, calcium, magnesium, iron, manganese, bromine, and rubidium are high. *M.sapientum* pulp showed high antimicrobial activity against 13 gram-positive and gram-negative bacteria

MATERIALS AND METHODS: The plant extract was prepared. Silver nanoparticles were taken in the form of AgNO₃. The plant extract was tested for its anti-inflammatory activity by protein denaturation assay. To assess the antimicrobial activity, the prepared extract was inoculated in different culture plates containing different microorganisms. The results obtained were collected and statistically analyzed in SPSS software and graphs were obtained.

RESULTS: Analysis of antimicrobial activity shows The zone of inhibition in Mueller Hinton agar showed *C.albicans* zone of inhibition in the cultured plate with the diameter of 10mm,12mm and 25mm in 25μL, 50μL, and 100μL respectively. *S.mutans* zone of inhibition in the cultured plate with the diameter of 15mm,18mm, and 22mm at 25μL, 50μL, and 100μL respectively.

CONCLUSION: From the results obtained and analyzed we may conclude that silver nanoparticles synthesized using *Musa sapientum* extract can be used as a good anti-microbial agent. Its anti-microbial activity can be enhanced by increasing the concentration. Hence further advancements in this study could lead to producing and marketing natural product-based antimicrobial and anti-inflammatory agents.

Keywords: Anti-,microbial, *Musa sapientum*, Mouth wash, Muller Hinton agar, silver nanoparticles, green synthesis.

INTRODUCTION

Musa sapientum is primarily grown in tropical and subtropical countries, and are commonly used around the world for their nutritional values(1) *Musa sapientum* popularly known as 'banana' is used predominantly in Indian folk medicine for the treatment of diabetes mellitus. (2) Peels were examined for mineral and nutritional content. The product of the mineral content is that the concentration of potassium, calcium, magnesium, iron, manganese, bromine, and rubidium are high. (3) The peels of *Musa sapientum* fruits have gained recognition as a natural source of antioxidants and phytochemicals abundant in free radical scavenging compounds. (4,5) *Musa sapientum* overcomes sprue, uremia, nephritis, and gout (6)*Musa sapientum* tends to have prominent antidiabetic anti-ulcer agents (7)antioxidant and anti-inflammatory effects. (8,9)

Metallic silver nanoparticles have made a remarkable return as a possible antimicrobial agent. (10)Antimicrobial action of silver nanoparticles against yeast, *Escherichia coli*, and *Staphylococcus aureus* has been investigated and is successful(11) Silver nanoparticles have gained recognition related to physical, chemical, and biological properties, which have been linked to catalytic action and bactericidal effects and have been used in nanobiotechnology study. (12)

Antimicrobial activity means the killing or inhibition of microbial disease (13) Both research samples had strong inhibitory effects on the bacteria community examined under the parameters. Unripe banana has a high antimicrobial activity for all test species with a zone of 8 mm.

M.sapientum pulp showed high antimicrobial activity against 13 gram-positive and gram-negative bacteria (14)

Both plankton and biofilm types present periodontal bacteria(15) Although bad oral hypothesis results in bacterial aggregation, the first step to successful oral hygiene is the elimination of these microbes. Usually, this is done by using mouthwash solutions. (16) Mouthwashes typically have bacteria-struggling components. Zinc gluconate, quaternary ammonium, and cetylpyridinium chloride, and essential oils are among these additives. (17) Mouthwash is usually an antiseptic solvent to cleanse or revitalize the mouth and teeth(18). The study aims to evaluate *Musa sapientum* mediated silver nanoparticle mouthwash and its antimicrobial activity.

MATERIALS AND METHODS

Preparation of plant extract

The samples were collected by using a Randomized sampling method. Dried, crushed, and powdered roots of *Musa sapientum* were used to prepare the extract (Figure 1). The plant extract was purchased in a readymade manner for this study. 0.5g of Preparation of plant extract *Musa sapientum* extract was added to 100ml of distilled water and was boiled for 5 minutes at 50 degrees Celsius. Now the solution was filtered. 1millimolar of Silver nitrate was dissolved in 80 ml of distilled water. Then 20% filtered plant extract was added with an 80% nano mixture to prepare the extract of *Musa sapientum* assisted with silver nanoparticles. (Figure 2,3) Then the sample of extracts was placed in different cultures plate to observe the zone of inhibition and was recorded.

Antimicrobial Activity

Antibacterial activity of respective nanoparticles against the strain *Staphylococcus aureus*, *Bacillus*, and *E.coli*. Muller Hinton agar (MHA) agar was utilized for this activity to determine the zone of inhibition. Muller Hinton agar was prepared and sterilized for 45 minutes at 120lbs. Media poured into the sterilized plates and let stabilize for solidification. The wells were cut using the good cutter and the test organisms were swabbed. The nanoparticles with different concentrations were loaded and the plates were incubated for 24 hours at 37 ° C. After the

incubation time the zone of inhibition was measured. Multiple culture plate study to be done for analyzing the zone of inhibition. Validation of the procedure was done by a nano research guide. The correlation analysis was done to analyze the antimicrobial activity of silver nanoparticles synthesized using *Musa sapientum* using SPSS. Also to analyze the cytotoxic and antitumor property of *Musa sapientum*.

Results and discussion



Figure 1: A) commercially available powdered extract of *Musa sapientum*, B) *Musa sapientum* plant extract

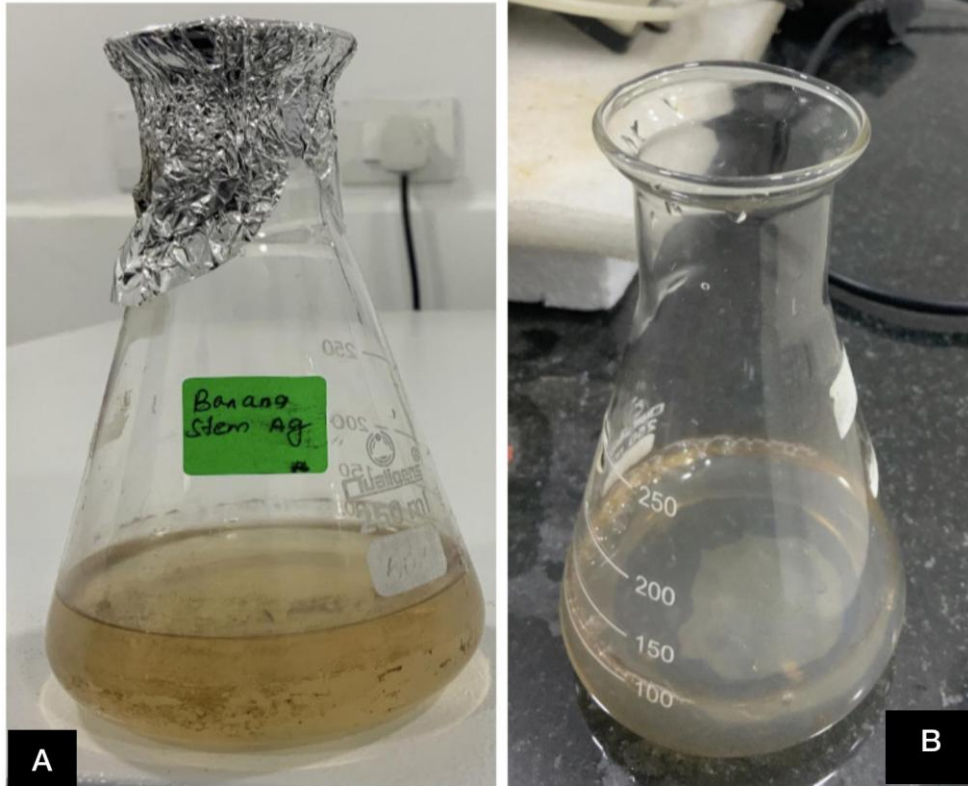


Figure 2: Image showing the preparation of *Musa sapientum* mediated silver nanoparticles. A - *Musa sapientum* extract. B - Silver nanoparticles solution.

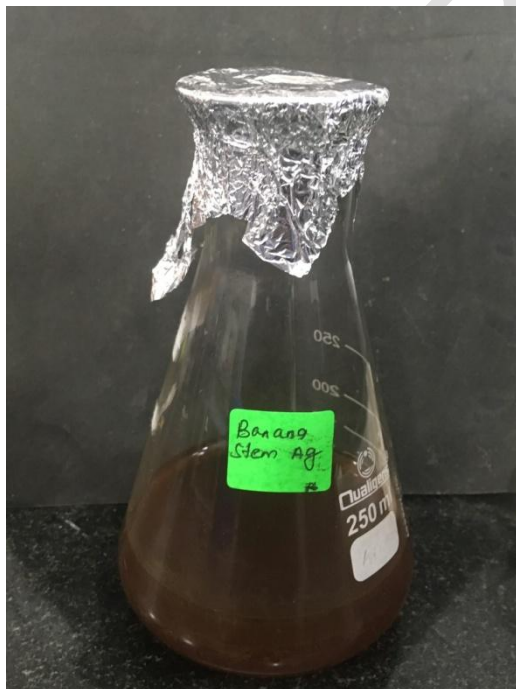


Figure 3: The characteristic color and property of the prepared extract are observed. There was also a characteristic color change observed after the filtration of the extract.

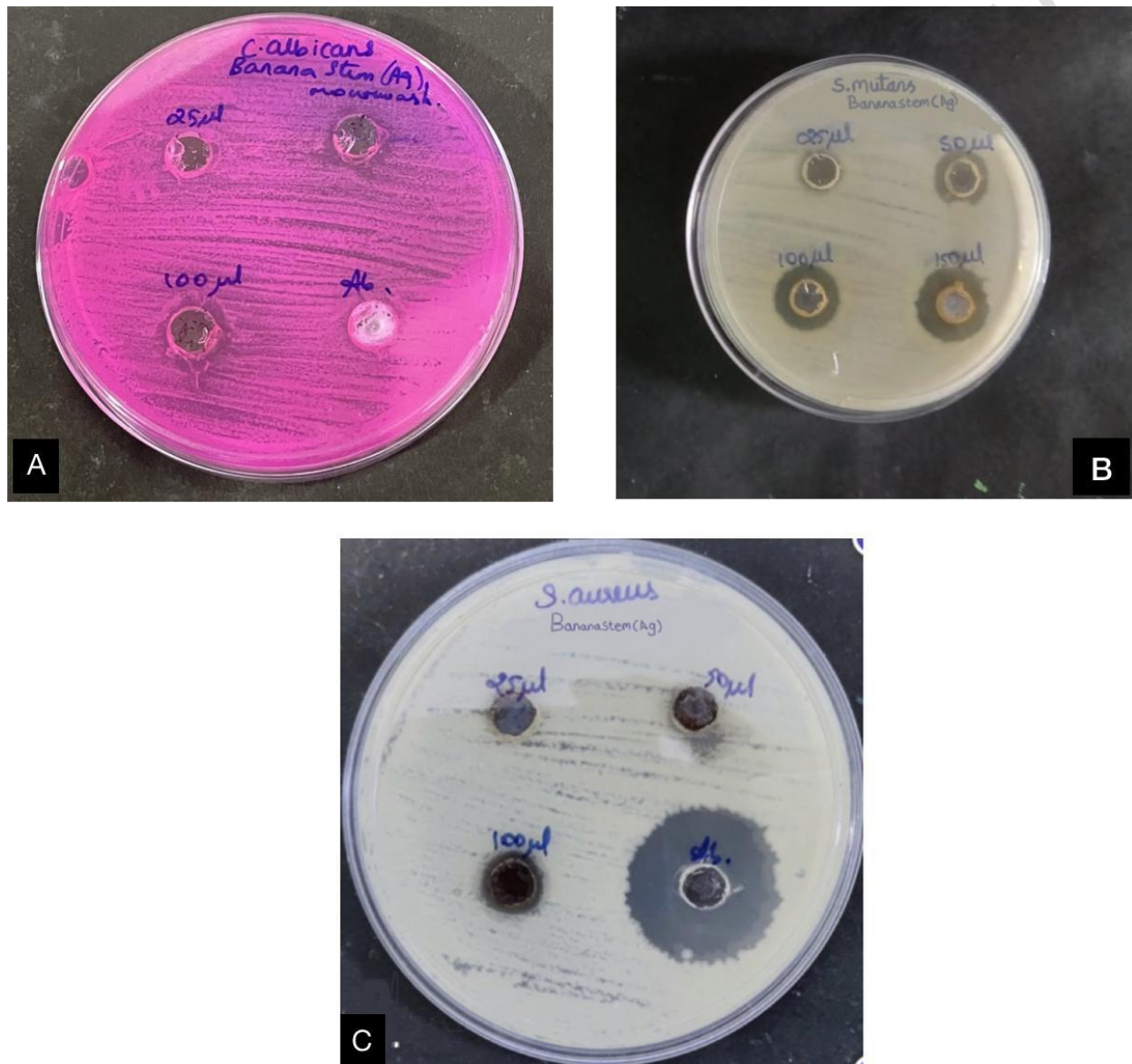


Figure 4: Antimicrobial activity seen in agar plates at different concentrations. In plate A, the zone of inhibition of *C. albicans* was observed at different concentrations. In plate B, the zone of

inhibition of *S.mutans* at different concentrations was observed. In plate C, the zone of inhibition of *S.aureus* was observed at different concentrations.

RESULTS AND DISCUSSION:

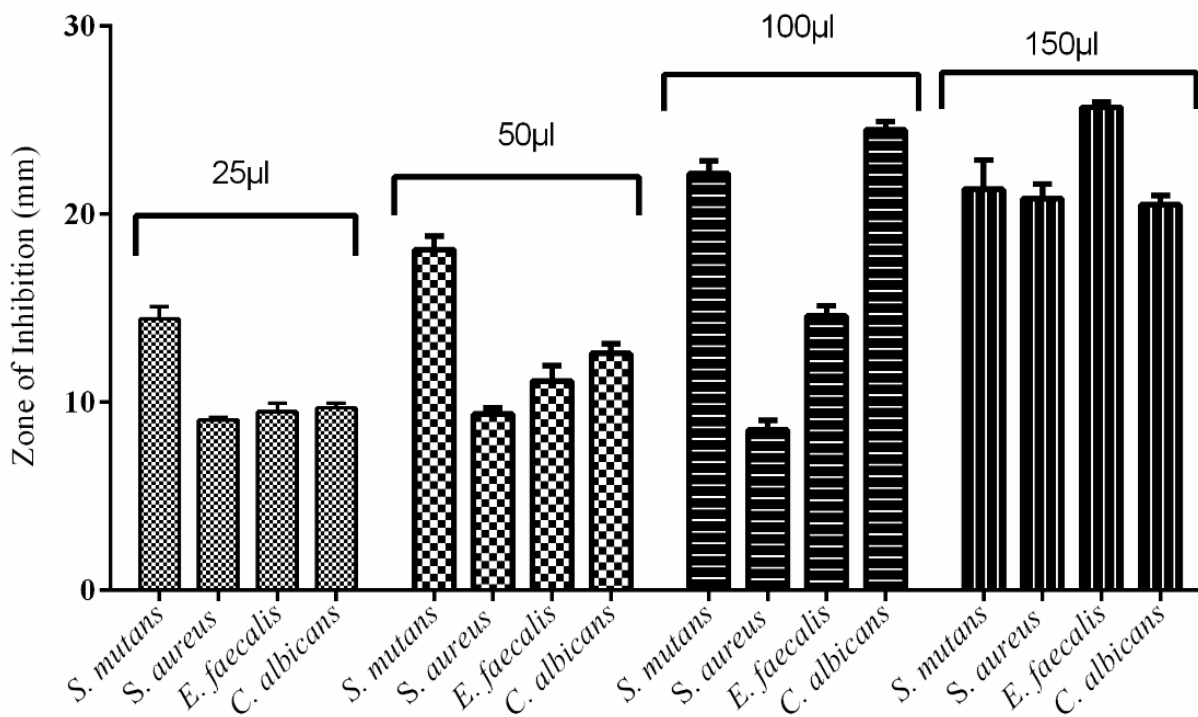


Figure 5: The graph represents the antimicrobial activity of *Musa sapientum* extract over different microorganisms. The X-axis represents different concentrations and the Y-axis represents a zone of inhibition of the extract of *C.albicans*, *S.mutans*, *S.aureus*, and the standard antibiotic at different concentrations. *C.albicans*, *S.mutans* showed an increased zone of inhibition at 50µL and 100µL.

By observing the prepared extract a characteristic color change was observed. The silver nanoparticles synthesized using *Musa sapientum* plant extract showed various color changes in the due course of shaking and mixing. The zone of inhibition in Mueller Hinton agar showed *C.albicans* zone of inhibition in the cultured plate with the diameter of 10mm,12mm, and 25mm in 25µL, 50µL, and 100µL respectively. *S.mutans* zone of inhibition in the cultured plate with the diameter of 15mm,18mm, and 22mm at 25µL, 50µL, and 100µL respectively. *S.aureus* zone

of inhibition in the cultured plate with the diameter of 9mm,9mm, and 9mm at 25 μ L, 50 μ L, and 100 μ L respectively (Figure 4). The extract of *Musa sapientum* with silver nanoparticles has shown better results in antimicrobial activity than the existing antibiotic against *C.albicans*. (figure 5) The antimicrobial activity against *C.albicans* shows a good zone of inhibition in the cultured plate with the diameter of 10mm,12mm and 25mm in 25 μ L, 50 μ L and 100 μ L respectively. The antibiotic-containing well showed inhibition of 20mm.

In a previous study by (19)the zone of inhibition of *C.albicans* against Ag-Nps increased with an increase in the concentration of Ag-Nps in agar well. Which is similar to our study. In a previous study by(20) the zones of inhibition were 9mm,10mm, and 12mm at 50 μ L,100 μ L, 150 μ L respectively against *C.albicans*. The antimicrobial effect kept increasing as concentration increased, similar to our study.

The extract of *Musa sapientum* with silver nanoparticles has shown better results in antimicrobial activity than the existing antibiotic against *S.mutans*. The antimicrobial activity against *S.mutans* shows a good zone of inhibition in the cultured plate with the diameter of 15mm,18mm and 22mm in 25 μ L, 50 μ L, and 100 μ L respectively. The antibiotic-containing well showed inhibition of 23mm

In previous research by (21) Ag-NPs zones of inhibition against *S.mutans* were measured as 14mm, 15mm, and 20mm in 50 μ L,100 μ L, 150 μ L. () our study had proved better than this study. In a previous study by Swarna Maiti et al Ag-NPs exhibited their antimicrobial activity (MIC) against *S.mutans* at 50 μ L.

The extract of *Musa sapientum* with silver nanoparticles has shown decreased antimicrobial activity than the existing antibiotic against *S.aureus*. The antimicrobial activity against *S.aureus* shows a good zone of inhibition in the cultured plate with the diameter of 9mm, 9mm, and 9mm in 25 μ L, 50 μ L, and 100 μ L respectively. The antibiotic-containing well showed inhibition of 20mm. The antimicrobial activity remains the same as the concentration increases.

In a previous study by (19)Ag-NPs activity against *S.aureus* was recorded as a zone of inhibition of 9mm, 18mm, and 20mm in 50 μ L,100 μ L, 150 μ L respectively(). Unlike our study,

the activity kept increasing as concentration increased. In a previous study by anima nanda activity of Ag-NPs remained constant even when concentrations of extract were increased(22)

According to the findings of the research study, unripe bananas have an antimicrobial function and are thus medicinal plants(23)The potency of unripe banana plants was found to be improved by the form of solvent used in this analysis, suggesting that some of the active ingredients in these medicinal plants dissolve better in ethanol than in water. When used with two different solvents (ethanol and water), unripe bananas had more antibacterial activity than lemongrass and turmeric, which had a strong antibacterial activity with the only ethanolic extract. Unripe bananas have antimicrobial properties, according to the researchers(24). This suggests that there is already a lot to be gained by using medicinal plants as an antimicrobial pointer to new sources of experimental medicines, which needs further study. Many plants are used in Nigeria to cure various diseases in the form of rudimentary oils, infusions, and plasters, with little clear proof of efficacy (25). Limitations of the study, Lower concentration of extract is taken, Multiple culture plates should be done and Multiple activity tests should be done. Future research with this combination can lead to effective mouthwash with a greater antimicrobial effect.

Our team has extensive knowledge and research experience that has translate into high quality publications (26).(27–40) ,(41–45)

Conclusion:

The combination of *Musa sapientum* with silver nanoparticles has shown an effective antimicrobial property against *Candida albicans* and *S.mutans* . But no effect was seen against *S.aureas*. Further research on this combination can lead to a better antibiotic with enhanced antimicrobial effect than the existing mouthwashes.

REFERENCES:

1. Devi CK, Department of Pharmacognosy, Narasarao peta Institute of Pharmaceutical Sciences, Pet N, Guntur-, Pradesh A, et al. Phyto Chemical Screening and Anti-Microbial

Activity of *Musa Paradisiaca*-Fruit Peel [Internet]. Vol. 3, Indian Journal of Applied Research. 2011. p. 248–9. Available from:

<http://dx.doi.org/10.15373/2249555x/july2013/77>

2. Pari L, Uma Maheswari J. Hypoglycaemic effect of *Musa sapientum* L. in alloxan-induced diabetic rats [Internet]. Vol. 68, Journal of Ethnopharmacology. 1999. p. 321–5. Available from: [http://dx.doi.org/10.1016/s0378-8741\(99\)00088-4](http://dx.doi.org/10.1016/s0378-8741(99)00088-4)
3. Surma N, Chemistry Department, College of Science, University of Agriculture, PMB, Makurdi, et al. FUEL GASES FROM WASTE HIGH DENSITY POLYETHYLENE (HDPE) VIA LOW TEMPERATURE CATALYTIC PYROLYSIS [Internet]. Vol. 3, Progress in Chemical and Biochemical Research. 2020. p. 20–30. Available from: <http://dx.doi.org/10.33945/sami/pcbr.2020.1.3>
4. Salako OA, Akindele AJ, Balogun AO, Adeyemi OO. Investigation of Antidepressant, Anxiolytic and Sedative Activities of the Aqueous Leaf Extract of *Musa sapientum* Linn. (Banana; Musaceae). Drug Res . 2019 Feb;69(3):136–43.
5. Timothy CN, Samyuktha PS, Brundha MP. Dental pulp Stem Cells in Regenerative Medicine--A Literature Review. Research Journal of Pharmacy and Technology. 2019;12(8):4052–6.
6. Marendra FRB, Colodel C, Canteri MHG, de Olivera Müller CM, Amante ER, de Oliveira Petkowicz CL, et al. Investigation of cell wall polysaccharides from flour made with waste peel from unripe banana (*Musa sapientum*) biomass. J Sci Food Agric. 2019 Jul;99(9):4363–72.
7. Siddique S, Nawaz S, Muhammad F, Akhtar B, Aslam B. Phytochemical screening and in-vitro evaluation of pharmacological activities of peels of *Musa sapientum* and *Carica* papaya fruit. Nat Prod Res. 2018 Jun;32(11):1333–6.
8. Dikshit P, Tyagi MK, Shukla K, Gambhir JK, Shukla R. Antihypercholesterolemic and antioxidant effect of sterol rich methanol extract of stem of *Musa sapientum* (banana) in cholesterol fed wistar rats. J Food Sci Technol. 2016 Mar;53(3):1690–7.

9. Preethikaa S, Brundha MP. Awareness of diabetes mellitus among general population. *Research Journal of Pharmacy and Technology*. 2018;11(5):1825–9.
10. Darvhekar V, Tripathi AS, Jyotishi SG, Mazumder PM, Shelke PG. Influence of *Musa sapientum* L. on pharmacokinetic of metformin in diabetic gastroparesis. *Chin J Integr Med*. 2016 Oct;22(10):783–8.
11. Adewoye EO, Adele BO. Effect of methanol extract of *Musa sapientum* leaves on protein glycation and erythrocyte antioxidant status in alloxan-induced diabetic Wistar rats. *Afr J Med Med Sci*. 2015 Sep;44(3):261–8.
12. Ross IA. *Musa sapientum* [Internet]. *Medicinal Plants of the World*. 2001. p. 319–31. Available from: http://dx.doi.org/10.1007/978-1-59259-237-1_17
13. Bährle-Rapp M. *Musa Sapientum* Extract [Internet]. *Springer Lexikon Kosmetik und Körperpflege*. 2007. p. 361–361. Available from: http://dx.doi.org/10.1007/978-3-540-71095-0_6723
14. Valladolid M, Garrido MA, García-Seminario R. Temporal distribution of “trips” and biological controllers in banana, *Musa sapientum* (C. Linneo, 1753) [Internet]. Vol. 17, *Manglar*. 2020. p. 113–8. Available from: <http://dx.doi.org/10.17268/manglar.2020.017>
15. Faria MML de, de Faria MML, Soares SBD, de Paula Vieira DA. DESIDRATAÇÃO OSMÓTICA E CONVENCIONAL DE BANANA DA TERRA (*MUSA SAPIENTUM*) / OSMOTIC AND CONVENTIONAL DEHYDRATION OF BANANA DA TERRA (*MUSA SAPIENTUM*) [Internet]. Vol. 6, *Brazilian Journal of Development*. 2020. p. 86506–18. Available from: <http://dx.doi.org/10.34117/bjdv6n11-174>
16. Alves H, Machado MT, Anaci Maria Nogueira. Análise Qualitativa do Processo de Reparo em Cicatriz Cirúrgica de Ratos Tratados com Extrato de *Musa Sapientum*, Aloe Vera e Colagenase/ Qualitative Analysis of the Repair Process in Surgical Scar in Treated Rats with *Musa Sapientum* Extract, Aloe Vera an [Internet]. Vol. 1, *REVISTA CIÊNCIAS EM SAÚDE*. 1970. p. 8–18. Available from: <http://dx.doi.org/10.21876/rcsfmit.v1i2.50>
17. Orhan İ, Küsmenoğlu Ş, Şener B. FATTY ACID PROFILE OF FRESH AND DRIED

BANANA (MUSA SAPIENTUM L. VAR. CAVENDISHII Lamb.) PEEL OILS : TAZE ve KURUTULMUŞ MUZ (Musa sapientum L. var. cavendishii Lamb.) KABUK YAĞLARININ YAĞ ASİTİ PROFİ [Internet]. Ankara Üniversitesi Eczacılık Fakültesi Dergisi. 2008. p. 0103–0019. Available from: http://dx.doi.org/10.1501/eczfak_0000000369

18. Harsha L, Brundha MP. Prevalence of Dental Developmental Anomalies among Men and Women and its Psychological Effect in a Given Population. *Journal of Pharmaceutical Sciences and Research*; Cuddalore. 2017 Jun 20;9(6):869–73.
19. Labh AK, Rajeshkumar S, Roy A, Santhoshkumar J, Lakshmi T. Herbal Formulation Mediated Synthesis of Silver Nanoparticles and Its Antifungal Activity Against *Candida albicans* [Internet]. Vol. 10, *Indian Journal of Public Health Research & Development*. 2019. p. 3454. Available from: <http://dx.doi.org/10.5958/0976-5506.2019.04117.2>
20. Nanda A, Saravanan M. Biosynthesis of silver nanoparticles from *Staphylococcus aureus* and its antimicrobial activity against MRSA and MRSE [Internet]. Vol. 5, *Nanomedicine: Nanotechnology, Biology and Medicine*. 2009. p. 452–6. Available from: <http://dx.doi.org/10.1016/j.nano.2009.01.012>
21. Sreenivasagan S, Subramanian AK, Rajeshkumar SRS. Assessment of antimicrobial activity and cytotoxic effect of green mediated silver nanoparticles and its coating onto mini-implants [Internet]. Vol. 9, *Annals of Phytomedicine: An International Journal*. 2020. Available from: <http://dx.doi.org/10.21276/ap.2020.9.1.27>
22. Katva S, Das S, Moti HS, Jyoti A, Kaushik S. Antibacterial Synergy of Silver Nanoparticles with Gentamicin and Chloramphenicol against. *Pharmacogn Mag*. 2018 Jan;13(Suppl 4):S828–33.
23. Bempah OA. Warning: Fruit-Sourced Carbohydrate, Banana *Musa sapientum* and Plantain *Musa paradisiaca* may endanger your Health if you are Diabetes Mellitus Patient [Internet]. Vol. 5, *Journal of Nutritional Health & Food Engineering*. 2016. Available from: <http://dx.doi.org/10.15406/jnhfe.2016.05.00158>
24. Olukunle OF, Adenola OJ. Comparative Antimicrobial Activity of Lemon Grass

- (*Cymbopogon citratus*) and Garlic (*Allium sativum*) Extracts on *Salmonella typhi* [Internet]. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2019. p. 1–9. Available from: <http://dx.doi.org/10.9734/jamps/2019/v20i230104>
25. Omonona BT. Consumer preference for medicinal plants in Oyo Metropolis, Nigeria [Internet]. Vol. 6, *Journal of Medicinal Plants Research*. 2012. Available from: <http://dx.doi.org/10.5897/jmpr11.1119>
 26. Anita R, Paramasivam A, Priyadharsini JV, Chitra S. The m6A readers YTHDF1 and YTHDF3 aberrations associated with metastasis and predict poor prognosis in breast cancer patients. *Am J Cancer Res*. 2020 Aug 1;10(8):2546–54.
 27. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. *Hypertens Res*. 2020 Dec;43(12):1459–61.
 28. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in *Streptococcus* sp., using computational approach. *Journal of King Saud University - Science*. 2020 Jan 1;32(1):904–9.
 29. Smiline Girija AS. Delineating the Immuno-Dominant Antigenic Vaccine Peptides Against gacS-Sensor Kinase in *Acinetobacter baumannii*: An in silico Investigational Approach. *Front Microbiol*. 2020 Sep 8;11:2078.
 30. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of *A. baumannii* and targeting with essential oil compounds from *Azadirachta indica*. *Journal of King Saud University - Science*. 2020 Dec 1;32(8):3380–7.
 31. Girija ASS. Fox3+ CD25+ CD4+ T-regulatory cells may transform the nCoV's final destiny to CNS! *J Med Virol* [Internet]. 2020 Sep 3; Available from: <http://dx.doi.org/10.1002/jmv.26482>
 32. Jayaseelan VP, Ramesh A, Arumugam P. Breast cancer and DDT: putative interactions, associated gene alterations, and molecular pathways. *Environ Sci Pollut Res Int*. 2021

Jun;28(21):27162–73.

33. 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*. 2021 Feb;122:105030.
34. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1-mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html>
35. Girija SA, Priyadharsini JV, Paramasivam A. Prevalence of carbapenem-hydrolyzing OXA-type β -lactamases among *Acinetobacter baumannii* in patients with severe urinary tract infection. *Acta Microbiol Immunol Hung*. 2019 Dec 9;67(1):49–55.
36. Priyadharsini JV, Paramasivam A. RNA editors: key regulators of viral response in cancer patients. *Epigenomics*. 2021 Feb;13(3):165–7.
37. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol*. 2020;64(1):93–9.
38. Girija As S, Priyadharsini J V, A P. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI). *Acta Clin Belg*. 2021 Apr;76(2):106–12.
39. Anchana SR, Girija SAS, Gunasekaran S, Priyadharsini VJ. Detection of csgA gene in carbapenem-resistant *Acinetobacter baumannii* strains and targeting with *Ocimum sanctum* biocompounds. *Iran J Basic Med Sci*. 2021 May;24(5):690–8.
40. Girija ASS, Shoba G, Priyadharsini JV. Accessing the T-Cell and B-Cell Immuno-Dominant Peptides from *A.baumannii* Biofilm Associated Protein (bap) as Vaccine Candidates: A Computational Approach. *Int J Pept Res Ther*. 2021 Mar 1;27(1):37–45.

41. Arvind P TR, Jain RK. Skeletally anchored forsus fatigue resistant device for correction of Class II malocclusions-A systematic review and meta-analysis. *Orthod Craniofac Res*. 2021 Feb;24(1):52–61.
42. Venugopal A, Vaid N, Bowman SJ. Outstanding, yet redundant? After all, you may be another Choluteca Bridge! *Semin Orthod*. 2021 Mar 1;27(1):53–6.
43. Ramadurai N, Gurunathan D, Samuel AV, Subramanian E, Rodrigues SJL. Effectiveness of 2% Articaine as an anesthetic agent in children: randomized controlled trial. *Clin Oral Investig*. 2019 Sep;23(9):3543–50.
44. 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.
45. Mathew MG, Samuel SR, Soni AJ, Roopa KB. Evaluation of adhesion of Streptococcus mutans, plaque accumulation on zirconia and stainless steel crowns, and surrounding gingival inflammation in primary molars: randomized controlled trial [Internet]. Vol. 24, *Clinical Oral Investigations*. 2020. p. 3275–80. Available from: <http://dx.doi.org/10.1007/s00784-020-03204-9>