CYTOTOXICITY OF TRIDAX PROCUMBENS LEAVES MEDIATED SILVER NANOPARTICLES

Vidusha.A[1], M. Jeevitha[2*]

Article History: Received: 12.12.2022 **Revised:** 29.01.2023 **Accepted:** 15.03.2023

Abstract

Background: Researchers are employing plant extract in the nanoparticle synthesis process to meet the growing demand for environmentally friendly nanoparticles. As it is more affordable, environmentally benign, and readily scaled up for large-scale synthesis, green synthesis offers an improvement over chemical and physical methods. The necessity for metal nanoparticles produced biologically from plant extracts over other hazardous technologies is therefore a better alternative. The synthesized silver nanoparticles (AgNPs) from traditional medicinal plants such as Tridax procumbens possess superior biological properties yet the cytotoxicity has to be assessed. The present study aims to evaluate the cytotoxic activity of AgNPs synthesized using T. procumbens leaf extract.

Materials and methods: In this study, silver nanoparticles from the leaf extract of T. procumbens were synthesized. The AgNPs were characterized using UV-visible spectrophotometer and then assessed its cytotoxic activity using the Brine Shrimp Lethality Assay (BSLA).

Results: The biosynthesized AgNPs has shown decreased cytotoxic activity at all concentrations (5 µL, 10 µL, 20 µL, 40 µL, 80 µL) and may be used to create innovative nanoformulations for pathogenic oral diseases.

Conclusion: Green synthesis of AgNPs utilizing T. procumbens possess lower cytotoxic activity and have more uses in treatment of oral diseases due to the growing demand for biosynthesized materials for various treatment modalities.

Keywords: cytotoxic activity, innovative, silver nanoparticles, sustainable, Tridax procumbens

[1]Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-77

[2*]Department of Periodontics, Saveetha dental college and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-77

DOI: 10.31838/ecb/2023.12.s2.058

1. Introduction

Nanobiotechnology focuses on the creation of biomaterials with a size smaller than 100 nanometers (Rao, Müller and Cheetham, 2007). To provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices, and systems that have novel properties and functions due to their small and/or intermediate size, nanotechnology entails research and technology development at the atomic, molecular, or macromolecular levels in the length scale of roughly 1 to 100 nanometer range (Fulekar, 2010). Applications to medicine and physiology involve materials and equipment with a high degree of specificity for subcellular (i.e., molecule) interactions with the body. This may result in cellular- and tissue-specific clinical applications with a focus on maximizing therapeutic effects while minimizing negative effects.

Reducing agents are used during the chemical synthesis of silver nanoparticles (AgNPs) to transform Ag^+ ions into AgNPs. In contrast to chemical and physical approaches, organic techniques for metal nanoparticles that use plant resources are viewed as practical and environmentally benign solutions (Abd-Elsalam, Periakaruppan and Rajeshkumar, 2021).

There are many recent investigations that were conducted using biological approaches. A range of resources are provided by biological agents such as fungi, bacteria, and plants in the biological technique for the creation of nanoparticles(Jeevitha and Rajeshkumar, 2019; Rajeshkumar et al., 2019; Begum et al., 2020; Devi et al., 2020; Ganta et al., 2020; K et al., 2020; Lakshme et al., 2020; Rajeshkumar and Jeevitha, 2021). At room temperature and pressure, the ratio of metal ion reduction utilizing biological agents is quicker than other methods. The number of harmful compounds to the environment and to human health can be decreased by the biological pathway of synthesis (Kanchi and Ahmed, 2018). Technological advancements in nanotechnology have made it possible to produce size-controlled combinations of various metal and metal oxide nanoparticles. These nanoparticles have characteristics to do particular tasks. Due to their antimicrobial properties and potential application in the medical field, AgNPs have recently attracted the attention of researchers (Wasilewska et al., 2023).

In various experiments, natural ingredients like alfalfa sprouts, chillies, green tea, rice, sunflower seeds, and jackfruit have been used to create AgNPs (Jain, Joshi and Sukhwal, 2014). Plant-mediated synthesis of metal nanoparticles is gaining popularity due to its simplicity, environmental friendliness, fast production of nanoparticles with a diversity of morphologies, and removal of the need for labor-intensive cell culture maintenance (Al-Ahmed, Isloor and Nasiruzzaman Shaikh, 2013).

The most basic and crucial method for ensuring the production of nanoparticles is UV vis spectroscopy. By observing swelling, precipitation, diffusion, and partitioning events, UV imaging provides a strong capacity for revealing insights into medication dissolution and formulation creation (Shukla and Iravani, 2018). It is possible to define cytotoxicity as the quality of harming cells. Using the larvae of the crustacean Artenia salina, the brine shrimp lethality assay (BSLA) is an increasingly popular technique for determining the cytotoxicity of bioactive substances. In the current experiment, T. procumbens (Tridax daisy or coat buttons) leaf extracts were used to synthesize AgNPs by a biological approach (Manjamalai, Kumar and Grace, 2012). A species of flowering plant belonging to the Asteraceae family is called T. procumbens. It is most recognised for being a common weed and nuisance plant. This herb has historically been used in India to treat wounds and as an insect, fungus, and anticoagulant (Jain, Nagar and Patel, 2012). Our team has extensive knowledge and research experience that has translated into high quality publications (Ramesh Kumar et al., 2011; Jain, Kumar and Manjula, 2014; Krishnan, Pandian and Kumar S, 2015; Keerthana and Thenmozhi, 2016; Sivamurthy and Sundari, 2016; Felicita, 2017a, 2017b; Kumar, 2017; Sekar et al., 2019; Johnson et al., 2020; Jeevitha et al., 2022). The present study aims to analyze the cytotoxicity of AgNPs synthesized from T. procumbens.

2. Materials and Methods

Preparation of plant extract

Fresh T. procumbens leaves were procured (Fig 1a) and powdered. The preparation of plant extract was done usingT. procumbens. 1 g of T. procumbens leaf powder was weighed. It was dissolved in 100 mL of distilled water.. Then it was boiled at 60°C to 70°C in the heating mantle for 5 to 10 minutes. The solution was filtered and the extract was obtained (Fig. 1b).

Figure 1: a. Fresh T. procumbens leaves b. T. procumbens leaf extract

Synthesis of silver nanoparticles using T. procumbens:

To make silver nitrate solution, 0.001 g of AgNO3 was dissolved in 70 mL of distilled water. 30 mL of leaf extract were added to this solution and shaken in an orbital shaker. Visual observations of the colour changes were made, and at predetermined intervals, pictures were taken.

Confirmation of AgNPs:

Absorption spectroscopy is referred to as UV visible spectroscopy which is the earliest technique and most frequently used in pharmaceutical analysis to analyze a substance in a solution on a qualitative, quantitative, and structural level. The synthesised AgNPs solution's presence was confirmed using UV-vis spectroscopy. Characterization was carried out using UV-vis spectroscopy, and the results were recorded.

Cytotoxic activity of T. procumbens mediated AgNPs:

AgNPs' cytotoxic effects were tested using a brine shrimp mortality experiment. Eggs of brine shrimp were purchased from Aquatic Remedies in Chennai. By mixing 2 g of iodine-free salt with 200 ml of distilled water, the rate of egg hatching was improved when it was added to artificial seawater. This artificial sea water was introduced to a room in the chamber that was divided into dark and light sections. In the chamber's shadowy section, shrimp eggs were inserted. It required 2 to 3 days for the eggs to develop into larvae. The larvae made their way to the light-filled area of the barrier. The cytotoxicity assessment was performed on the hatched nauplii. 10mL to 12mL of saline water was added to a 6 well ELISA plate. To that, 10 nauplii were gradually added to each of the wells which were tested at different concentrations of test solution (5L, 10L, 20L, 40L, 80L) and a control. 48 hours were spent incubating the plates. The number of live nauplii present on the ELISA plates was counted after 24 h and 48 h of observation.

3. Results And Discussion

Visual observation:

T. procumbens was employed in the current study to create AgNPs, which changed color from light green to dark green within 24 hours and then remained that color, showing the synthesis process was successful.(Fig. 2). The complete creation of nanoparticles can be seen visually by observing a change in color (Niederberger and Pinna, 2009). Previous research has documented how the color of metal nanoparticles made using plant extracts changes during the synthesis process (Makhlouf and Barhoum, 2018). Similar to the previous studies, the silver nanoparticles' indication of a light-green to dark-green color shift was observed (Karthik et al., 2019).

Figure 2: Reduction of silver ions to silver nanoparticles visually identified by color change

UV vis spectrophotometer analysis

The technique of UV-visible spectroscopy is frequently used to characterize nanoparticles. The characteristic visible-range absorption is caused by the metal nanoparticles' surface plasmon resonance. The surface plasmon resonance band peak of the current study is located at a wavelength of 430 nm, according to the UV-visible spectroscopy analysis (Fig. 3). Previous studies verified that the absorbance pattern of AgNPs synthesis was identical. In a previous study, T. procumbens was used to create an aqueous medium containing AgNPs, and the UV visible spectrum showed an absorbance peak at about 440 nm (Zulfiqar et al., 2022). Similarly, peaks between 400 nm and 450 nm in the UV visible spectrum indicated the surface plasmon resonance for the synthesized AgNPs from leaf and flower extracts of T. procumbens (J.soni et al., 2022; Rieshy, V et al., 2022).

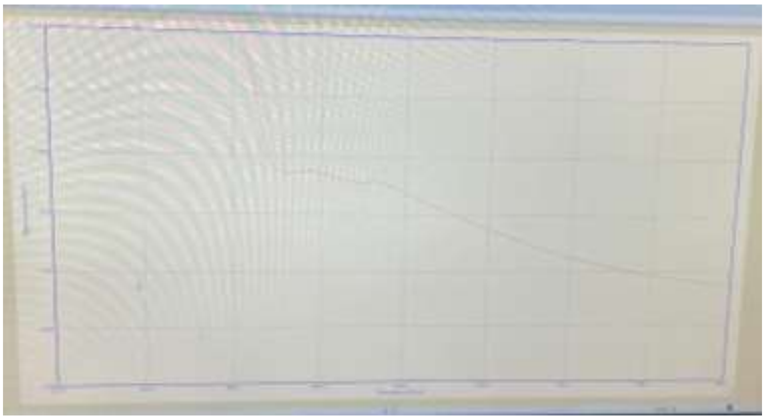

Figure 3: UV- Vis Spectroscopic analyses of AgNPs synthesized from T. procumbens recorded as function of time

Cytotoxic analysis:

In order to determine how hazardous a bioactive chemical is to cells, the Brine shrimp lethality assays are essential tools in the research of cytotoxicity (34). The survivability of the nauplii for various AgNPs concentrations synthesized from T. procumbens was recorded (Table. 1). 100% of the nauplii in concentrations of 5µL, 10µL, 20µL, 40µL and 80µL were still alive after 24 hours. Additionally, the control revealed that all nauplii were alive. 90% of the nauplii were discovered to be alive after 48 hours at the concentration of 10µL and at the highest concentration of 80µL. At concentrations of 5µL, 20µL and 40µL and control, it was visible that all of the nauplii were alive. From the results it is inferred that at the concentrations of 5µL, 20µL and 40µL, there was no cytotoxicity observed and at 10µL and 80µL, reduced cytotoxicity was seen (Fig. 4) AgNPs mediated by Ricinus communis demonstrated cytotoxicity, demonstrating that doses < 20 g/mL were biologically acceptable. With an LD50 value of 514.50 g/mL, AgNPs made from Lantana camara exhibited cytotoxic effects on brine shrimp (A. salina nauplii) (Sampath et al., 2022). Numerous researches have looked into the toxicity of AgNPs to eukaryotic cells, bacteria, and multicellular organisms, however the majority ignore key concerns. With more severe morphological defects, more cells arrested in the

G2/M phase, and more cells going through apoptosis, the AgNPs demonstrated increased cytotoxicity. These findings suggested that the nano-size of AgNPs had a role in the in vitro cytotoxicity process. The development of a dependable and environmentally benign method for the synthesis of metallic nanoparticles is a top priority in the field of nanotechnology. Because plant extracts, especially those from the leaves contain vital phytochemicals, they have been employed extensively for the creation of metal and metal oxide nanoparticles. The development of nanoformulations may serve as treatment for a variety of ailments that can be facilitated by studying the biological properties of AgNPs mediated by T. procumbens leaf extract such as its antibacterial, anti-inflammatory, and antioxidant activities.

Table 1: Brine shrimp lethality assay of T. procumbens mediated AgNPs at different concentrations compared with control

	No. of live nauplii	
Concentration	Day 1 (After 24 h)	Day 2 (After 48 h)
5 µL	10	10
10 µL	10	9
20 µL	10	10
40 µL	10	10
80 µL	10	9
Control	10	10

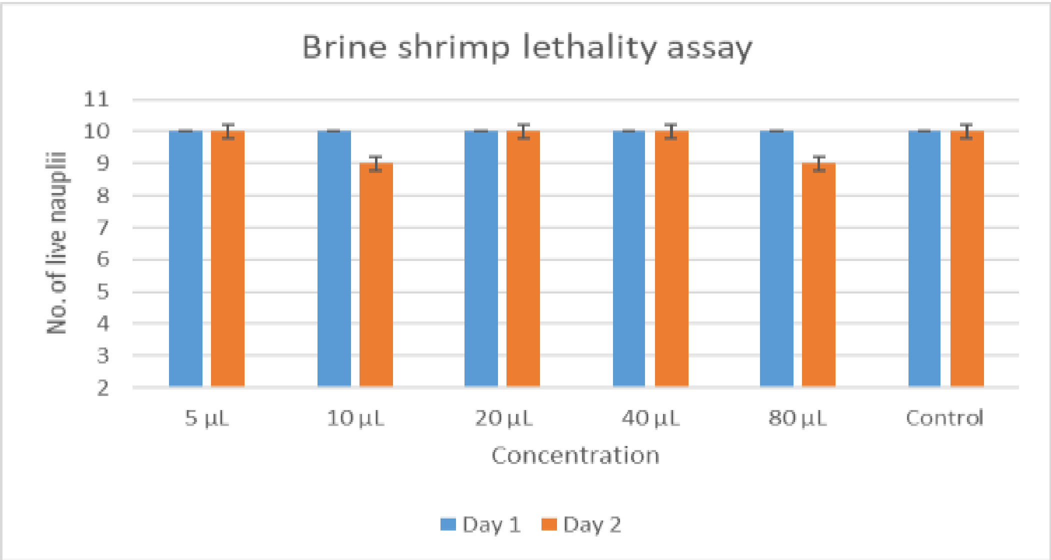

Fig. 4

Figure 4. Cytotoxic effect of T. procumbens mediated AgNPs at different concentrations compared with control

4. Conclusion

The study has shown that T. procumbens leaf extract derived AgNPs have less cytotoxicity. As a result, a variety of safe, environmentally acceptable, and cost-effective nanoformulations can be created at optimal concentrations of these nanoparticles. Future studies of AgNPs nanoparticles synthesized from T. procumbens leaf extract that evaluate their biological characteristics, such as their antibacterial, anti-inflammatory, and antioxidant activity, may lead to the creation of nanoformulations that might be used as therapeutics for a variety of pathogenic oral diseases.

Acknowledgement

We would like to thank Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University for providing us support to carry out the study.

Conflict of Interest:
The authors declare that there were no conflicts of interest in the present study.

Source of Funding:
The present project is supported by
- Saveetha Institute of Medical and Technical Sciences
- Saveetha Dental College and Hospitals, Saveetha University
- Aishwaryam Speciality Hospital

5. References

Abd-Elsalam, K.A., Periakaruppan, R. and Rajeshkumar, S. (2021) Agri-Waste and Microbes for Production of Sustainable Nanomaterials. Elsevier.

Al-Ahmed, A., Isloor, A.M. and Nasiruzzaman Shaikh, M. (2013) Inorganic Nanomedicine: Synthesis, Characterization and Application. Trans Tech Publications Ltd.

Begum, A. et al. (2020) 'Cytotoxicity of Iron Nanoparticles Synthesized Using Dried Ginger', Journal of Pharmaceutical Research International, pp. 112–118. Available at: https://doi.org/10.9734/jpri/2020/v32i2530829.

Devi, R.S. et al. (2020) 'Free Radical Scavenging Activity of Copper Nanoparticles Synthesized from Dried Ginger', Journal of Pharmaceutical Research International, pp. 1–7. Available at: https://doi.org/10.9734/jpri/2020/v32i1930703.

Felicita, A.S. (2017a) 'Orthodontic management of a dilacerated central incisor and partially impacted canine with unilateral extraction - A case report', The Saudi dental journal, 29(4), pp. 185–193.

Felicita, A.S. (2017b) 'Quantification of intrusive/retraction force and moment generated during en-masse retraction of maxillary anterior teeth using mini-implants: A conceptual approach', Dental press journal of orthodontics, 22(5), pp. 47–55.

Fulekar, M.H. (2010) Nanotechnology: Importance and Applications. I. K. International Pvt Ltd.

Ganta, S.S.L. et al. (2020) 'Anti-Inflammatory Activity of Dried Ginger Mediated Iron Nanoparticles', Journal of Pharmaceutical Research International, pp. 14–19. Available at: https://doi.org/10.9734/jpri/2020/v32i2830866.

Jain, D., Joshi, A. and Sukhwal, A. (2014) Biosynthesis of Silver Nanoparticles Using Tagetes Patula Leaf Extract. LAP Lambert Academic Publishing.

Jain, D.K., Nagar, H. and Patel, N. (2012) Analgesic,Antipyretic, Anti-arthritic Activity of T.Procumbens Leaves: Evaluation of Analgesic, Antipyretic and Antiarthritic Activity of Tridax Procumbens Leaves Extract.

Jain, R.K., Kumar, S.P. and Manjula, W.S. (2014) 'Comparison of intrusion effects on maxillary incisors among mini implant anchorage, j-hook headgear and utility arch', Journal of clinical and diagnostic research: JCDR, 8(7), pp. ZC21–4.

Jeevitha, M. et al. (2022) 'Clinical Evaluation of Lateral Pedicle Flap Stabilized with Cyanoacrylate Tissue Adhesive: A Randomized Controlled Clinical Trial', Contemporary clinical dentistry, 13(1), pp. 24–29.

Jeevitha, M. and Rajeshkumar, S. (2019) 'Antimicrobial Activity of Silver Nanoparticles Synthesized Using Marine Brown Seaweed Spatoglossum Asperum Against Oral Pathogens', Indian Journal of Public Health Research & Development, p. 3568. Available at: https://doi.org/10.5958/0976-5506.2019.04140.8.

Johnson, J. et al. (2020) 'Computational identification of MiRNA-7110 from pulmonary arterial hypertension (PAH) ESTs: a new microRNA that links diabetes and PAH', Hypertension research: official journal of the Japanese Society of Hypertension, 43(4), pp. 360–362.

Kanchi, S. and Ahmed, S. (2018) Green Metal Nanoparticles: Synthesis, Characterization and their Applications. John Wiley & Sons.

Karthik, L. et al. (2019) Biological Synthesis of Nanoparticles and Their Applications. CRC Press.

Keerthana, B. and Thenmozhi, M.S. (2016) 'Occurrence of foramen of huschke and its clinical significance', Research Journal of Pharmacy and Technology, 9(11), pp. 1835–1836.

K, J. et al. (2020) 'Green synthesis of Selenium nanoparticles using Capparis decidua and its anti-inflammatory activity', International Journal of Research in Pharmaceutical Sciences, pp. 6211–6215. Available at: https://doi.org/10.26452/ijrps.v11i4.3298.

Krishnan, S., Pandian, S. and Kumar S, A. (2015) 'Effect of bisphosphonates on orthodontic tooth movement-an update', Journal of clinical and diagnostic research: JCDR, 9(4), pp. ZE01–5.

Kumar, S. (2017) 'The emerging role of botulinum toxin in the treatment of orofacial disorders: Literature update', Asian journal of pharmaceutical and clinical research, 10(9), p. 21.

Lakshme, P.S.T. et al. (2020) 'Evaluation of Antioxidant and Cytotoxic Effect of Selenium Nanoparticles Synthesised Using Capparis decidua', Journal of Pharmaceutical Research International, pp. 60–66. Available at: https://doi.org/10.9734/jpri/2020/v32i1930709.

Makhlouf, A.S.H. and Barhoum, A. (2018) Emerging Applications of Nanoparticles and Architectural Nanostructures: Current Prospects and Future Trends. William Andrew.

Manjamalai, A., Kumar, M.J.M. and Grace, V.M.B. (2012) 'Essential oil of Tridax procumbens L induces apoptosis and suppresses angiogenesis and lung metastasis of the B16F-10 cell line in C57BL/6 mice', Asian Pacific journal of cancer prevention: APJCP, 13(11), pp. 5887–5895.

Niederberger, M. and Pinna, N. (2009) Metal Oxide Nanoparticles in Organic Solvents: Synthesis, Formation, Assembly and Application. Springer Science & Business Media.

Rajeshkumar, S. et al. (2019) 'Anticariogenic Activity of Fresh Aloe Vera Gel Mediated Copper Oxide Nanoparticles', Indian Journal of Public Health Research & Development, p. 3664. Available at: https://doi.org/10.5958/0976-5506.2019.04158.5.

Rajeshkumar, S. and Jeevitha, M. (2021) 'Plant-mediated biosynthesis and characterization of zinc oxide nanoparticles', Zinc-Based Nanostructures for Environmental and Agricultural Applications, pp. 37–51. Available at: https://doi.org/10.1016/b978-0-12-822836-4.00023-9.

Ramesh Kumar, K.R. et al. (2011) 'Depth of resin penetration into enamel with 3 types of enamel conditioning methods: a confocal microscopic study', American journal of orthodontics and dentofacial orthopedics: official publication of the American Association of Orthodontists, its constituent societies, and the American Board of Orthodontics, 140(4), pp. 479–485.

Rao, C.N.R., Müller, A. and Cheetham, A.K. (2007) Nanomaterials Chemistry: Recent Developments and New Directions. John Wiley & Sons.

Rieshy, V et al (2022) 'Preparation of Tridax procumbens leaves based chitosan gel and its antimicrobial activity', Journal of Pharmaceutical Negative Results, 13(7), pp. 2593-2599

Sampath, G. et al. (2022) 'Biologically Synthesized Silver Nanoparticles and Their Diverse Applications', Nanomaterials (Basel, Switzerland), 12(18). Available at: https://doi.org/10.3390/nano12183126.

Sekar, D. et al. (2019) 'Methylation-dependent circulating microRNA 510 in preeclampsia patients', Hypertension research: official journal of the Japanese Society of Hypertension, 42(10), pp. 1647–1648.

Shukla, A.K. and Iravani, S. (2018) Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier.

Sivamurthy, G. and Sundari, S. (2016) 'Stress distribution patterns at mini-implant site during retraction and intrusion—a three-dimensional finite element study', Progress in orthodontics, 17(1), pp. 1–11.

Soni J, Jeevitha M (2022). Cytotoxicity of Tridax procumbens flower mediated silver nanoparticles. Journal of Pharmaceutical Negative Results, 13(7), pp. 2600-2608.

Wasilewska, A. et al. (2023) 'Physico-chemical properties and antimicrobial activity of silver nanoparticles fabricated by green synthesis', Food chemistry, 400, p. 133960.

Zulfiqar, H. et al. (2022) 'Antibacterial, Antioxidant, and Phytotoxic Potential of Phytosynthesized Silver Nanoparticles Using Fruit Extract', Molecules , 27(18). Available at: https://doi.org/10.3390/molecules27185847.