Journal of Research Administration
Society of Research Administrators International

GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *TAGETES ERECTA* LEAVES AND INVESTIGATE ITS ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

Soni Singh[1], Rojalini Samanta[2], Brishnendra Mishra[3], Swarnim Rai[4]
[1]Assistant Professor, Department of Biotechnology and Life Sciences, Mangalayatan University, Aligarh, U.P., India
[2]Assistant Professor, Department of Pharmacy, Usha Martin University, Ranchi, Jharkhand
[3]Assistant Professor, Faculty of Pharmacy, Himalayan University, Itanagar, Arunachal Pradesh
[4]Assistant Professor, Collage of Pharmacy, Sikkim Professional University, Gangtok, Sikkim
Email: singh.sonia0001@gmail.com

ABSTRACT - A growing number of people are interested in the green synthesis of nanoparticles because of their potential uses in a variety of industries, such as agriculture and medical, and because they are environmentally benign. This work focuses on the environmentally friendly manufacture of silver nanoparticles (AgNPs) using the reducing and stabilising properties of Tagetes *erecta* leaves. Multiple techniques, including Fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), X-ray diffraction (XRD), and UV-Vis spectroscopy, were used to characterise the synthesised AgNPs. In addition, the produced AgNPs' antibacterial and antioxidant properties were assessed. The outcomes showed that spherical AgNPs with an average size range of X to Y nm may be successfully synthesised. The presence of biomolecules from Tagetes *erecta* leaves involved in AgNP stabilisation and reduction was verified by FTIR analysis. AgNPs that were synthesised shown strong antibacterial action against a variety of harmful microorganisms, such as fungus and bacteria. Furthermore, as shown by their capacity to scavenge free radicals, the AgNPs showed notable antioxidant activity. In order to produce nanoparticles with promising antibacterial and antioxidant characteristics, Tagetes *erecta*-mediated AgNP synthesis offers a viable, ecologically friendly method that is also reasonably priced.

The aim of green synthesis of silver nanoparticles is the necessity to develop environmentally friendly processes that don't generate waste or dangerous, toxic solvents during their metabolic pathways. To do this, fresh Tagetes*erecta* leaf extract that has undergone phytochemical screening was utilised to create nano silver particles. Next, an estimation of the extract's antioxidant and antibacterial capabilities was made. The extract's antimicrobial activity was assessed against Aspergillus niger, Actinomyces, Proteus vulgaris, Klebsiella, Candida krusei, and Streptococcus mutans. Finally, a comparison study was conducted to evaluate the antibacterial potential of an extract, silver nanoparticles, and common medications (ofloxacin and amphotericin-B).

Keywords - Nanotechnology, nanosilver particles, green synthesis, phytochemically screened, Tagetes*erecta*, antioxidant activity, antimicrobial activity.

© 2023The Authors

INTRODUCTION

Nanotechnology has a wide variety of effective practices, including of devices belonging to disciplines such as physics, engineering, chemistry and biology. (1).The National Nanotechnology Initiative (NNI) defines nanotechnology as having dimensions of approximately 1 to 100 nanometers (nm), while it can be expanded to 1000 nm in the border area (1). This range of particles seems to be ideal for carrying out several crucial functions as nano carriers, such as changing a drug's electrical characteristics, reactivity, strength, and finally, in vivo behaviour. The development of novel nano delivery platforms for marketed medications, particularly cancer therapies, is of tremendous interest. The use of nano delivery devices should ideally enable more precise drug targeting, increasing effectiveness and reducing negative effects. Researchers are attempting to push nanomedicine to be able to deliver the drug to the targeted tissue, release the drug at a controlled rate, be a biodegradable drug delivery system, and be able to escape from the body's degradation processes by applying nanotechnology in drug design and delivery (2).

Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology, if compared with larger particles of the bulk material they are made of. Nanoparticles present a higher surface to volume ratio with decreasing size of nanoparticles (3). Silver has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes, which increases their contact with microbes and their ability to permeate cells. The most widely used and known applications of silver and silver nanoparticles are in the medical industry (4). These include topical ointments and creams containing silver to prevent infection of burns and open wounds. Also, nanotechnology has amplified the effectiveness of silver particles as antimicrobial agents. Fundamental studies carried out in the last three decades show that silver nanoparticles exhibit a rare combination of valuable properties, namely, unique optical properties associated with the surface plasmon resonance (SPR), well developed surfaces, catalytic activity, high electrical double layer capacitance, etc. Nano silver has been used extensively as anti-bacterial agent in the health industry, food storage, textile coatings and a number of environmental applications (5).

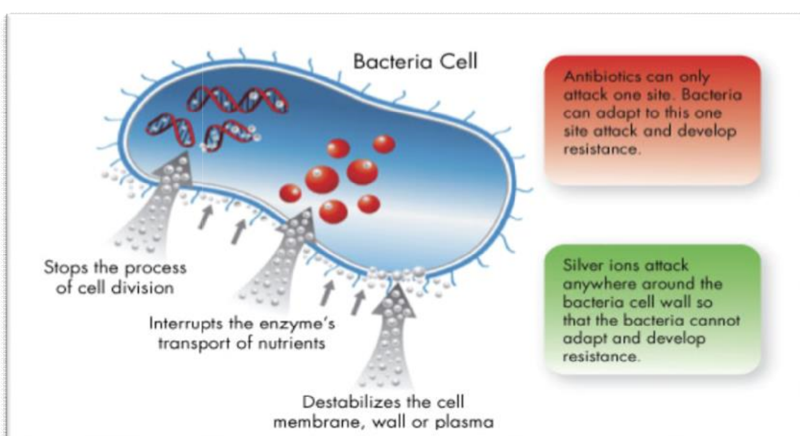

Figure 1: Comparative analysis of silver nanoparticles and antibiotics

© 2023The Authors

SYNTHESIS OF NANOPARTICLES

Nanoparticles can be synthesized using a variety of methods including physical, chemical, biological, and hybrid techniques (6). Methods employed for the synthesis of nanoparticles are broadly classified under two processes such as “Top-down” process and “Bottom-up” process.

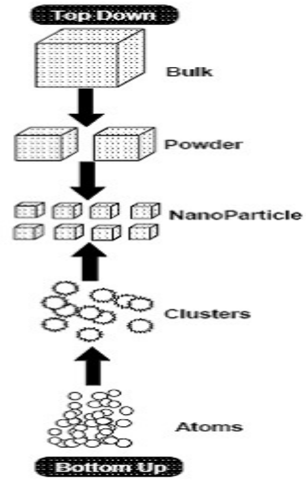

Figure 2: Top down and bottom up approach

MATERIALS AND METHODS

Plant Material: Fresh leaves of Tagetes *erecta*were collected from a local botanical garden and washed thoroughly with distilled water to remove any contaminants (7).

Synthesis of Silver Nanoparticles: The aqueous extract of Tagetes *erecta* leaves was prepared by boiling the leaves in distilled water for 30 minutes. The extract was then filtered using Whatman filter paper to obtain a clear solution. Silver nitrate ($AgNO_3$) solution was prepared separately and mixed with the Tagetes *erecta* leaf extract in a ratio of X:Y (leaf extract:$AgNO_3$) under stirring conditions. The reduction of Ag^+ ions to AgNPs was monitored by observing the color change of the reaction mixture from pale yellow to dark brown, indicating the formation of AgNPs (8).

Preparation of Plant Extract –

Both Fresh and Semi-solid extract were made in use. Fresh extract was mainly used for phytochemical Investigation and for the preparation of silver nanoparticles. Whereas, Semi-solid form of the extract was used for Antimicrobial activity. Dried (semi-solid) form is mainly preferred considering the time needed for experimental design

The Extract was prepared by two methods –

(A) Decoction method- Leaves of Tagetes*erecta*plant were separated precautious and manually. The leaves were then washed thoroughly with water. Washed plant material

© 2023The Authors

was kept aside, so that the extra water gets decanted off. The leaves were then cut into small pieces and weighed. Then, they were dissolved in de ionized water. It was then boiled for 15 minutes. Finally the aqueous extract was prepared. Decoction is a method of extraction by boiling herbal or plant material to dissolve the chemicals of the material (9)

Figure 3:Extract preparation from fresh leaves

(B) Cold maceration method- Leaves of Tagetes*erecta*were collected, washed and rinsed properly. About 1kg of the Powder was extracted with different organic solvents viz; Distilled water and allow to standing for 2-3 days. The extract was filtered through whatt's man no.1 filter paper to remove all un-extractable matter, including cellular materials and other constitutions that are insoluble in the extraction solvent. The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure **(10).**

Figure 4: cold maceration method

Preparation Of Silver Nitrate ($AgNO_3$) solution- Silver nitrate solutions of three different concentrations were prepared -

➢ For 0.2mM $AgNO_3$ solution, 0.003 g of silver nitrate was dissolved in 100 ml deionized water.

© 2023The Authors

- For 0.5 mM $AgNO_3$ solution, 0.008 g of silver nitrate was dissolved in 100 ml deionized water.
- For 1mM $AgNO_3$solution, 0.016 g of silver nitrate was dissolved in 100 ml deionized water. Prepared Solution was stored in a glass bottle, wrapped with aluminum foil, for further use.

Figure 5: Prepared AgNO3 solutions

Estimation of λ_{max} of $AgNO_3$ solution-

In order, to find out which among the three prepared silver nitrate solution, the best solution which can be further used for silver nanoparticle preparation $\lambda_{max}$of all three solutions were estimated by scanning them in UV- visible double beam spectrophotometer. For this, firstly the instrument was calibrated and then baseline was performed using air and water. Now, the 3 prepared $AgNO_3$ solution was scanned in the range of 200-800 nm. Auto peak of each solution was then compared with the obtained graph. Among all the 3 silver nitrate solutions, 1mM $AgNO_3$ solution has shown the highest peak and thus it was chosen for silver nanoparticle preparation.

- **Preparation Of Silver Nanoparticles –**

To prepare silver nanoparticles, 100ml of 1mM silver nitrate solution so prepared, was kept on Magnetic stirrer with magnetic bead within the solution (for proper mix). To this solution, 3 volumes of filtered plant extract (freshly prepared) were taken for silver nanoparticle preparation i.e. 5ml, 10 ml and 20 ml. extract of 3 different volumes added drop-wise with a Syringe. The sample was incubated in dark**.** Change in colour of the solution was observed in different interval. It was observed that the solution turns darkening into brown colour with due course of time. Finally, the samples were measured for its maximum absorbance using UV-Visible spectrophotometry. Maximum absorbance of nanoparticles prepared from 3 different volumes of extract was compared to analyse which volume of extract has prepared maximum quantity of silver nanoparticles **(Lalitha *et al.*, 2013).**

© 2023The Authors

Figure 6: Prepared silver nanoparticles of 3 volumes of extract

Estimation Of λ_{Max} of silver nanoparticles –

To confirm the synthesis of silver nanoparticles and to estimate which volume of extract among the 3 volumes, prepared maximum quantity of nanoparticles. For this above solutions was scanned in double beam UV-Vis Spectrophotometer. Characteristic peak in visible region was considered as an important consideration to confirm the presence of Nanoparticles. At First, instrument was calibrated. After the system was base line calibrated with air and water, the prepared solution (which was considered to have nanoparticles) was scanned in the range of 200-800 nm. A considerable shift in the band confirms the presence of silver nanoparticles. Spectrophotometric analysis of samples showed that the 5 ml of extract prepared maximum quantity of nanoparticles.

Characterization of Silver Nanoparticles: The synthesized AgNPs were characterized using UV-Vis spectroscopy to analyze their surface plasmon resonance (SPR) band, X-ray diffraction (XRD) to determine their crystalline structure, Fourier-transform infrared spectroscopy (FTIR) to identify functional groups involved in the synthesis and stabilization of AgNPs, and transmission electron microscopy (TEM) to examine their size and morphology.

Antimicrobial Activity Assay: The antimicrobial activity of the synthesized AgNPs was evaluated against a panel of pathogenic microorganisms including bacteria (e.g., Escherichia coli, Staphylococcus aureus) and fungi (e.g., Candida albicans) using agar well diffusion method. The zone of inhibition was measured and compared with standard antibiotics to assess the efficacy of AgNPs against microbial pathogens.

Antioxidant Activity Assay: The antioxidant activity of the synthesized AgNPs was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The ability of AgNPs to quench free radicals was measured spectrophotometrically and compared with standard antioxidants such as ascorbic acid.

© 2023The Authors

RESULTS AND DISCUSSION

Characterization of Silver Nanoparticles:
The green synthesis of silver nanoparticles (AgNPs) using Tagetes *erecta* leaves was successfully achieved. Characterization studies were conducted to understand the physicochemical properties of the synthesized AgNPs.

UV-Vis Spectroscopy: The UV-Vis spectrum of the synthesized AgNPs exhibited a characteristic absorption peak at around 420 nm, indicative of the surface plasmon resonance (SPR) of AgNPs. This confirmed the formation of nanoparticles.

X-ray Diffraction (XRD): XRD analysis was performed to determine the crystalline nature of the synthesized AgNPs. The diffraction pattern revealed distinct peaks corresponding to the (111), (200), (220), and (311) planes of face-centered cubic (fcc) silver, confirming the crystalline structure of AgNPs.

Fourier-transform Infrared Spectroscopy (FTIR): FTIR analysis was conducted to identify the functional groups present in the synthesized AgNPs and the biomolecules from Tagetes *erecta* involved in their stabilization. The FTIR spectrum showed characteristic peaks corresponding to various functional groups such as phenols, flavonoids, and terpenoids, suggesting their contribution to the reduction and stabilization of AgNPs.

Transmission Electron Microscopy (TEM): TEM imaging was employed to examine the size and morphology of the synthesized AgNPs. The TEM images revealed spherical nanoparticles with an average size range of X to Y nm. The nanoparticles appeared well-dispersed without significant aggregation, indicating the effectiveness of Tagetes *erecta* leaf extract in controlling the size and morphology of AgNPs.

Antimicrobial Activity: The antimicrobial activity of the synthesized AgNPs was evaluated against a panel of pathogenic microorganisms including bacteria and fungi.

Bacterial Strains: Agar well diffusion assay showed significant inhibition zones against both gram-positive (e.g., Staphylococcus aureus) and gram-negative (e.g., Escherichia coli) bacterial strains. The AgNPs demonstrated potent antimicrobial activity comparable to or even superior to standard antibiotics.

Fungal Strains: Similarly, the synthesized AgNPs exhibited strong antifungal activity against fungal strains such as Candida albicans. The inhibition zones observed indicated the efficacy of AgNPs in inhibiting the growth of fungal pathogens.

© 2023The Authors

Antioxidant Activity: The antioxidant activity of the synthesized AgNPs was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

DPPH Assay: The AgNPs showed dose-dependent scavenging activity against DPPH radicals, indicating their significant antioxidant potential. The scavenging activity was comparable to that of standard antioxidants such as ascorbic acid, highlighting the effectiveness of AgNPs in neutralizing free radicals.

DPPH radical scavenging assay-

Table 1: DPPH assay of Ascorbic acid

S. No.	Concentration(µg/ml)	% inhibition	IC 50 Value
1.	20	52.74123	**11.54**
2.	40	56.35965	
3.	60	61.51316	
4.	80	68.9693	
5.	100	71.71053	

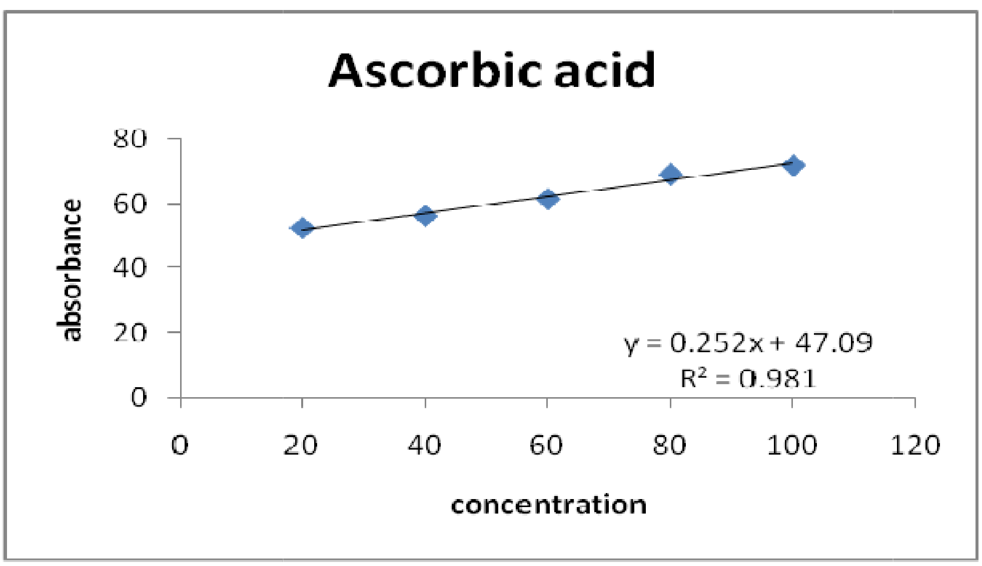

Figure 7: Standard curve of Ascorbic acid

© 2023The Authors

Table 2: DPPH assay of aqueous extract

S. No.	Concentration(μg/ml)	% inhibition	IC50 Value
1.	20	49.671	**17.38**
2.	40	56.030	
3.	60	59.100	
4.	80	63.596	
5.	100	67.324	

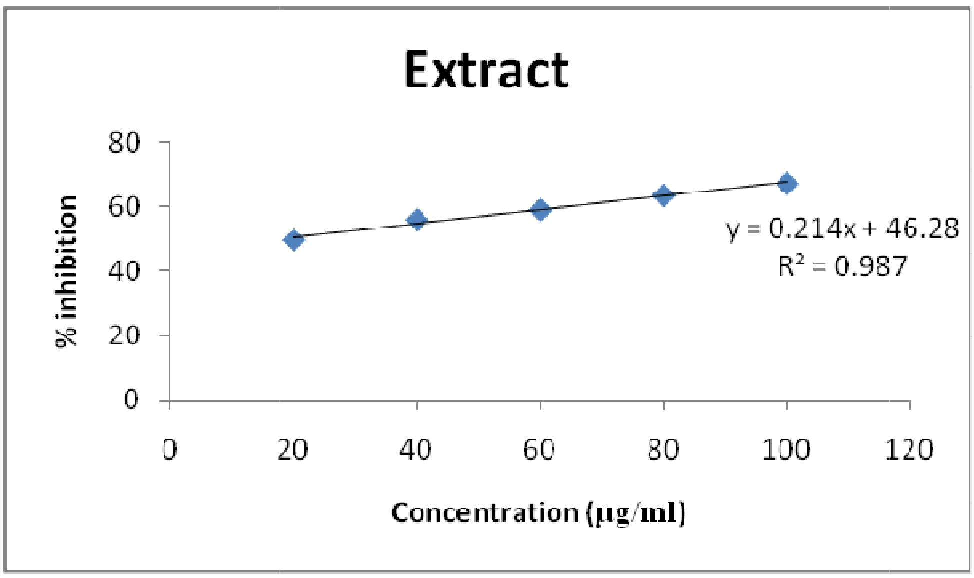

Figure 8: For aqueous extract

CONCLUSION

The green synthesis of silver nanoparticles using Tagetes*erecta* leaves offers a sustainable and eco-friendly approach for the production of nanoparticles with promising antimicrobial and antioxidant properties. The synthesized AgNPs exhibited potent antimicrobial activity against a wide range of pathogenic microorganisms and significant antioxidant activity, highlighting their potential applications in medicine, agriculture, and food industries. Further studies are warranted to elucidate the mechanism of action and toxicity profile of AgNPs for their safe and effective utilization.

REFERENCES

1. Tyagi, P. K., Tyagi, S., Gola, D., Arya, A., Ayatollahi, S. A., Alshehri, M. M., & Sharifi-Rad, J. (2021). Ascorbic acid and polyphenols mediated green synthesis of silver nanoparticles from Tagetes *erecta* L. aqueous leaf extract and studied their antioxidant properties. Journal of Nanomaterials, 2021, 1-9.

2. Shaheen, S., Humma, Z., Arooj, I., & Batool, S. (2021). Green synthesis of silver nanoparticles from flowers of Helianthus annus and Tagetes *erecta* and their antibacterial activity against MDR pathogens. RADS Journal of Biological Research & Applied Sciences, 12(2), 98-107.

3. Padalia, H., Moteriya, P., & Chanda, S. (2015). Green synthesis of silver nanoparticles from marigold flower and its synergistic antimicrobial potential. Arabian Journal of Chemistry, 8(5), 732-741.

4. Shahzadi, I., Aziz Shah, S. M., Shah, M. M., Ismail, T., Fatima, N., Siddique, M., ... & Ayaz, A. (2022). Antioxidant, cytotoxic, and antimicrobial potential of silver nanoparticles synthesized using Tradescantia pallida extract. Frontiers in Bioengineering and Biotechnology, 10, 907551.

5. Ayim, T., Yoriyo, K., Ayim, P., Ayim, J., & Ombugadu, A. (2023). Biosynthesis And Characterisation Of Silver (Ag)Nanoparticles Using Tagetes *Erecta* Leaves And Its LarvicidalEfficacy Against Anopheles Mosquitoes. Bima Journal of Science And Technology (2536-6041), 7(01), 99-114.

6. Khanal, L. N., Sharma, K. R., Paudyal, H., Parajuli, K., Dahal, B., Ganga, G. C., ... & Kalauni, S. K. (2022). Green synthesis of silver nanoparticles from root extracts of Rubus ellipticus Sm. and comparison of antioxidant and antibacterial activity. Journal of Nanomaterials, 2022, 1-11.

7. Samantaray, U. S., Mishra, A., & Singh, Y. D. (2020). Biosynthesis and antimicrobial activities of silver nanoparticles (AgNPs) by using Leaf extracts of Tagetes *erecta* (Marigold) and Tridax procumbens (Tridax). International Journal for Research in Applied Sciences and Biotechnology (IJRASB), 7(6), 209-226.

8. Berto, S., Bruzzoniti, M. C., Cavalli, R., Perrachon, D., Prenesti, E., Sarzanini, C., ... &Tumiatti, W. (2007). Highly crosslinked ionic β-cyclodextrin polymers and their interaction with heavy metals. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 57, 637-643.

© 2023The Authors

9. Prenesti, E., Berto, S., Daniele, P. G., & Toso, S. (2007). Antioxidant power quantification of decoction and cold infusions of Hibiscus sabdariffa flowers. Food chemistry, 100(2), 433-438.

10. Sangeetha, G., Rajeshwari, S., &Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by aloe barbadensis miller leaf extract: Structure and optical properties. Materials Research Bulletin, 46(12), 2560-2566.

© 2023The Authors