Journal of Pharmaceutical Research International

33(47B): 621-629, 2021; Article no.JPRI.74402
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Biosynthesis of Copper Nanoparticles using Mucuna Pruriens and its Antioxidant and Antidiabetic Activity

Shifa Jawahar Ali[1], R. V. Geetha[2*] and S. Rajeshkumar[3]

[1]Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha university, Chennai- 600077, India.
[2]Department of Microbiology, Saveetha Dental College and Hospitals Saveetha Institute of Medical and Technical Sciences, Saveetha university, Chennai- 600077, India.
[3]Department of Pharmacology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha university, Chennai- 600077, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author SJA carried out the literature search, data collection, data analysis and manuscript writing. Author RVG conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. All the authors have equally contributed in developing the manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i47B33163
Editor(s):
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.
Reviewers:
(1) Murthy Chavali, Alliance University, India.
(2) Manu Mitra, University of Bridgeport, USA.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74402

Original Research Article

Received 01 August 2021
Accepted 09 October 2021
Published 04 November 2021

ABSTRACT

Introduction: The field of Nanotechnology has gained importance since last century. Nanoparticles can be used in medicine due to its increased interaction with microbes and has less side effects than drugs. Antioxidant compounds scavenge free radicals and inhibit the oxidative mechanisms that lead to degenerative diseases. There is a growing number of diabetes patients all over the world. Wide varieties of synthetic drugs are being used for the treatment of Type 2 diabetes mellitus, most of them possess side effects in the long run such as hepatotoxicity, abdominal pain, flatulence and diarrhea. Therefore, there is a need for a search of an alternate antidiabetic agent
Aim: The aim of the study is to synthesize Copper nanoparticles from *Mucuna pruriens* and to evaluate its antioxidant and antidiabetic activity.

**Corresponding author: E-mail: geetha@saveetha.com;*

Materials and methods: Plant extract of *Mucuna pruriens* was prepared and filtered by Whatman No 1 filter paper. Copper sulphate was added to the plant extract and kept in a magnetic stirrer for nanoparticle synthesis. The synthesized nanoparticle was preliminarily analysed using UV visible spectroscopy. Finally the left over solution was taken to calculate antioxidant activity and antidiabetic activity.
Results: Antioxidant activity was calculated by DPPH method and the percentage of inhibition of copper nanoparticles synthesised from *Mucuna pruriens* was 58.5% for 10µL, 59.6% for 20µL, 67.5% for 30µL, 71.4% for 40µL and 72.3% for 50µL. Antidiabetic activity was calculated by alpha-amylase inhibitory assay and the percentage of inhibition of copper nanoparticles synthesised from *Mucuna pruriens* was 66% for 10µL, 69% for 20µL, 73% for 30µL, 79% for 40µL and 80% for 50µL.
Conclusion: We can conclude that copper nanoparticles synthesised from *Mucuna pruriens* are a potent antioxidant and antidiabetic agent. Since it shows a good activity in free radical scavenging, copper nanoparticles can be used in a clinical therapeutic application and also in the management of type 2 diabetes mellitus.

Keywords: Antioxidant activity; antidiabetic activity; copper nanoparticles; Mucuna pruriens.

1. INTRODUCTION

The field of Nanotechnology has gained importance since last century. Nanotechnology has given particles of various nanoscale levels. These nanoparticles are within the size of 1 to 100 nanometers in diameter [1]. Nanoparticles can be used in medicine due to its increased interaction with microbes and has less side effects than drugs [2,3]. Nanoscience and nanotechnology has become a priority field of research for today's researchers around the world [1,4,5]. Copper nanoparticles have important applications in diverse fields such as catalysis, water treatment, solar cells and a significant role in advanced electronic circuits due to its good electrical conductivity. It is also used as a disinfectant due to antibacterial properties and has wide use in pharmaceutical and health care [6,7]. Biological synthesis of copper nanoparticles are ecological, economical and easily scalable when compared to physical and chemical methods [8].

The use of *Mucuna pruriens* seed extract for the synthesis of copper nanoparticles has been investigated for the first time. *Mucuna pruriens* is a herb which belongs to the family fabaceae and is used for the management of nervous disorders, male infertility, as an antidepressant and also for treating Parkinson's disease [9]. Among the various wild legumes, the velvet bean Mucuna pruriens is found throughout the world's tropical and subtropical regions. *Mucuna pruriens* is a viable source of dietary proteins due to its high protein concentration and hence it is a good source of food. It is a popular Indian medicinal plant and used in Ayurveda as a powerful aphrodisiac used for treating nervous disorders and arthritis. The main phenolic compound of *Mucuna pruriens* seeds is the L-Dopa, this substance is used as a first line treatment for Parkinson's disease [10]. L-Dopa obtained from *Mucuna pruriens* have less or no side effects when compared with the synthetic L-Dopa administered to Parkinson's patients [11].

Oxidative stress is one of the important risk factors in the pathogenesis of many chronic diseases [12–14]. Free radicals and other reactive oxygen species are recognized as agents responsible for human aging [15]. An antioxidant is a substance that inhibits oxidative damage to a target molecule [16]. The characteristic property of an antioxidant is its ability to trap free radicals. Antioxidant compounds scavenge free radicals and inhibit the oxidative mechanisms that lead to degenerative diseases [17,18]. Diabetes Mellitus is a metabolic syndrome, associated with severe physiological imbalances. It is characterized by chronic hyperglycemia that leads to multiple biochemical impairments and oxidative stress. There is a growing number of diabetes patients all over the world. Wide varieties of synthetic drugs are being used for the treatment of Type 2 diabetes mellitus , most of them possess side effects in the long run such as hepatotoxicity, abdominal pain, flatulence and diarrhea. Therefore, there is a need for a search of an alternate antidiabetic agent [19]. Evidence has shown that copper nanoparticles synthesised from medicinal plant *Dioscorea bulbifera* tuber extract showed antioxidant and antidiabetic activity [20].Our team has extensive knowledge and research experience that has translate into high quality publications[21–32],[33–37]. [38] [39] [40]. [41–45] The aim of the study is to evaluate

the antioxidant and antidiabetic activity of Copper nanoparticles synthesised from *Mucuna pruriens* which have not yet been investigated.

2. MATERIALS AND METHODS

2.1 Extract Preparation

In the present study, 1gm of powder of *Mucuna pruriens* seed was added in 100 ml of distilled water and boiled for 10-15 minutes at 70 degree celsius. After boiling, the plant extract was filtered by Whatman No 1 filter paper. 60 ml of 20 milli molar copper sulphate was prepared in 250 ml of conical flask, 40 ml of filtered plant extract was mixed to it and kept in a magnetic stirrer for nanoparticle synthesis (figure1). The synthesized nanoparticle was preliminarily analysed using a UV visible spectrophotometer (figure 2). Prior to the final step the nanoparticle solution was centrifuged at 8000 rpm to prepare nanoparticle pellet powder, it was dried in a hot air oven at 80 degree celsius. The dried powder was sent for characterisation. Finally the left over solution was taken to calculate antioxidant and antidiabetic activity. All the results were taken photographs and recorded in the excel sheets.

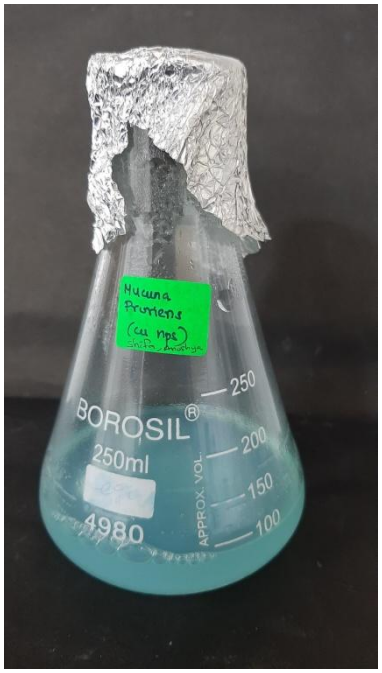


Fig. 1. The figure shows the plant extract after nanoparticle synthesis

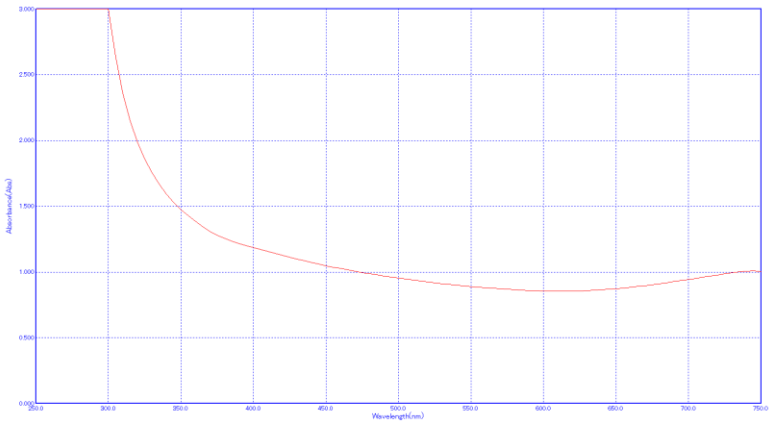


Fig. 2. The figure shows the UV-Visible spectra of *Mucuna pruriens* extract mediated copper nanoparticles

2.2 Antioxidant Assay- DPPH Method

DPPH assay was used to test the antioxidant activity of biogenic synthesized copper nanoparticles. Diverse concentrations (2-10 µg/ml) of *Mucuna pruriens* plant extract interceded copper nanoparticles were mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. The percentage of inhibition was determined from the following equation.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

2.3 Antidiabetic Assay- Alpha-Amylase Inhibitory Assay

The in-vitro antidiabetic assay was performed using Alpha-amylase inhibitory assay.
The amount of maltose liberated during the experiment was used to determine alpha - amylase inhibition. The method described by Bhutkar and Bhise was used. (Bhutkar and Bhise, 2012). Different concentration of nanoparticles (10, 20, 30, 40, 50 mU/L) was pre-incubated with 100 mU/L of alpha- amylase solution (1% w/v) was further added to it and the mixture was incubated at room temperature for 10 minutes. 100 mU/L of 96 mM (3,5-Dinitrosalicylic acid solution) DNSA reagent was added to it to stop the reaction and the solution was heated in a water bath for 5 minutes. A control was maintained in which an equal amount of enzyme extract was replaced by a sodium phosphate buffer kept at a pH of 6.9. Reading was measured at 540 nm. The experiment was performed in triplicate. Acarbose was used as a positive control.

% inhibition was calculated using the formulae:

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

Where, C= control, T= test sample.

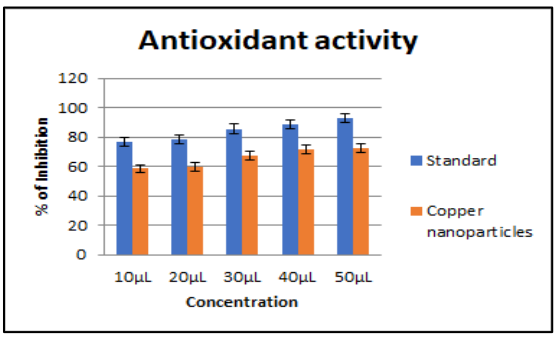
3. RESULTS

Table 1. The table represents the antioxidant activity of copper nanoparticles synthesised from *Mucuna pruriens* compared to the standard

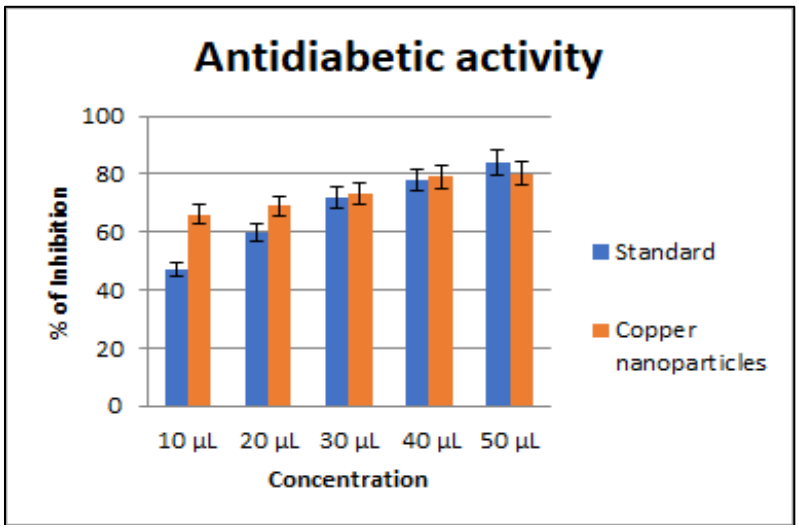
No.	Concentration	Standard- % of inhibition	Copper nanoparticles- % of inhibition
1	10µL	76.56	58.5
2	20µL	78.52	59.6
3	30µL	85.63	67.5
4	40µL	88.68	71.4
5	50µL	93.15	72.3

Table 2. The table represents the antidiabetic activity of copper nanoparticles synthesised from *Mucuna pruriens* compared to the standard

No.	Concentration	Standard- % of inhibition	Copper nanoparticles- % of inhibition
1	10µL	47	66
2	20µL	60	69
3	30µL	72	73
4	40µL	78	79
5	50µL	84	80



Graph 1. The graph represents the antioxidant activity of copper nanoparticles synthesised from *Mucuna pruriens* compared to the standard. Blue colour denotes the standard. Orange colour denotes the copper nanoparticles synthesised from *Mucuna pruriens*



Graph 2. The graph represents the antidiabetic activity of copper nanoparticles synthesised from *Mucuna pruriens* compared to the standard. Blue colour denotes the standard. Orange colour denotes the copper nanoparticles synthesised from *Mucuna pruriens*

3. DISCUSSION

Antioxidant activity was calculated by DPPH method and the percentage of inhibition of copper nanoparticles synthesised from *Mucuna pruriens* was 58.5% for 10µL, 59.6% for 20µL, 67.5% for 30µL, 71.4% for 40µL and 72.3% for 50µL. The percentage of inhibition of the standard was 76.56% for 10µL, 78.52% for 20µL, 85.63% for 30µL, 88.68% for 40µL and 93.15% for 50µL. Hence maximum inhibition was observed at 50µL or at higher concentration (table 1)(Graph 1). Antidiabetic activity was calculated by alpha-amylase inhibitory assay and the percentage of inhibition of copper nanoparticles synthesised from *Mucuna pruriens* was 66% for 10µL, 69% for 20µL, 73% for 30µL, 79% for 40µL and 80% for 50µL. The percentage of inhibition of the standard was 47% for 10µL, 60% for 20µL, 72% for 30µL, 78% for 40µL and 84% for 50µL (table 2)(Graph 2).

From the results, we can conclude that copper nanoparticles inhibit the function of alpha-amylase enzyme. Inhibition of this enzyme has a therapeutic effect on diabetes mellitus by controlling the level of glucose in the blood [46].Hyperglycemia due to chronic diabetes generates reactive oxygen species leading to oxidative stress which have a crucial role in lipid peroxidation and membrane damage. Preventing oxidative damage with free radical scavengers and inhibiting digestive enzymes such as α-amylase and α-glucosidase are the two important therapeutic strategies for prevention of diabetes.

Previous research works have reported on the various activities exhibited by the nanoparticles synthesised from natural sources such as cytotoxic, antimicrobial activity [47–51]. The antifungal effect of copper nanoparticles isolated from white fish (Rutilus frisii kutum) eggs were tested against the fungus *Saprolegnia sp*. Copper nanoparticles at a concentration of 10 ppm have been found to have antifungal effects on Saprolegnia species. Antifungal activity of copper nanoparticles was found to be positively correlated with their concentration and exposure time. This makes them a good alternative to malachite green which is also carcinogenic. [52]. A study on copper nanoparticles synthesised using medicinal plants such as Gnidia glauca and Plumbago zeylanica were then tested for anti-diabetic activity by inhibiting porcine pancreatic -amylase and -glucosidase. CuNPs were able to inhibit porcine pancreatic α-amylase by 30 to 50 %, while α-glucosidase was inhibited by 70 to 88 %. This research demonstrates that phytogenic CuNPs synthesised with *G. glauca* and *P. zeylanica* can be used to develop anti-diabetic nanomedicines [53]. In our study, antidiabetic activity was checked for the copper nanoparticles synthesised from *Mucuna pruriens* by alpha amylase inhibitory assay and it showed good antidiabetic activity.

In future, copper nanoparticles synthesised from *Mucuna pruriens* can be assessed for its anticancer, antiinflammatory, antifungal and antibacterial activity and clinical trials can be

carried out. The study's limitation was that it was a preliminary study and conducted in vitro, so it cannot be assumed that the results of antidiabetic and antioxidant activity could be translated into clinical effectiveness.

4. CONCLUSION

The copper nanoparticles biosynthesised from seed extract of *Mucuna pruriens* have good antioxidant and antidiabetic activity. We can conclude that copper nanoparticles are a potent antioxidant and antidiabetic agent. Since it shows a good activity in free radical scavenging, copper nanoparticles can be used in a clinical therapeutic application and also in the management of type 2 diabetes mellitus.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGMENT

- Saveetha Dental College
- Ateeq al Dhahery Trading est.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ganta SSL, Jeevitha M, Preetha S, Rajeshkumar S. Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles. Journal of Pharmaceutical Research International 2020:14–9.
2. Ali SJ, Preetha S, Jeevitha M, Prathap L, S. R. Antifungal Activity of Selenium Nanoparticles Extracted from Capparis decidua Fruit against Candida albicans. Journal of Evolution of Medical and Dental Sciences 2020;9: 2452–5. Avaialble:https://doi.org/10.14260/jemds/2020/533.
3. Rajeshkumar S, Malarkodi C, Al Farraj DA, Elshikh MS, Roopan SM. Employing sulphated polysaccharide (fucoidan) as medium for gold nanoparticles preparation and its anticancer study against HepG2 cell lines. Materials Today Communications 2021;26:101975. Avaialble:https://doi.org/10.1016/j.mtcomm.2020.101975.
4. 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 hydroxy apatite nanoparticles against oral pathogens. Bioinformation 2020;16:863–8. Avaialble:https://doi.org/10.6026/97320630016863.
5. Nasim I, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, India. Cytotoxicity and anti-microbial analysis of silver and graphene oxide bio nanoparticles. Bioinformation 2020;16:831–6. Avaialble:https://doi.org/10.6026/97320630016831.
6. Hassanien R, Husein DZ, Al-Hakkani MF. Biosynthesis of copper nanoparticles using aqueous Tilia extract: antimicrobial and anticancer activities. Heliyon 2018;4:e01077. Avaialble:https://doi.org/10.1016/j.heliyon.2018.e01077.
7. 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 2020;48:1153–8.
8. Noor S, Shah Z, Javed A, Ali A, Hussain SB, Zafar S, et al. A fungal based synthesis method for copper nanoparticles with the determination of anticancer, antidiabetic and antibacterial activities. J Microbiol Methods 2020;174:105966.
9. Rana DG, Galani VJ. Dopamine mediated antidepressant effect of Mucuna pruriens seeds in various experimental models of depression. Ayu 2014;35:90–7.
10. Katzenschlager R, Evans A, Manson A, Patsalos PN, Ratnaraj N, Watt H, et al. Mucuna pruriens in Parkinson's disease: a double blind clinical and pharmacological study. J Neurol Neurosurg Psychiatry 2004;75:1672–7.
11. Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. The Magic Velvet

Bean of Mucuna pruriens. Afr J Tradit Complement Altern Med 2012;2:331–9.
12. Shunmugam R, Renukadevi Balusamy S, Kumar V, Menon S, Lakshmi T, Perumalsamy H. Biosynthesis of gold nanoparticles using marine microbe (Vibrio alginolyticus) and its anticancer and antioxidant analysis. Journal of King Saud University - Science 2021;33:101260.
13. Mohapatra S, Leelavathi L, Rajeshkumar S, Sri Sakthi D, Jayashri P. Assessment of cytotoxicity, anti-inflammatory and antioxidant activity of zinc oxide nanoparticles synthesized using clove and cinnamon formulation--an in-vitro study. J Evol Med Dent Sci 2020;9:1859+.
14. Chellapa LR, Shanmugam R, Indiran MA, Samuel SR. Biogenic nanoselenium synthesis, its antimicrobial, antioxidant activity and toxicity. Bioinspired, Biomimetic and Nanobiomaterials 2020;9:184–9.
15. Shifa J, Gayathri R, Priya VV. Preliminary phytochemical analysis and total phenol content of aqueous fruit extract of Sambucus nigra. Drug Invention Today 2020.
16. Yamagishi S-I, Matsui T. Nitric oxide, a janus-faced therapeutic target for diabetic microangiopathy—Friend or foe? Pharmacol Res 2011;64:187–94.
17. Mahdi-Pour B, Jothy SL, Latha LY, Chen Y, Sasidharan S. Antioxidant activity of methanol extracts of different parts of Lantana camara. Asian Pac J Trop Biomed 2012;2:960–5.
18. Rajakumari R, Volova T, Oluwafemi OS, Rajesh Kumar S, Thomas S, Kalarikkal N. Grape seed extract-soluplus dispersion and its antioxidant activity. Drug Dev Ind Pharm 2020;46:1219–29.
19. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, et al. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. Front Endocrinol 2017;8:6.
20. More SGP, Jagtap RNS, Chippalkatti R. Antidiabetic and Antioxidant Properties of Copper Nanoparticles Synthesized by Medicinal Plant Dioscorea bulbifera. Journal of Nanomedicine & Nanotechnology 2015;s6. Avaialble:https://doi.org/10.4172/2157-7439.s6-007.
21. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species. Archives of Oral Biology 2018;94:93–8. Avaialble:https://doi.org/10.1016/j.archoralbio.2018.07.001.
22. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol 2019;90:1441–8.
23. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res 2020;43:153–4.
24. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. Heliyon. 2018;4:e01051.
25. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol 2020;17:668–9.
26. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol 2020;17:550–1.
27. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? Front Immunol 2020;11:1206.
28. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res 2020;43:74–5.
29. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Nat Prod Res 2021;35:1893–8.
30. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from Porphyromonas gingivalis with the bioactive compounds from Rosmarinus officinalis. Asian Biomed 2019;13:197–203.
31. 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 2020;82. Avaialble:https://doi.org/10.36468/pharmaceutical-sciences.650.
32. 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;64:93–9.
33. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent 2021;31:436–41.
34. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent 2021;31:285–6.
35. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol 2021;126:105132.
36. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci 2020;15:562–3.
37. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent 2020;18:379–86.
38. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res 2020;43:1459–61.
39. 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;32:3380–7.
40. Girija AS. Fox3 (+) CD25 (+) CD4 (+) T-regulatory cells may transform the nCoV's final destiny to CNS! COMMENT; 2021.
41. 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. Pesquisa Brasileira Em Odontopediatria E Clínica Integrada 2019;19:1–10. Avaialble:https://doi.org/10.4034/pboci.2019.191.61.
42. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease. Clin Exp Pharmacol Physiol 2017;44:327–34.
43. Sureshbabu NM, Selvarasu K, Jayanth KV, 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 Reports in Dentistry 2019;2019:1–6. Avaialble:https://doi.org/10.1155/2019/7046203.
44. Mohan M, Jagannathan N. Oral field cancerization: an update on current concepts. Oncol Rev 2014;8:244.
45. Menon S, Ks SD, R S, S R, S VK. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces 2018;170:280–92.
46. Sathvika K, Rajeshkumar S, Lakshmi T. Antidiabetic activity of silver nanoparticles synthesized using neem and Aloe vera plant formulation. Drug Invention 2019.
47. 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 2020;27:8166–75.
48. Rajeshkumar S, Sherif MH, Malarkodi C, Ponnanikajamideen M, Arasu MV, Al-Dhabi NA, et al. Cytotoxicity behaviour of response surface model optimized gold nanoparticles by utilizing fucoidan extracted from padina tetrastromatica. J Mol Struct 2021;1228:129440.
49. Roy A, Shanmugam R, Warrier D, Others. Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp Lethality Assay. Journal of Evolution of Medical and Dental Sciences 2020;9:2894–8.
50. Francis T, Rajeshkumar S, Roy A, Lakshmi T. Anti-inflammatory and Cytotoxic Effect of Arrow Root Mediated Selenium Nanoparticles. Pharmacognosy Journal 2020;12.
51. Sreenivasagan S, Subramanian AK, Rajeshkumar SRS. Assessment of antimicrobial activity and cytotoxic effect of green mediated silver nanoparticles and its

coating onto mini-implants. Annals of Phytomedicine: An International Journal 2020;9.
Avaialble:https://doi.org/10.21276/ap.2020.9.1.27.

52. Kalatehjari P, Yousefian M, Khalilzadeh MA. Assessment of antifungal effects of copper nanoparticles on the growth of the fungus Saprolegnia sp. on white fish (Rutilus frisii kutum) eggs. Egypt J Aquat Res 2015;41:303–6.

53. Jamdade DA, Rajpali D, Joshi KA, Kitture R, Kulkarni AS, Shinde VS, et al. Gnidia glauca- and Plumbago zeylanica-Mediated Synthesis of Novel Copper Nanoparticles as Promising Antidiabetic Agents. Adv Pharmacol Sci 2019;2019:9080279.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74402