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GREEN SYNTHESIS OF SILVER NANOPARTICLES, THEIR CHARACTERIZATION USING LEAF EXTRACTS OF *Tridax procumbens* L.

Govind Dhulgande[1], Datta Dhale[2] and Datta Ghogare[3]

Abstract

Nature has been source of various medicinal agents for thousands of years. An impressive number of modern drugs have been isolated from natural resources mainly plants. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. Now a days an essential values and uses of some plants have been workout and published, but many of them remain unexplored to date. Therefore there is necessity to explore their medicinal uses. The recent development and implementation of new technologies have led to new era towards nanotechnology which unfolds role of plants in bio and green synthesis of nanoparticles. It seem to have drawn quite attention with a view of synthesized stable nanoparticles. The recent emergence of nanotechnology has provided a new pharmacological modality for silver nanoparticles to be used in different medicines. In the present study, *Tridax procumbens* leaf has been used to produce the silver nanoparticles (AGNPs) from two solvent systems (distilled water and 50% alcohol). Synthesis of AGNPs from leaf extracts was carried out and the characterization of the synthesized AGNPs was done using UV-visible spectroscopy. Both the extracts exhibited significant results for the biosynthesis of AGNPs by using silver nitrate as a reducing agent, the synthesis of AGNPs was found by colour change from yellowish green to dark brown. The UV-visible spectroscopy revealed the absorption maxima at 200nm to 700nm for both solvent systems. The nanoparticle size were in the range of 30nm to 100nm. Hence the use of nanotechnology in various industries have increased rapidly due to its unique and valuable properties. It also gives considerable potential for profitable applications in various industries.

Key words: *Tridax procumbens*, AGNPs, biosynthesis, nanoparticles, pharmacognosy

[1]Post-Graduate Department of Botany, Sir Parahurambhau College, Pune (MS), [2]Post-Graduate Department of Botany, SSVP Santha's L.K. Dr. P. R. Ghogrey Science College, Dhule (MS), [3]Department of Botany, Matsyodari Arts, Science and Commerce College, Ambad Dist. Jalna (MS), India
E-mail: drgovind2012@gmail.com

Introduction

Now a day's nanotechnology improves existing industrial processes, materials and applications by scaling them down to the Nano scale in order to full exploration. It has been many applications in various industries. In short, nanostructures in all forms capture a very active area of research and development in the fields of nanotechnology. Metal nanoparticles have a great value due to its unique feature. They have catalytic, magnetic, electrical and optical properties. Silver nanoparticles are largely used in various fields, including medical food, cosmetics, etc. due to its unique physical, chemical and biological properties. Earlier various plants are known to their medicinal value and that's why they were used in traditional medicine. Recently most of the plants are used to produce nanoparticles because their easy availability in nature. Also they are safe to handle and non-hazardous. Also they have various important biomolecules such as phenols, alkaloids, tannins, etc. and these biomolecules are known for synthesis of nanoparticles. *Tridax procumbens* L., commonly known as coat buttons or daisy, is a species of flowering plant in the Asteraceae family. It is well known weed and widely occurred in tropical America, but now it has been introduced everywhere. The plant have daisy like yellow centred white flowers with toothed ray florets. The leaves are toothed and arrow shaped. The seeds are of achene type with stiff hairs. Traditionally it is used as natural wound healer in India (Vastrad et.al, 2016). It has been used as anticoagulant, antifungal and natural insect repellent. In Ayurveda it has been used for liver disorders, gastritis and heartburn (Wani et.al, 2016). It has been also used in the treatment of boils, blisters and cuts by traditional peoples of India (Nallella et.al, 2013). In present investigation the synthesis and characterization have been used to prepare silver nanoparticles from leaf extracts of *Tridax procumbens* plant to explore the traditional value. Chemical approach of silver nanoparticles with size distribution have been studied. AGNPs also revealed notable attention towards antimicrobial activity and have been shown to be more effective antimicrobial agent.

Materials and Methods

Collection of Plant Material

Fresh leaves of *T. procumbens* L. were collected from the campus of S. P. College, Pune-30 (MS), India for the present study.

Chemicals

All chemicals and reagents used were of analytical grade. Silver nitrate was procured from Himedia, Thomas Baker, Mumbai (MS), India.

Extraction of Biomolecules

The fresh leaves of *T. procumbens* were cleaned with distilled water and dried for 3hrs to remove moisture content. 10gms of fresh leaves were weighed, cut into fine pieces and ground in mortar and pestle. Then prepared leaf extract were mixed in the solvents (50% alcohol and distilled water). All solvents centrifuged at 4^0C temperature for 15 minutes at 10000 rpm (Remi centrifuge, C24 Plus, Mumbai) and supernatant was

collected. Residue was re-extracted with fresh 25ml of the respective solvent and process was repeated for twice. The obtained supernatant was collected and stored at 8^0C temperature in refrigerator for further analysis.

Synthesis of Silver Nanoparticles

100 ml of 1mM $AgNO_3$ solution and 10 ml of leaf extract of each solvent (50% alcohol and aqueous solution) was added into two conical flasks separately. Stirred it for 2hrs at 200 rpm using magnetic stirrer and kept under dark condition at room temperature for 24hrs to record the colour change. The gradual change in colour indicates the complete reduction of $AgNO_3$ to Ag+ ions.

Spectrophotometric Analysis

The UV-visible spectrophotometer (Beckman- DU50 Fullerton CA, USA) was used for spectrophotometry analysis. The complete bio-reduction of $AgNO_3$ to Ag+ ions in the solvent extract was carried out by periodic evaluation of the samples for 24 hrs. Subsequently 1 ml of sample of each solvent diluted with 2 ml of deionized water and scanned at UV-visible spectra between wavelengths of 200nm to 700nm.

Scanning Electron Microscope Analysis

SEM analysis was carried out to know the size and shape of the synthesized silver nanoparticles using *T. procumbens* leaf extract. Thin films of the sample were prepared on glass slide followed by coverslip. Then the images of silver nanoparticles were obtained by scanning electron microscope (ZEISS EVO-MA 10, Germany).

Estimation of Antibacterial Activity

For antibacterial activity two strains of bacteria namely, *Bacillus subtilis* (Gram +ve) and another *E. coli* (Gram -ve) were used. These strains were procured from NCIM, Resource Centre, CSIR-NCL, Pune, India. Disk diffusion method was used for testing. (Anonymous, 1986). The plates containing bacterial agar media were divided into four equal parts and previously prepared discs were placed in following manner;

i) Disc soaked with deionized water as control,
ii) Disc soaked with plant leaf extract,
iii) Disc soaked with 1Mm AgNO3 solution and
iv) Disc soaked with synthesized AgNPs.

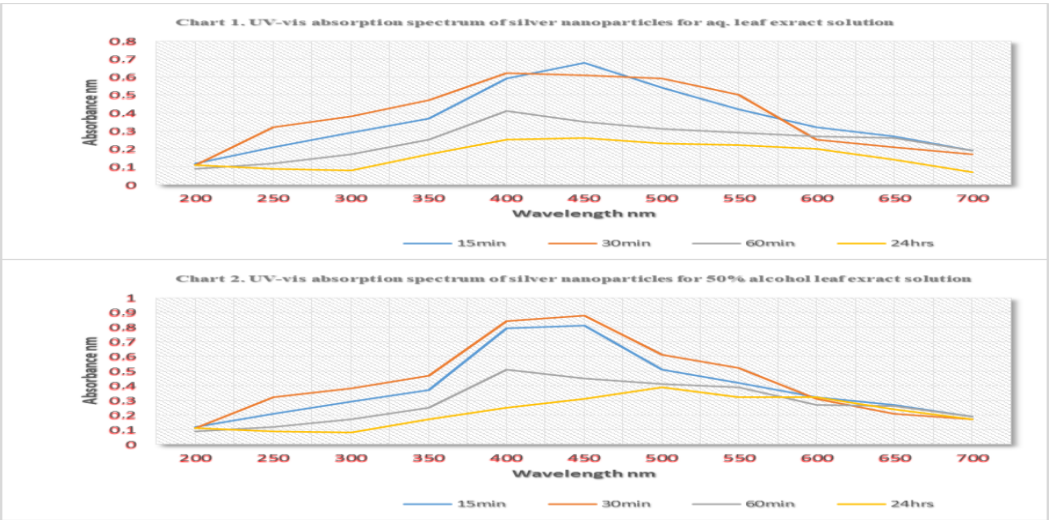
After that plates were incubated for 48hrs at room temperature. Antibacterial activity was measured by diameter of inhibition zone.

Results and Discussion

Characterization of AGNPs

UV-visible spectroscopy analysis of synthesized silver nanoparticles for both solvent systems of *T. procumbens* were evaluated. The gradual change in colour indicated the formation of silver nanoparticles. The colour of the solution changed from faint light

yellowish green to dark brown. This was confirmed that there is completion of reaction between plant leaf extract and silver nitrate. Similar findings were recorded by Shukla et.al, 2010; Namratha N and Monica PV, 2013 and Lalitha et.al, 2013. The UV-visible spectra recorded after time intervals of 15min, 30min, 60min and 24hrs of reaction time. The absorption spectra of AGNPs has maximum at 350nm to 500nm for both the solvents. It revealed that the formation of AGNPs is more within first 30min. Comparatively, the AGNPs of 50% alcohol solvent showed strong absorption spectrum than aqueous solvent (Chart 1 and 2). The change in colour of AGNPs due to excitation of surface plasmon vibrations in AGNPs and have been recorded by Shankar et. al., 2004 and Ankamwar et.al, 2005.



SEM Analysis

SEM analysis of synthesized AGNPs were distinguished on the basis of their variable size and shapes. It showed different shapes such as spherical, cuboid and triangular. It may be due to availability of different quantity and nature of capping agents present in both leaf extract solvents. An aqueous leaf extract showed spherical and cuboid shape and size ranges between 45.25nm to 90.34nm (Fig.1.). While 50% alcohol leaf extract showed triangular shape and size ranges between 45.21nm to 99.57nm (Fig.2.a and b). The alcohol leaf extract revealed comparatively smaller AGNPs size than aqueous leaf extract solvent. Same results were recorded by Vastrad et.al, 2016.


Fig.1.

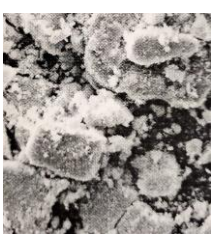


Fig.2 a.


Fig.2.b.

Antibacterial Activity Analysis

The antibacterial property of both the leaf extracts were assessed on the basis of diameter zone of inhibition. Both the leaf extracts were found to be more efficient. The synthesized nanoparticles of alcohol leaf extract showed more zone of inhibition than aqueous leaf extract against both the strains of bacteria. Also similar findings have been recorded by Banerjee et.al, 2014.

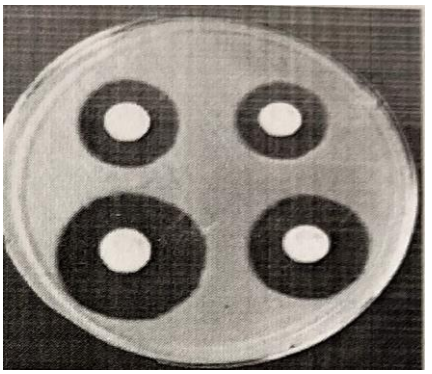


Plate 1. Zone of inhibition for 50% alcohol solution *E .coli* (Gram -ve)

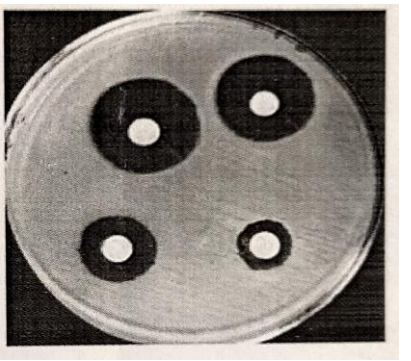


Plate 2. Zone of inhibition for 50% alcohol solution *B. subtilis* (Gram +ve)

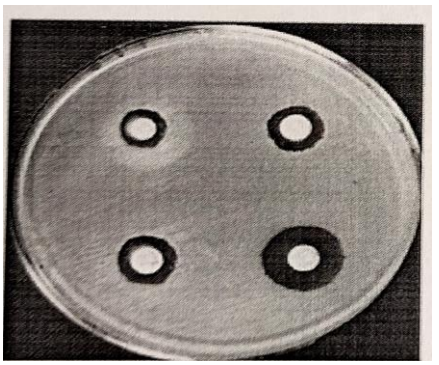


Plate 3. Zone of inhibition for 50% aq. solution *E. coli* (Gram -ve)

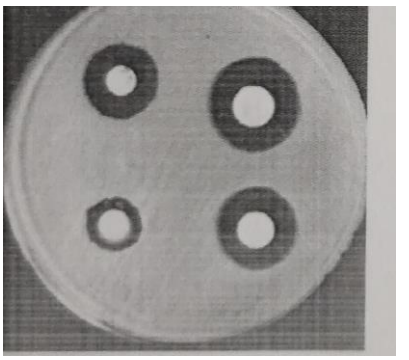


Plate 4. Zone of inhibition for 50% aq. solution *B. subtilis* (Gram +ve)

Conclusion

In the present study, a simple and medicinal approach has been considered to obtain an eco-friendly biosynthesis of AGNPs which was obtained from bio-reduction of *T. procumbens* L. leaf extracts with $AgNO_3$ solution. The synthesized AGNPs showed very significant results towards carried out analysis. The results of present study suggested that *T. procumbens* L. can be used as a potential antibacterial source in medicine. It can be also used as a drug source as a wound healer.

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