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Research Article

Green Synthesis of Selenium Nanoparticles Mediated Aqueous extract of Euphorbia Tirucalli and its Antimicrobial and Cytotoxic Activities

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Abstract

Background: Nanotechnology is focused on the development of nanoparticles of various sizes and chemical compositions as well as their use in health research for human benefits. Selenium nanoparticles (SeNPs) are well-known bioactive compounds. Selenium nanoparticles are synthesized by various chemical and biological methods. We have formulated Euphorbia tirucalli-mediated selenium nanoparticles and studied their antimicrobial and cytotoxic activities.

Aim: This study aims to formulate a selenium-mediated aqueous extract of Euphorbia tirucalli and to evaluate the antimicrobial activity and cytotoxicity against oral pathogens.

Materials and methods: 0.5 mg of Sodium selenite in distilled water was added to 40 ml of Euphorbia tirucalli plant extract. The preparation of the selenium-mediated aqueous extract was examined by UV Vis - Spectrometry at the wavelength of 300-650 nm. Further evaluation for its antimicrobial activity was performed by measuring the zone of inhibition against oral pathogens. By using Brine Shrimp Lethality Assay (BSLA), the cytotoxicity was determined.

Results: The selenium-mediated aqueous Euphorbia tirucalli extract characterization showed maximum absorbance at 525 nm. The Agar well diffusion method showed the maximum ZOI in Staphylococcus aureus at 18mm at 50 ug/ml and in Lactobacillus at 18 mm at 50 ug/ml. Using the BSLA method, the cytotoxicity increased with increasing concentration of the extract, showing 10 alive nauplii at 5ul and 8 alive nauplii at 80ul.

Conclusion: The green synthesis of selenium-mediated Euphorbia tirucalli showed a potent antimicrobial activity and reduced lethality.

Keywords: Selenium nanoparticles; Euphorbia tirucalli; Antimicrobial; Cytotoxicity

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Introduction

Over the past ten years, the application of nanotechnology in medicine has recently made significant advances, and recent advancements in this field have thrown up interesting possibilities for the future of healthcare (1)(2). Nanoparticles have drawn a lot of interest because of their electrical, optical, photoresponsive, and catalytic properties (3). Selenium is a crucial trace element for humans. When combined with other nanoparticles, selenium nanoparticles show improved biocompatibility, bio adequacy, decreased toxicity, excellent cell reinforcement movement, and disease-preventive features (4). Previous study reported by Zhang et al showed the synthesis of SeNp biogenic with Bacillus pumilus and H2O2 (5). Few other studies demonstrated the anticancer activity of Se-NP by Spirulina platensis synthesis and the antimicrobial activity of Stenotrophomonas maltophilia and Bacillus mycoides (6,7). Nanoparticles are synthesized by various methods like physical, chemical, and biological methods. However, the synthesis by conventional methods, including physical and chemical methods has many drawbacks, such as being time-consuming, high costs, and producing toxic by-products (8)(9). There is a need to develop non-toxic eco-friendly, safe, cost-effective nanoparticles. This green synthesis is carried out by using fruits, plants, bacteria, fungi, and algae (3). The reaction involved in this process is a bottom-up approach with oxidation and reduction of the biomolecules (7). Several researchers have reported the synthesis of SeNPs using fenugreek extract, Cassia auriculata extract, E. officinalis fruit extract, microbial polymers, Boulardii yeast cells, and Penicillium chrysogenum (4)(8). There is less evidence of SeNP synthesis in Euphorbia Tirucalli extract. A new technique for producing selenium nanoparticles rapidly using the plant latex of E. Tirucalli has been reported in the current study.

E. tirucalli belongs to the family Euphorbiaceae, otherwise known as Alveloz, Pencil cactus. This plant is a shrub with twigs resembling pencils, hence named pencil cactus (10). Several investigators have identified the pharmacological effects of E. tirucalli, including its molluscicidal, antibacterial, anti-herpes, and antimutagenic activity (10). Moreover, latex also exhibits anti-carcinogenic properties (11,12). The latex of E. tirucalli is used in northeastern Brazil as a laxative, antibiotic, and parasite control agent as well as to cure verrucae, cancer, chancre, epithelioma, sarcoma, skin tumors, and as a traditional medicine for syphilis (12). Previous study reports concluded that the organic extract of E.tirucalli was more effective against the dental pathogen when compared to the aqueous extract (12). Previous literature by Sudheer Kumar et al succeeded in synthesizing zinc nanoparticles by using Euphorbia tirucalli latex extract through the green synthesis method (13). Also, Kgosiemang et al described that biosynthesized CuNPs using latex of Euphorbia tirucalli showed antiproliferative activity against breast cancer (14). Hence, the present study aimed to synthesize selenium nanoparticles using E. tirucalli and assess their Antimicrobial and Cytotoxic activity through the green synthesis method. This was novel and the first to report the synthesis of SeNP using Euphorbia tirucalli stem extract.

Materials and Methods

Preparation of stem extract

Fresh stems of Euphorbia tirucalli were collected from the Herbal garden of Saveetha Dental College and Hospital, and thoroughly cleaned three times with running tap water and once with distilled water. The stem was cut into pieces of approximately 15 gms. Then, it was crushed using a mortar and pestle to which 100 ml of distilled water was added. Followed by it, the extract was boiled for 15 min at 60°C

Synthesis of E. tirucalli-mediated selenium nanoparticle extract

Accurately measured 0.5 mg of sodium selenite was added to the 60 ml of distilled water. It was then kept in an orbital shaker with a magnetic stirrer for nanoparticle synthesis. 40 ml of filtered E.tirucalli aqueous extract was added to it gradually and stirred at 800 rpm in a magnetic stirrer. (Figure 1) The color change was observed visually and photographs were obtained. (Figure 2) Using a Lark refrigerated centrifuge, the finished product was centrifuged for 10 minutes at 8000 rpm. The pellets were collected, and the purified pellet was dried at 60°C for 2 hours.

Figure 1: Preparation of aqueous extract of Euphorbia tirucalli

Figure 2: The color change noticed to dark brown after the reaction with the sodium selenite solution

Characterization of nanoparticle UV visible spectrophotometer of selenium nanoparticle

Double UV-visible spectroscopy was used to analyze the optical properties of synthesized nanoparticles at 300- 650 nm. The results were recorded and a graphical analysis was done.

Antimicrobial activity of SeNP-induced stem extract

The Agar well diffusion method was used to determine the antimicrobial activity of E.tirucalli extract nanoparticles. Mueller Hinton Agar was produced and autoclaved at 121'C for 15 to 20 minutes. On the sterile Petri plates, the sterile MHA media was poured and allowed to solidify. After solidification, bacteria such as Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis, and Lactobacillus were swabbed with sterile cotton buds. With the help of well cutter wells, we're made. In a plate with four wells, three wells contained SeNP-mediated Euphorbia tirucalli pellet solution at concentrations of 25, 50, and 100 µl, while the fourth well contained a standard antibiotic (Amoxicillin). Following that, the plates were incubated for 24 hours at 37°C. Following incubation, the plates were examined and the Zone of Inhibition around the wells loaded with nanoparticles was measured in mm. The Rose Bengal Agar was prepared for Candida albicans and the inoculated plates were incubated at 37°C for 48 hours. The measured value was tabulated. (Figure 3 & 4)

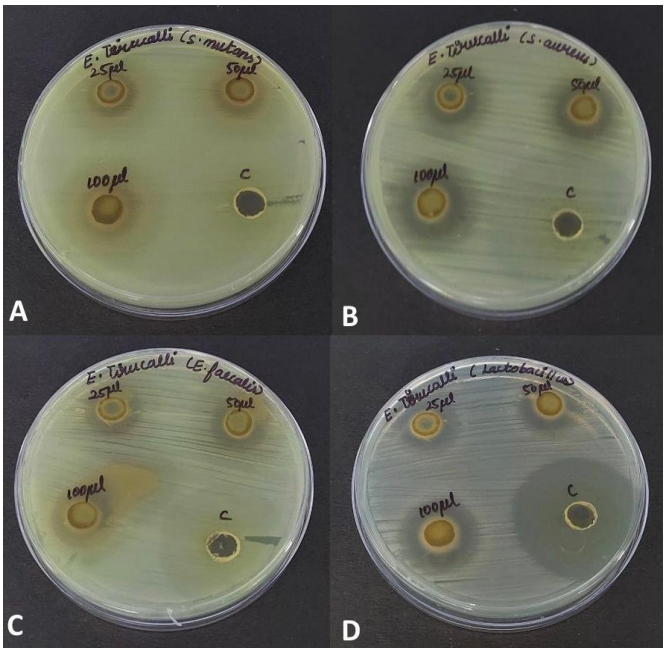

Figure 3: Represents the zone of inhibition of the SeNPs mediated Euphorbia Tirucalli extract against the oral microbiome inoculated in agar diffusion plate with 5mm wells (a) Streptococcus mutans (b) Staphylococcus aureus, (c) Enterococcus faecalis (d) Lactobacillus

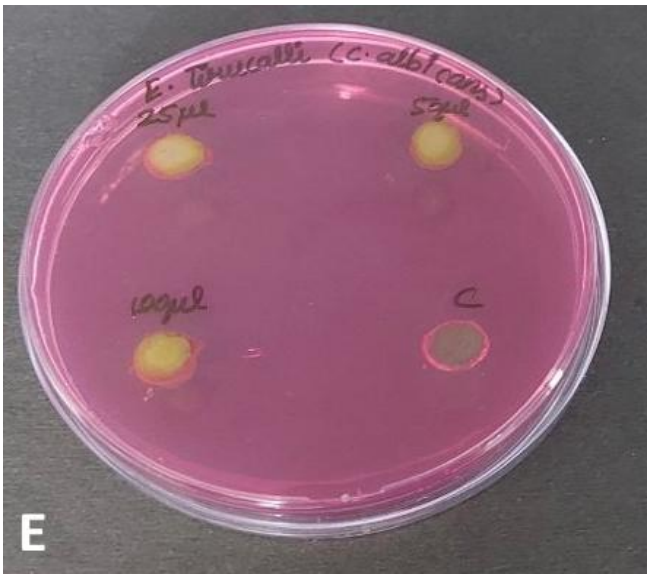

Figure 4: Represents the zone of inhibition of SeNPs mediated Euphorbia Tirucalli extract against fungal organism candida albicans.

Cytotoxicity by Brine Shrimp lethality assay (BSLA)

6 wells that had 10 newly hatched larvae (Nauplii) of the brine shrimp (Artemia salina) were used. Each well was exposed to a different concentration of the synthesized E.tirucalli SeNP aqueous extract 5, 10, 20, 40, and 80 µl. One well containing live nauplii served as the standard sample, as this well was not treated with selenium nanoparticles. It was undisturbed for 48 hours. After 48 hours, the number of live nauplii was recorded, and the data was graphed. (Figure 5 &7)(Table 1).

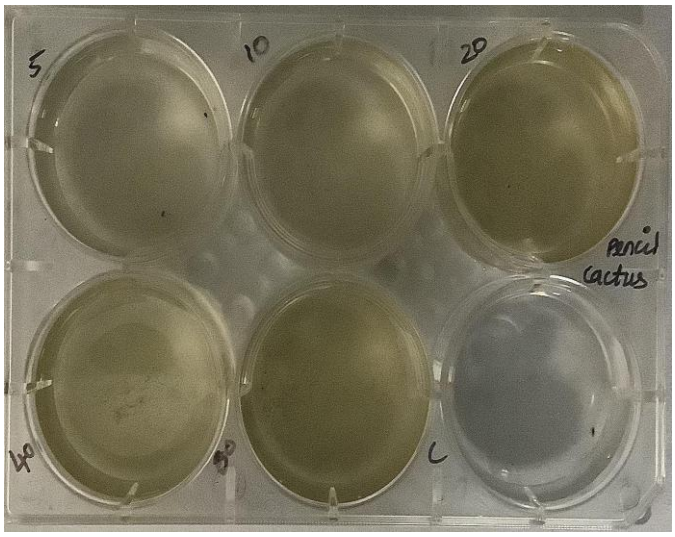

Figure 5: Represents the Brine shrimp lethality assay (BSLA) used to evaluate the cytotoxicity of the extract

Table 1: Cytotoxic Activity (BSLA)	
Concentration	No. of live nauplii
control	10
5ul	10
10ul	10
20ul	9
40ul	8
80ul	8

Table 2: Zone of inhibition against the evaluated oral microbiome					
	Zone of inhibition pathogens (mm)				
Concentration	Streptococcus mutans	Staphylococcus aureus	Enterococcus faecalis	Candida albicans	Lactobacillus
control	9	40	36	16	38
25ul	14	16	16	12	18
50ul	15	18	14	14	16
100ul	13	16	13	12	14

Results

UV visible spectrophotometric analysis

The maximum absorbance was determined by using a spectrophotometer between the wavelength range of 200 and 800 nm. The presence of selenium in the samples was confirmed by the absorbance scan, which was based on the observation of a significant Plasmon resonance peak at 525 nm. Previous investigations have noted that the strong Plasmon peak of spherical Se-NPs was seen throughout the UV-visible spectrum in the range of 250-400 nm.

Antimicrobial activity

The antimicrobial activity of the E.tirucalli-mediated SeNP was evaluated in this study by using agar well diffusion methods. (Table 2) shows the antimicrobial activities of E.tirucalli expressed in the size of the inhibition zone of microbial growth. The zone of inhibition was mainly measured against the oral pathogens including antimicrobial and antifungal organisms. The prepared E.tirucalli-mediated SeNps extracts showed a dose-dependent area of inhibition in all the test microorganisms. The maximum ZOI was noted in gram-positive organisms in Staphylococcus aureus with 18mm at 50 µl and Lactobacillus at 18mm at 50 µl. The ZOI for Enterococcus faecalis and Streptococcus mutans was 16mm and 15 mm respectively. The lowest inhibitory value was noted with Candida albicans with 12 mm at 25 µl conc. (Figure 6)

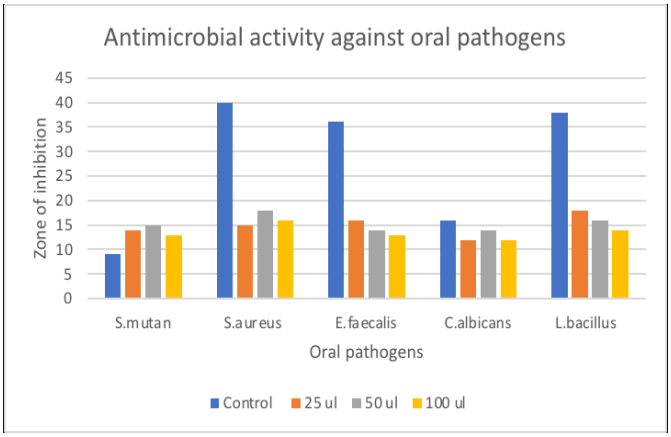

Figure 6: Graphical representation of the ZOI of the extract against the oral microorganism.

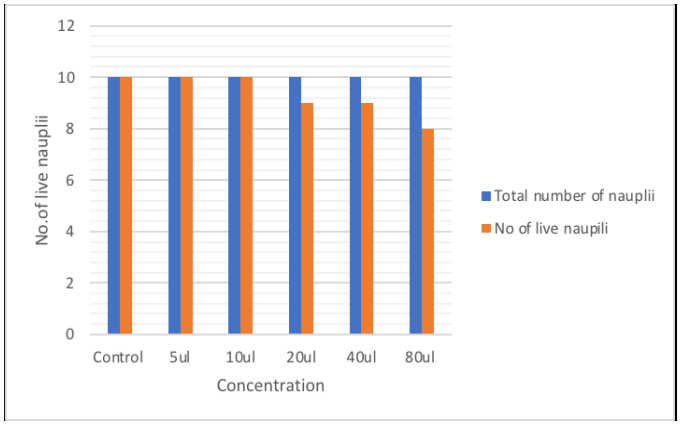

Figure 7: Graphical representation of the cytotoxic activity of the SeNPS stem extract of E.tirucalli

Discussion

Nanotechnology has emerged as an important and dynamic area of scientific interest in the world. Nanotechnology is becoming more popular among researchers because of its advantages in the medical and pharmaceutical industries, such as reduced risk and side effects, rapid action with lower dosages due to higher bioavailability, increased therapeutic effects, and medical problems like cancers (15)(16). One of the most important problems faced by contemporary medicine is the discovery of efficient drug delivery techniques that might enhance the therapeutic profile and efficacy of therapeutic agents (17). According to reports given by Global Public Health in 2019, 2.3 billion people worldwide have permanent tooth decay, and 530 million children have primary tooth decay (18). There is ample evidence associating oral diseases with the risk of colorectal cancer, bleeding gums, toothaches, preterm birth in expectant mothers, chronic renal disease, myocardial infarction, and stroke (18,19). These oral pathogens frequently include Gram-positive and Gram-negative bacteria that are associated with the development and progression of caries, including Veillonella species, Atopobium species, Prevotella species, Streptococcus mutans, Lactobacillus species, Enterococcus faecalis, and several non-mutants streptococcus (20). It has become necessary to look for alternative treatments that are relatively less expensive, easily accessible, effective, and safe due to the prevalence of oral disease, increased microbial resistance, and fewer adverse effects associated with some conventional oral care agents currently used in oral care (21). Antimicrobial resistance has become the biggest global health threat and it threatens people of all ages. The increasing resistance of various infections to common drugs has led to an increase in the number of infections.

In biological systems, selenium is a crucial micronutrient. SeNPs have extensive applications in nanomedicine due to their antibacterial, anticancer, and antioxidant properties, and their cytotoxicity is lower than that of the most commonly used silver nanoparticles (22)(23)(22). Nanoscale selenium, an essential trace element, has demonstrated potent antibacterial and anticancer activities (23). The herb Euphorbia L, otherwise called pencil cactus. This plant is a member of the Euphobiaceae family, well-known for producing milky latex which is characteristic of this family. It's widely known that it has gained importance in traditional medicine for the treatment of a variety of medical conditions, including ear infections, diarrhea, coughs, and dysentery (10). Mali et al concluded that the methanolic and ethanolic extracts of the plant Euphorbia Tirucalli have been shown to have antibacterial and antifungal activity. (24) Annamalai et al reported the antibacterial activity of E.tirucalli mediated by AuNps against bacterial strains of Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumonia was highly effective (25). However, there is no study reported showing the combined activity of selenium and Euphorbia tirucalli. This study was done to evaluate the

antimicrobial and cytotoxic activity of selenium nanoparticles mediated E.tirucalli aqueous stem extract.

In our study, the UV-Vis spectra confirmed the biosynthesis of SeNPs, since the color of the product changed to brown color due to selenious acid, which has an absorption maximum (kmax) at 525 nm. Similar study by Miglani et al reported that the highest absorbance peak was observed at 388 nm with selenium nanoparticles mediated with guava leaf extract against enterococcus (26). The wide surface Plasmon resonance peak strongly demonstrates the polydispersity of the SeNPs. This color shift could be the result of a broad peak in the surface plasmon resonance (SPR), and as the time progressed from 5 to 1,440 min, the peak intensity of the color change increased (Fig.2)

Antimicrobial activity in the present study revealed that the zone of inhibition was maximum in the gram-positive organism S.aureus with 18mm at 50 µl concentration. A lower zone of inhibition has been seen in fungal organisms in C.albicans with 12 mm at 25 µl concentration. Our results were in concordance with previous literature showing maximum inhibition concentration at 1000 µg concentration for the methanolic extract of E. tirucalli was reported against S. aureus and B. subtilis (27)(28). Similar results have been found with acetone extract of E.tirucalli showing MIC of 500 µg for C. albicans and 750 µg for A. niger and A. fumigatus (27). The antimicrobial activity can be due to the presence of tannins which are the surfactant and may inactivate the microbial adhesion and are also complex with membrane polysaccharides.

The cytotoxic activity test of E.tirucalli selenium nanoparticles shows that at lower concentrations, the cytotoxic activity of the nanoparticles is reduced. The nauplii alive at 5 µl is 8 which was the maximum and the lowest was 8 at 80 µl. This is consistent with a previous study of SeNP with 7 live nauplii at 5 µl (29). This demonstrates that E.tirucalli-mediated selenium nanoparticle compositions that are dosage dependent may prove to be less toxic and safe for medicinal treatments.

From the results illustrated the synthesized aqueous extract of Euphorbia tirucalli mediated by selenium nanoparticles showed an effective antimicrobial activity against the oral pathogens and showed reduced lethality with the number of live nauplii at lower concentrations was increased when compared to the higher concentration. Hence this extract along with selenium nanoparticles could be used as an antimicrobial agent in oral care products. The development of caries-causing bacteria may be reduced by adding selenium nanoparticles to toothpaste, chewing gum, and mouthwashes.

Conclusion

Being a significant class of nanoparticles, selenium nanoparticles have numerous medical and commercial uses. We concluded that selenium nanoparticles mediated E.tirucalli extract has antibacterial effects against the important oral bacteria. Further studies are required to find these effects to replace synthetic medications with natural remedies and compare the different extracts of E.tirucalli against different bacterial species using various nanoparticles.

Conflict of interest: Nil

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Reference

Anjum S, Ishaque S, Fatima H, Farooq W, Hano C, Abbasi BH, et al. Emerging Applications of Nanotechnology in Healthcare Systems: Grand Challenges and Perspectives. Pharmaceuticals [Internet]. 2021 Jul 21;14(8). Available from: http://dx.doi.org/10.3390/ph14080707

Lal RKS. Synthesis of Organic Nanoparticles and their Applications in Drug Delivery and Food Nanotechnology: A Review [Internet]. Vol. 03, Journal of Nanomaterials & Molecular Nanotechnology. 2014. Available from: http://dx.doi.org/10.4172/2324-8777.1000150

Website [Internet]. Available from: Singh, J., Dutta, T., Kim, KH. et al. "Green" synthesis of metals and their oxide nanoparticles: applications for environmental remediation. J Nanobiotechnol 16, 84 (2018). https://doi.org/10.1186/s12951-018-0408-4

Bisht N, Phalswal P, Khanna PK. Selenium nanoparticles: a review on synthesis and biomedical applications [Internet]. Vol. 3, Materials Advances. 2022. p. 1415–31. Available from: http://dx.doi.org/10.1039/d1ma00639h

Rasouli M. (2019). Biosynthesis of Selenium Nanoparticles using yeast Nematospora coryli and examination of their anti-candida and anti-oxidant activities. IET nanobiotechnology, 13(2), 214–218. https://doi.org/10.1049/iet-nbt.2018.5187

Cremonini, E., Zonaro, E., Donini, M., Lampis, S., Boaretti, M., Dusi, S., Melotti, P., Lleo, M. M., & Vallini, G. (2016). Biogenic selenium nanoparticles: characterization, antimicrobial activity and effects on human dendritic cells and fibroblasts. Microbial biotechnology, 9(6), 758–771. https://doi.org/10.1111/1751-7915.12374

Yang, F., Tang, Q., Zhong, X., Bai, Y., Chen, T., Zhang, Y., Li, Y., & Zheng, W. (2012). Surface decoration by Spirulina polysaccharide enhances the cellular uptake and anticancer efficacy of selenium nanoparticles. International journal of nanomedicine, 7, 835–844. https://doi.org/10.2147/IJN.S28278

Johnson J, Shanmugam R, Lakshmi T. A review on plant-mediated selenium nanoparticles and its applications. J Popul Ther Clin Pharmacol. 2022 Jan 6;28(2):e29–40.

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. Clin Oral Investig. 2020 Sep;24(9):3275–80.

Mali PY, Panchal SS. Euphorbia tirucalli L.: Review on morphology, medicinal uses, phytochemistry and pharmacological activities [Internet]. Vol. 7, Asian Pacific Journal of Tropical Biomedicine. 2017. p. 603–13. Available from: http://dx.doi.org/10.1016/j.apjtb.2017.06.002

Maroyi A. Diospyros lycioides Desf.: Review of its botany, medicinal uses, pharmacological activities, and phytochemistry [Internet]. Vol. 8, Asian Pacific Journal of Tropical

Biomedicine. 2018. p. 130. Available from: http://dx.doi.org/10.4103/2221-1691.225617

Gupta N, Vishnoi G, Wal A, Wal P. Medicinal Value of Euphorbia Tirucalli [Internet]. Vol. 4, Systematic Reviews in Pharmacy. 2013. p. 40. Available from: http://dx.doi.org/10.4103/0975-8453.135843

K.H. Sudheer Kumar, N. Dhananjaya, L.S. Reddy Yadav, E. tirucalli plant latex mediated green combustion synthesis of ZnO nanoparticles: Structure, photoluminescence and photo-catalytic activities, Journal of Science: Advanced Materials and Devices, Volume 3, Issue 3, 2018, Pages 303-309, ISSN 2468-2179, https://doi.org/10.1016/j.jsamd.2018.07.005.

Kgosiemang IK, Lefojane R, Direko P, Madlanga Z, Mashele S, Sekhoacha M. Green synthesis of magnesium and cobalt oxide nanoparticles using *Euphorbia tirucalli*: Characterization and potential application for breast cancer inhibition. Inorg Nano-met Chem. 2020 Nov 1;50(11):1070–80.

Mohammadzadeh V, Barani M, Amiri MS, Yazdi MET, Hassanisaadi M, Rahdar A, et al. Applications of plant-based nanoparticles in nanomedicine: A review [Internet]. Vol. 25, Sustainable Chemistry and Pharmacy. 2022. p. 100606. Available from: http://dx.doi.org/10.1016/j.scp.2022.100606

Joudeh, N., Linke, D. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. J Nanobiotechnol 20, 262 (2022). https://doi.org/10.1186/s12951-022-01477-8

Dua K, Mehta M, de Jesus Andreoli Pinto T, Pont LG, Williams KA, Rathbone M. Advanced Drug Delivery Systems in the Management of Cancer. Elsevier; 2021. 572 p.

Peres, M. A., Macpherson, L. M. D., Weyant, R. J., Daly, B., Venturelli, R., Mathur, M. R., Listl, S., Celeste, R. K., Guarnizo-Herreño, C. C., Kearns, C., Benzian, H., Allison, P., & Watt, R. G. (2019). Oral diseases: a global public health challenge. Lancet (London, England), 394(10194), 249–260. https://doi.org/10.1016/S0140-6736(19)31146-8

Hamza SA, Asif S, Khurshid Z, Zafar MS, Bokhari SAH. Emerging Role of Epigenetics in Explaining Relationship of Periodontitis and Cardiovascular Diseases. Diseases [Internet]. 2021 Jun 29;9(3). Available from: http://dx.doi.org/10.3390/diseases9030048

Chattopadhyay I, Verma M, Panda M. Role of Oral Microbiome Signatures in Diagnosis and Prognosis of Oral Cancer. Technol Cancer Res Treat. 2019 Jan 1;18:1533033819867354.

Razzaque MS. Commentary: Microbial Resistance Movements: An Overview of Global Public Health Threats Posed by Antimicrobial Resistance, and How Best to Counter. Front Public Health. 2020;8:629120.

Amit Khurana, Sravani Tekula, Mohd Aslam Saifi, Pooladanda Venkatesh, Chandraiah Godugu, Therapeutic applications of selenium nanoparticles, Biomedicine & Pharmacotherapy, Volume 111, 2019, Pages 802-812, ISSN 0753-3322, https://doi.org/10.1016/j.biopha.2018.12.146.

Johnson, J., Shanmugam, R., & Lakshmi, T. (2022). A review on plant-mediated selenium nanoparticles and its applications. Journal of population therapeutics and clinical pharmacology = Journal de la therapeutique des populations et de la pharmacologie clinique, 28(2), e29–e40. https://doi.org/10.47750/jptcp.2022.870

Mali PY, Panchal SS. Euphorbia neriifolia L.: Review on botany, ethnomedicinal uses, phytochemistry and biological activities [Internet]. Vol. 10, Asian Pacific Journal of Tropical Medicine. 2017. p. 430–8. Available from: http://dx.doi.org/10.1016/j.apjtm.2017.05.003

Sharma A, Chandraker S, Patel VK, Ramteke P. Antibacterial Activity of Medicinal Plants Against Pathogens causing Complicated Urinary Tract Infections. Indian J Pharm Sci. 2009 Mar;71(2):136–9.

Miglani, S., & Tani-Ishii, N. (2021). Biosynthesized selenium nanoparticles: characterization, antimicrobial, and antibiofilm activity against Enterococcus faecalis. PeerJ, 9, e11653. https://doi.org/10.7717/peerj.11653

Kirbag, S., Erecevit, P., Zengin, F., & Guvenc, A. N. (2013). Antimicrobial activities of some Euphorbia species. African journal of traditional, complementary, and alternative medicines : AJTCAM, 10(5), 305–309. https://doi.org/10.4314/ajtcam.v10i5.13

Yu J, Li M, Zhan D, Shi C, Fang L, Ban C, et al. Inhibitory effects of triterpenoid betulin on inflammatory mediators inducible nitric oxide synthase, cyclooxygenase-2, tumor necrosis factor-alpha, interleukin-6, and proliferating cell nuclear antigen in 1, 2-dimethylhydrazine-induced rat colon carcinogenesis. Pharmacogn Mag. 2020;16(72):836.

Mithra S, Rajesh Kumar S, Ramani P. Antimicrobial Activity and Cytotoxic Effectof Cinnamon and Clove Mediated Gold Nanoparticles Based Mouthwash: An in vitro Study. Journal of Nanosciences: Current Research. 2021 Nov 29;6(4):1–4.