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Green Synthesis of Silver Nanoparticles from Aloe Vera and Neem Leaf Extract and their Cytotoxic Effect evaluation

Joshua Narde[1], Nabeel Ahmed[2]*, Subhabrata Maiti[3], S Rajeshkumar[4], Dhanraj Ganapathy[5]

[1]Post Graduate Student, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077, Tamil Nadu,India

[2]Assistant Professor, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu,India

[3]Associate Professor, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu,India

[4]Department of Pharmacology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu,India

[5]Professor & Head, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu,India

***Corresponding author:** Nabeel Ahmed, Assistant Professor, Department of Prosthodontics and Implantology, Saveetha Dental College And Hospitals, Saveetha Institute Of Medical And Technical Sciences, Saveetha University, Chennai-600077,Tamil Nadu,India, Email: nabeel.5610@gmail.com

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ABSTRACT

Background: Traditional processes are used to create nanoparticles. Hazardous compounds are required as capping agents to maintain stability, resulting in environmental toxicity. As a result, "green synthesis" is required. As a result, the goal of this study was to see how cytotoxic selenium nanoparticles supplemented with aloe vera and neem leaf extract were used. The various medicinal properties of neem and aloe vera prompted us to use them both.

Aim: Aim of the study was green preparation of silver nanoparticles from aloe vera and neem leaf extracts and to check its cytotoxicity evaluation.

Material and methods: Cytotoxic effect of prepared selenium nanoparticles from banana stem was assessed using Brine Shrimp Assay respectively at 5 µL, 10 µL, 20 µL, 40 µL and 80µL.

Results: The selenium nanoparticle is safe to use in dental materials up to a concentration of 20 µL. Although cytotoxicity was observed at 40µL and 80µL concentrations.

Conclusion: Within the scope of the study, it is possible to conclude that selenium nanoparticles can be used safely in concentrations of up to 20µL.

Keywords: *silver nanoparticle, aloe vera, neem, cytotoxic effect, innovation*

INTRODUCTION

Nanotechnology is a relatively recent science that has ushered in a new era of scientific. The area of nanotechnology has attracted a lot of attention in the scientific community since Norio Taniguchi originally described it in 1974 and two IBM researchers who created the scanning tunnelling microscope experimentally confirmed it in 1981[1]. The introduction of atoms and molecule research has a significant impact on this scientific field.In recent years, nanoparticles have received a lot of attention in the scientific world. a great deal of coverage in recent years in the scentc community. Additionally, optical, electronics, biological, and materials sciences have benefited from this technique[2]. Lately the antioxidant, anti-inflammatory, antimicrobial and cytotoxic effects of the nanoparticle formulation have become an area of interest[2, 3].

The traditional investigational models have been modified because of nanomaterials. The need for lightweight, sustainable products, focused treatments, and improved diagnostic tools has made it possible to identify the useful contributions of nanomaterial[4]s. These nano-dimensions are being researched more and more in medicine. Their most crucial characteristic, a higher surface-volume ratio in comparison to the bulk size, boosts their activity in biological systems[5].

Pure, well-defined nanoparticles can be created by chemical and physical methods, but these processes are more expensive, energy-intensive, and potentially hazardous to the environment[5, 6] For the synthesis of nanoparticles, biosynthetic approaches can use either microorganism cells or plant extract[7]. The use of plant extracts in the biosynthesis reaction is a fascinating area of nanoparticle biosynthesis[8]. Recent years have seen the development of green techniques for nanoparticle synthesis into a significant area of nanotechnology[9][10][11]. The selection of plants for this paper was based on their potential for use in medicine.

Since ancient times, medicinal herbs have been a crucial source of medication[12]. Despite the availability of contemporary pharmaceuticals, a sizable percentage of the Indian population still prudently manage their health utilising herbal treatments. Neem leaves, or Azadirachta indica, offer a wide range of beneficial qualities, including immunological modulation, anti-inflammation, anti-hyperglycemic, anti-malarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic, and anticarcinogenic effects [13, 14][15].

Due to its anti-inflammatory, antibacterial, and wound-healing characteristics, aloe vera has long been used to treat digestive issues as well as skin injuries ranging from burns, wounds, insect bites to even eczema[16].The goal of this medicinal plant's research has been to confirm its traditional usage, understand its mode of action, and pinpoint the chemicals that are responsible for these effect since it has a wide of spectrum of functions from antimicrobial, anticancer, antidiabetic properties and also becoming a popular component of dental disease related medicine[17, 18]. Hence it seemed correct to formulate a mixture using these two plants.Our team has extensive knowledge and research experience that has translate into high quality publications [19–36] This study was done to assess the cytotoxicity of green sysnthesised silver nanoparticles with aloe vera and neem leaf extract.

MATERIALS AND METHODS

Aloe Vera and neem Leaf Extract Prepapartion

Organic aloe vera and neem leaf were hand-picked and rinsed several times in distilled water. A sterile knife was used to cut the leaves into little pieces, which were then crushed into fine particles with a mortar and pestle. The aloe vera and neem extract was concentrated to 1 M using 1g of this extract in 100ml of distilled water. The boiled extract was filtered using Whatmann No. 1 filter paper. For future experiments, the filtrates were stored at 5 °C.

Synthesis of Silver Nanoparticles

Aloe vera and neem leaf extract were used as aqueous extracts for the bioreduction process. In order to promote the biosynthesis of AgNPS, 0.1mM silver nitrate was dissolved in 100ml of distilled water and swirled for a brief length of time. Then, drop by drop, 5ml of filtered neem

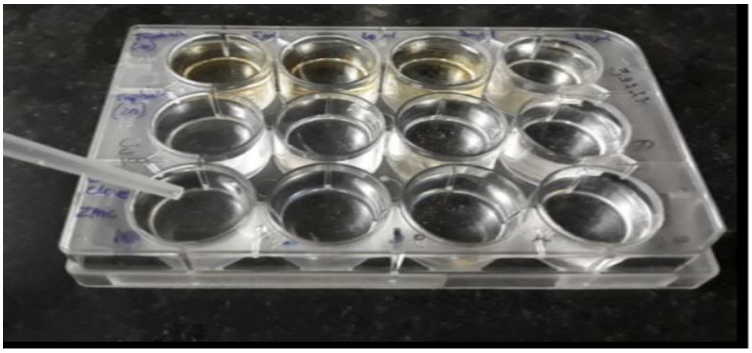
leaf extract and aloe vera extract were added. The solution combination was swirled in a magnetic stirrer at a speed of 650–800 rpm for 72 hours. The reaction mixture's colour changes were continuously monitored using a twin beam UV visible spectrophotometer at various wavelengths between 25 and 650 nm. Neem leaf extract and aloe vera extract were used to make silver nanoparticles, which were then centrifuged at 8000 rpm for ten minutes.

UV-vis Spectroscopy

The integrated oscillations of conduction band electrons on the surface of metal nanoparticles in resonance with light led to the discovery of the Surface Plasmon Resonance absorption band. A UV-vis spectrometer was used to examine how the nanocomposite developed.

Cytotoxic Effect

Using the brine shrimp assay, the cytotoxicity of silver nanoparticle extract was assessed. The culture of the shrimp eggs were done in iodine-free salt in water to which sodium bicarbonate was added as a source of nutrition.12 well ELISA(Figure 1) plates were utilized and to it were added 10 nauplii to the well after adding 6-8 ml of saltwater to each dish. Each well received different amounts of silver nanoparticles (5 litres, 10 litres, 20 litres, 40 litres, and 80 litres), which were then incubated for 24 hours[37]



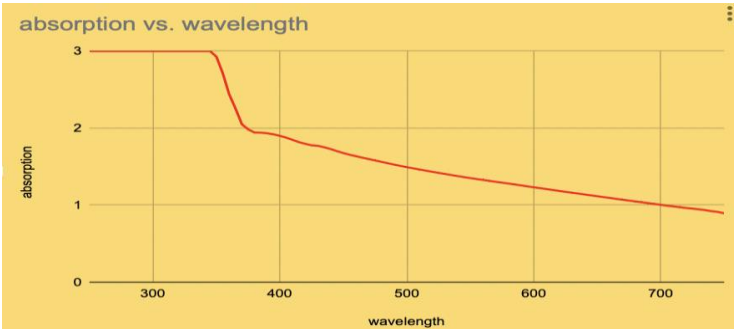
After 24 hours, the mortality rate was determined by counting all live and dead nauplii.The formula used was:

Cytotoxicity= number of dead nauplii/number of dead nauplii+number of live nauplii×100

RESULTS

UV-vis absorption study

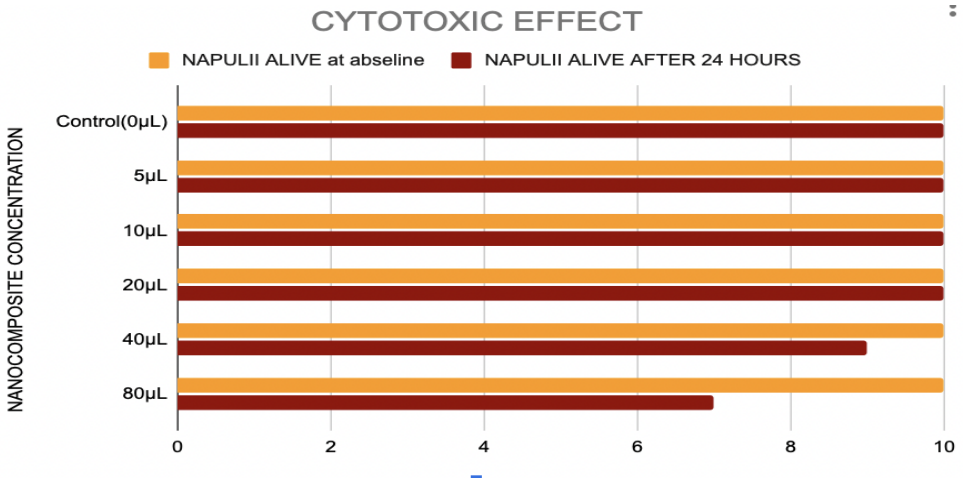
The figure shows the colloidal solution of leaf extract and aqueous silver nitrate's UV-vis absorption spectra readings. The silver salt solution was colourless at first and then changed to brown when aloe vera and neem leaf extract was added to the reaction mixture. The production of Ag NPs in the aqueous medium is firmly confirmed by this change in colour of the silver solution, which is clearly visible. In accordance with other studies, the values were seen closer to the 370-400nm point at the 6 hour mark[38].(Figure 2)



Cytotoxicity studies

The cytotoxicity of silver nanoparticles enhanced with aloe vera and neem leaf is shown in Table 1. Up to a concentration of 20µL, 100% of the nauplii samples that were alive at the end of the test time died. At 40µL, a 10% death rate was seen, while at 80 L, a 30% death rate was observed(Figure 3)The cytotoxicity of the nanoparticles increased as the concentration was raised.

Nanocomposite Concentration	Napulii Alive After 24 Hours	Death Percentage
Control(0µL)	10	0%
5µL	10	0%
10µL	10	0%
20µL	10	0%
40µL	9	10%
80µL	7	30%



DISCUSSION

Nanotoxicity, a booming scientific subject, has sprung up as a result of the increasing use of nanoparticles, mostly in commercially available things. The dangers of nanomaterials are yet unknown (Shanmugam Rajeshkumar et al. 2013). For this purpose, extensive procedures are being carried out to analyse the harmful impacts of these nanomaterials. Nanotoxicity has emerged as a vigorous research area as a result of the increased use of nanoparticles, primarily in commercially available products. Nanomaterials' risk factors are still unknown. The ability of certain chemicals or mediator cells to destroy living cells is referred to as cell cytotoxicity[39]

Healthy living cells can be induced to undergo necrosis (accidental cell death) or apoptosis by using a cytotoxic compound (programmed cell death). Given this information, the ability to precisely measure cytotoxicity can be a very useful tool in identifying compounds that may pose certain health risks in humans[40]).This is especially important during the research phase of developing new pharmaceutical treatments to ensure end-user safety[41] Because of the low cost, ease of use, long life span, and rapid screening technique of brine shrimp lethality assays, many researchers have recently focused on them[42].Artemia salina, a brine shrimp, is commonly used in drug research to determine the toxicity of various components. The brine shrimp assay was proposed by Michael et al. (1956), and it was later developed by Vanhaecke et al.

Previously, conventional methods were used to create nanoparticles. Even though conventional physical and chemical methods require less time to synthesise large quantities of nanoparticles, toxic chemicals are required as capping agents to maintain stability. Because of the use of toxic chemicals, these methods resulted in

environmental toxicity[43] The Green Synthesis method was proposed to avoid the use of such chemicals, and it is now widely used all over the world. It is both environmentally friendly and cost effective[44][45]. As a result, we conducted this study to assess the cytotoxicity of silver nanoparticles with the aloe vera and neem leaf extract.

The aloe vera and neem leaf extracts, which contain functional substances such as cyclic peptides, sorbic acid, citric acid, euphol, polyhydroxy limonoids, ascorbic acid, retinoic acid, tannins, ellagic acid, and gallic acid, are thought to play an important role in the bioreduction and stabilisation of nanoparticles[46][47].Neem leaf extract can produce silver nanoparticles that are stable in solution and have a good surface Plasmon resonance behaviour when exposed to UV-VIS light[48]

Dental disease is caused by an immune reaction between pathogenic bacteria and the host. Periodontal tissue destruction causes an overproduction of lipid peroxides, inflammatory mediators, and oxidised proteins. More reactive oxygen species are produced by macrophages, neutrophils, and fibroblasts when these substances are present (ROS). Because it scavenges reactive oxygen species and protects antioxidant enzymes like SOD, which are vital for cellular defence, aloe vera and neem leaf extract is a good antioxidant[49].

CONCLUSION

Silver nanoparticles derived from aloe vera and neem leaf stem revealed promising cytotoxic effect against live shrimp eggs within the study's parameters. More research on cytotoxicity and antibacterial efficacy against periodontal pathogens is required.

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REFERENCES

1. Khan I, Saeed K, Khan I (2019) Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry 12:908–931
2. Rico CM, Majumdar S, Duarte-Gardea M, Peralta-Videa JR, Gardea-Torresdey JL (2011) Interaction of Nanoparticles with Edible Plants and Their Possible Implications in the Food Chain. Journal of Agricultural and Food Chemistry 59:3485–3498
3. Shanmugam R, Subramaniam R, Kathirason SG, Ali D, Balusamy SR, Gurusamy A, Arunachalam K, Sellami H (2021) Curcumin-Chitosan Nanocomposite Formulation Containing - Mediated Silver Nanoparticles, Wound Pathogen Control, and Anti-Inflammatory Potential. Biomed Res Int 2021:3091587
4. Nicolae-Maranciuc A, Chicea D, Chicea LM (2022) Ag Nanoparticles for Biomedical Applications-Synthesis and Characterization-A Review. Int J Mol Sci. https://doi.org/10.3390/ijms23105778
5. Hussain CM (2018) Handbook of Nanomaterials for Industrial Applications. Elsevier
6. Okafor F, Janen A, Kukhtareva T, Edwards V, Curley M (2013) Green Synthesis of Silver Nanoparticles, Their Characterization, Application and Antibacterial Activity. International Journal of Environmental Research and Public Health 10:5221–5238
7. Kathiraven T, Sundaramanickam A, Shanmugam N, Balasubramanian T (2015) Green synthesis of silver nanoparticles using marine algae Caulerpa racemosa and their antibacterial activity against some human pathogens. Applied Nanoscience 5:499–504
8. Orbaek AW, McHale MM, Barron AR (2015) Synthesis and Characterization of Silver Nanoparticles for an Undergraduate Laboratory. Journal of Chemical Education 92:339–344
9. Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R, Narayana Rao D (2010) Biosynthesis of Silver Nanoparticles Using Coriandrum Sativum Leaf Extract and Their Application in Nonlinear Optics. Advanced Science Letters 3:138–143
10. Gardea-Torresdey JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose-Yacaman M (2003) Alfalfa Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles. Langmuir 19:1357–1361
11. Umashankari J, Inbakandan D, Ajithkumar TT, Balasubramanian T (2012) Mangrove plant, Rhizophora mucronata (Lamk, 1804) mediated one pot green synthesis of silver nanoparticles

and its antibacterial activity against aquatic pathogens. Aquatic Biosystems. https://doi.org/10.1186/2046-9063-8-11
12. Hoque MDM, Mahfuzul Hoque MD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S (2007) Antibacterial Activity of Guava (Psidium guajavaL.) and Neem (Azadirachta indicaA. Juss.) Extracts Against Foodborne Pathogens and Spoilage Bacteria. Foodborne Pathogens and Disease 4:481–488
13. Sandanasamy JD (2013) Fatty Acid Composition and Antibacterial Activity of Neem (Azadirachta indica) Seed Oil. The Open Conference Proceedings Journal 4:43–48
14. Mahmood MS, Shahid Mahmood M (2018) Evaluation of Antiviral Activity of Azadirachta indica (Neem) Bark Extract against Newcastle Disease Virus. Pakistan Veterinary Journal 38:25–28
15. Alzohairy MA (2016) Therapeutics Role ofAzadirachta indica(Neem) and Their Active Constituents in Diseases Prevention and Treatment. Evidence-Based Complementary and Alternative Medicine 2016:1–11
16. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP (2020) Pharmacological Update Properties of and its Major Active Constituents. Molecules. https://doi.org/10.3390/molecules25061324
17. Pradeep AR, Garg V, Raju A, Singh P (2016) Adjunctive Local Delivery of Aloe Vera Gel in Patients With Type 2 Diabetes and Chronic Periodontitis: A Randomized, Controlled Clinical Trial. J Periodontol 87:268–274
18. Yeturu SK, Acharya S, Urala AS, Pentapati KC (2016) Effect of Aloe vera, chlorine dioxide, and chlorhexidine mouth rinses on plaque and gingivitis: A randomized controlled trial. J Oral Biol Craniofac Res 6:54–58
19. Neelakantan P, Grotra D, Sharma S (2013) Retreatability of 2 mineral trioxide aggregate-based root canal sealers: a cone-beam computed tomography analysis. J Endod 39:893–896
20. Aldhuwayhi S, Mallineni SK, Sakhamuri S, Thakare AA, Mallineni S, Sajja R, Sethi M, Nettam V, Mohammad AM (2021) Covid-19 Knowledge and Perceptions Among Dental Specialists: A Cross-Sectional Online Questionnaire Survey. Risk Manag Healthc Policy 14:2851–2861
21. Sheriff KAH, Ahmed Hilal Sheriff K, Santhanam A (2018) Knowledge and Awareness towards Oral Biopsy among Students of Saveetha Dental College. Research Journal of Pharmacy and Technology 11:543
22. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, Pathak Y, Marofi F, Shamlou S, Hassanzadeh A (2021) Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. Stem Cell Res Ther 12:192
23. Jayaraj G, Ramani P, Herald J. Sherlin, Premkumar P, Anuja N (2015) Inter-observer agreement in grading oral epithelial dysplasia – A systematic review. Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology 27:112–116
24. Paramasivam A, Priyadharsini JV, Raghunandhakumar S, Elumalai P (2020) A novel COVID-19 and its effects on cardiovascular disease. Hypertens Res 43:729–730
25. Li Z, Veeraraghavan VP, Mohan SK, et al (2020) Apoptotic induction and anti-metastatic activity of eugenol encapsulated chitosan nanopolymer on rat glioma C6 cells via alleviating the MMP signaling pathway. Journal of Photochemistry and Photobiology B: Biology 203:111773
26. Gan H, Zhang Y, Zhou Q, Zheng L, Xie X, Veeraraghavan VP, Mohan SK (2019) Zingerone induced caspase-dependent apoptosis in MCF-7 cells and prevents 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis in experimental rats. J Biochem Mol Toxicol 33:e22387
27. Dua K, Wadhwa R, Singhvi G, et al (2019) The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. Drug Dev Res 80:714–730
28. Mohan M, Jagannathan N (2014) Oral field cancerization: an update on current concepts. Oncol Rev 8:244
29. Agarwal S, Ashok V, Maiti S, Agarwal V (2022) Dentists' Preference toward Fixed Versus Removable Implant Prosthesis on Edentulous Jaws to Improve Quality of Life. J Long Term Eff Med Implants 33:83–89
30. Kushali R, Maiti S, Girija SAS, Jessy P (2022) Evaluation of Microbial Leakage at Implant Abutment Interfact for Different Implant Systems: An In Vitro Study. J Long Term Eff Med Implants 32:87–93
31. Aparna J, Maiti S, Jessy P (2021) Polyether ether ketone - As an alternative biomaterial for Metal Richmond crown-3-dimensional finite element analysis. J Conserv Dent 24:553–557
32. Ponnanna AA, Maiti S, Rai N, Jessy P (2021) Three-dimensional-Printed Malo Bridge: Digital Fixed Prosthesis for the Partially Edentulous Maxilla. Contemp Clin Dent 12:451–453

33. Venugopalan S, R S, N T, Maiti S (2022) An Analysis of Implant Prosthesis and Its Dynamic Occlusal Contacts. J Long Term Eff Med Implants 33:1–7
34. Merchant A, Ganapathy DM, Maiti S (2022) Effectiveness of local and topical anesthesia during gingival retraction. Brazilian Dental Science 25:e2591
35. Agarwal S, Ashok V, Maiti S (2020) Open- or Closed-Tray Impression Technique in Implant Prosthesis: A Dentist's Perspective. J Long Term Eff Med Implants 30:193–198
36. Agarwal V, Nallaswamy D, Suresh V, Ganapathy D, Sivasamy V, Agarwal S (2022) Effectiveness Of Cad Cam Milled Vs Dmls Titanium Framework for Hybrid Denture Prosthesis - A Systematic Review. Journal of Pharmaceutical Negative Results 544–571
37. Kanagesan S, Aziz SBA, Hashim M, Ismail I, Tamilselvan S, Alitheen NBBM, Swamy MK, Purna Chandra Rao B (2016) Synthesis, Characterization and in Vitro Evaluation of Manganese Ferrite (MnFe2O4) Nanoparticles for Their Biocompatibility with Murine Breast Cancer Cells (4T1). Molecules 21:312
38. Alim-Al-Razy M, Asik Bayazid GM, Rahman RU, Bosu R, Shamma SS (2020) Silver nanoparticle synthesis, UV-Vis spectroscopy to find particle size and measure resistance of colloidal solution. Journal of Physics: Conference Series 1706:012020
39. Mohapatra S, Leelavathi L, Rajeshkumar S, D. SS, P. J (2020) Assessment of Cytotoxicity, Anti-Inflammatory and Antioxidant Activity of Zinc Oxide Nanoparticles Synthesized Using Clove and Cinnamon Formulation - An In-Vitro Study. Journal of Evolution of Medical and Dental Sciences 9:1859–1864
40. Rajan S, Marimuthu K, Balaji Ayyanar C, Khan A, Siengchin S, Rangappa SM (2022) In-vitro cytotoxicity of zinc oxide, graphene oxide, and calcium carbonate nano particulates reinforced high-density polyethylene composite. Journal of Materials Research and Technology 18:921–930
41. El-Rafie HM, Abd El-Aziz SM, Zahran MK (2022) In vitro cytotoxicity against breast cancer using biogenically synthesized gold and iron oxide nanoparticles derived from the hydroethanolic extract of Salvia officinalis L. Chemical Papers. https://doi.org/10.1007/s11696-022-02464-x
42. Francis T, Rajeshkumar S, Roy A, Lakshmi T (2020) Anti-inflammatory and Cytotoxic Effect of Arrow Root Mediated Selenium Nanoparticles. Pharmacognosy Journal 12:1363–1367
43. Chandran SP, Chaudhary M, Pasricha R, Ahmad A, Sastry M (2006) Synthesis of Gold Nanotriangles and Silver Nanoparticles Using Aloe vera Plant Extract. Biotechnology Progress 22:577–583
44. Sadowski Z (2013) Green synthesis of silver and gold nanoparticles using plant extracts. Green biosynthesis of nanoparticles: mechanisms and applications 79–91
45. Mariychuk R, Yakovlieva V, Porubska J, Eliasova A, Lisnyak VV (2019) Green Synthesis of Silver and Gold Nanoparticles Using Extract of Blackcurrant. 2019 International Council on Technologies of Environmental Protection (ICTEP). https://doi.org/10.1109/ictep48662.2019.8968965
46. (2015) Plant extract synthesized silver nanoparticles: An ongoing source of novel biocompatible materials. Ind Crops Prod 70:356–373
47. Krishnaraj C, Jagan EG, Ramachandran R, Abirami SM, Mohan N, Kalaichelvan PT (2012) Effect of biologically synthesized silver nanoparticles on Bacopa monnieri (Linn.) Wettst. plant growth metabolism. Process Biochemistry 47:651–658
48. Deka NJ, Nath R, Tamuly S, Hazorika M, Pegu SR, Deka SM (2021) Green synthesis and characterization of silver nanoparticles using leaves extract of Neem (Azadirachta indica L.) and assessment of its in vitro antioxidant and antibacterial activity. Annals of Phytomedicine: An International Journal. https://doi.org/10.21276/ap.2021.10.1.17
49. Bahuguna G, Kumar A, Mishra NK, Kumar C, Bahlwal A, Chaudhary P, Singh R (2016) Green synthesis and characterization of silver nanoparticles using aqueous petal extract of the medicinal plantCombretum indicum. Materials Research Express 3:075003