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GREEN APPROACH TO SYNTHESIS, CHARACTERIZATION OF SILVER NANOPARTICLES BY USING TRIDAX PROCUMBENS LEAF EXTRACT AND THEIR ANTIBACTERIAL ACTIVITY

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Abstract:
In the last few years, it has been seen that the importance of silver nanoparticles has gained much interest by many chemists and biologists. Therefore, Indian medicinal plants has yet to bring numerous sources of profitable, healthy, mostly reduced and stabilized compounds utilized in the biosynthesis of silver nanoparticle. The main aim of this study is to investigate the viable and sustainable ways for the biosynthesis of AgNPs from 1 mM aqueous $AgNO_3$ using leaf extracts of widely available plants such as Tridax procumbens (Tridax), which are well known for their wide availability and medicinal property. AgNPs were synthesized by the reaction of 1 mM $AgNO_3$ and 5% leaf extracts of each plant separately. The formations of the AgNPs were confirmed by the colour changes of the mixture solution and duly characterized by UV-Visible spectrophotometric analysis. The synthesized silver nanoparticles were subjected to Ultraviolet-Visible (UV-Vis) Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscope (SEM), their antibacterial and antifungal activities were tested against two bacterial strains and one fungal strain. Finally, the AgNPs showing better antimicrobial activity was tested for their water disinfection study against three water samples collected from River, Pond and Cannel. Obtained AgNPs from the different leaf extracts indicated higher antimicrobial activities against Escherichia coli and Bacillus spp. in comparison to both $AgNO_3$ and the raw plant extracts of Tridax. The final results showed that Tridax extract silver nanoparticles are showing significant antimicrobial activities, So the bacterial inhibition zone by the silver nanoparticles prepared from the Tridax leaves extract show maximum inhibition for Gram +ve *S. aureus* and *K. Pnemoniae* and Gram negative *E. coli* as well as *A. niger*.

Keywords: Green Synthesis, Silver Nanoparticles, Plant Extraction, Antimicrobial Activity

1. Introduction:

Nanoparticles are any type of particles which have their grain size less than 100 nanometer at least in any one of its dimension [1]. Nanoparticles have greater surface to volume ratio due to their reduced grain size. The higher surface to volume ratio results in enhanced physical and chemical properties of nanoparticles. Nanoparticles compromise the gap between material properties between bulk and atomic/molecular levels. The nanoscience spread its application in various fields such as electronics, materials, medicine and bio-technology towards the edge of science evolution [2,3]. Nanotechnology makes the usage of nanoparticles through design, production of devices and systems by controlling the grain size and shape at nanometre scale [4].Nowadays metal nanoparticles have greater impact in medicine, sensing, etc., because of their characteristic properties. Among metals, Silver nanoparticles gain much attention because of their peculiar physical and chemical properties. They have antibacterial and anti-viral properties and throw applications in medical and other industries. All the above properties of silver nanomaterials can be controlled by controlling their grain size.Silver nanoparticles can be synthesized by various physical and chemical methods and these methods are not environment friendly. Some of these methods may impart for adsorption of toxic substances on to the surface of nanomaterials. In order to avoid this, toxic free, environment friendly green methods are employed nowadays. Green synthesis methods employ certain principles that will reduce the usage of hazardous substances during the synthesis of nanomaterials [5].

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Nanoparticles, especially inorganic nanoparticles have been a subject of research for few years, because of their versatile applications and advantages. Gold and silver nanoparticles have a broad spectrum of applications particularly in the medical field. The therapeutic uses of these nanoparticles are being researched. The availability, compatibility and capability of these nanoparticles to functionas effective tools for medical imaging as well as treatment are being established [6,7]. The biological method of synthesis of nanoparticles have proved to be a better method than the chemical methods as the former has prominent advantages like low capital involved in production and less energy consuming.

The use of hazardous chemicals such as hydrazine eliminates the method from being an eco-friendly one while, green synthesis using plant extracts is environmentally benign. Moreover, nano-crystalline silver colloids produced by such aqua-chemical routes exhibit aggregation with time, thereby compromising with the size factor upon storage [8]. Green synthesis of nanoparticles is environment friendly. Due to slower kinetics, they offer better manipulation and control over crystal growth and their stabilization. Green synthesis is advantageous over chemical and physical method as it is cost effective, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [9, 10]. It sidelines various undesired deleterious effects of the chemical synthesis process and is thus environment friendly. Also, the active constituents of the plants used for green synthesis of nanoparticles adhere to or cap the nanoparticles and thereby improve their therapeutic efficacy. Nanosilver has been extensively used in wound management, treatment of ulcers and lesions, healing of skin-graft sites and preventing infection [11].

Tridax procumbens Linn. is a specie of flowering plantnand a local weed, which occurs in tropical as well as subtropical regions in all the seasons. This is a perennial herb with a creeping stem; leaves are simple, ovate-elliptical and coarsely serrate or lobed. Flowers are yellow, in heads each on a long stalk and fruits are small. Tridax procumbens is traditionally known for its antiviral and antioxidant efficacies, wound healing activity, insecticidal and anti-inflammatory activity. It also possesses significant medicinal properties against blood pressure, malaria, dysentery, diarrhoea,

stomach-ache and headache [12]. The extracts of the leaves of T. procumbens have been proved to decrease the time taken for blood clotting and thus helps in hemostasis. The objective of this study was the biosynthesis of silver nanoparticles using the extract of T. procumbens leaf. The biologically synthesized silver nanoparticles were tested for blood coagulation activity.

2. Materials And Methods:

All chemical and reagents used were of analytical grade.Silver nitrate was procured from Himedia. Liquiceilin-E wasprocured from Tulip Diagnostics Pvt Ltd., India.

2.1 Collection of plant material:

Tridax procumbens plant leaves were collected from the campus of Shivaji Art's, Comm. & Science College Kannad Dist. Aurangabad Maharashtra.

2.2 Tridax procumbens extract preparation:

A 10 g portion ofthoroughly washed fresh T. procumbens leaves were cut intofine pieces and 200 mL of sterile distilled water was added andexposed to microwave irradiation for 3 min to subdue theactive plant constituents [13]. The solution was then filtered in hot condition. The clear filtrate which is the extract of the leaf was used for nanoparticles synthesis.

2.3 Preparation of silver nitrate solution:

Analytical grade silvernitrate procured from Hi Media labs (RM 638) was prepared as10^{-3} and 10^{-6} M solutions and used in this experiment.

2.4 Experimental methods:

In this experimental method, the aqueous plant extracts were mixed with an aqueous solution with appropriate metal salt. Then the synthesis of nanoparticles occurred at room temperature involving with the below pathways. (schematic diagram is shown in Fig. 1).

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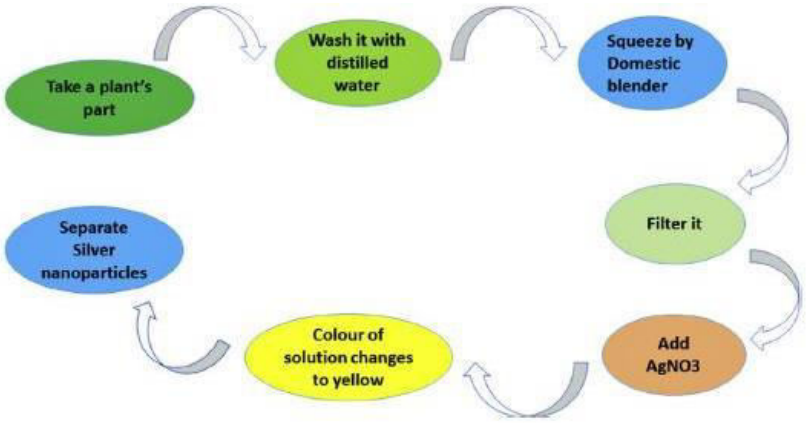

Fig. 1: Schematic representation of synthesis of $AgNO_3$ synthesis

3.5 Formation of leaf extracts:

Two commonly available Indian medicinal plants such as Tridax procumbens (Tridax daisy) (Fig.2) and (Details description was given in Table 1), was collected from the local area of Shivaji Art's, Comm. & Science College Kannad Dist. Aurangabad Maharashtra, India, on the basis of their ancient medicinal history as well as their ease of availability. Firstly, fresh, young and healthy green leaves were collected and washed carefully with tap water followed by distilled water to wipe out all the dust and unwanted particles and then cut into small pieces and allowed to dry at room temperature. Then the dried leaves were finely chopped and 20 g of each plant were weighed separately and transferred into the 250 mL Erlenmeyer flask containing 100 mL distilled water and allowed to heat at 60 ^{o}C for about 1 hour [14]. Then the extracts were filtered through Whatmann's No 1 filter paper, which resulted in the elimination of the particulate matters as well as in the collection of clear solutions. Now the clear solutions with proper covering were kept in refrigeration at 40C for further experiments. The processes of sterilization were maintained throughout the whole experiment to get results of high accuracy level and negligible errors without any contamination.

Fig, 2: Tridax procumbens (Tridax daisy)

Table 1: Detailed description of two medicinal plants used in the present study

Sr.No.	Plant common names	Odia name	Biological name	Family	Sub family	Codes
1	Coatbuttons/ tridax daisy	Bisalya karani	*Tridax procumbens*	*Asteraceae*	*Heliantheae*	TP

2.6 Synthesis of silver nanoparticles:

To synthesize the silver nanoparticles, 45 mL of the 1 mM silver nitrate ($AgNO_3$) solution was added to 5

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mL of each leaf extracts separately in 250 mL Erlenmeyer flasks. The synthesis of AgNPs was carried out separately with each type of plant extract by continuous stirring by a magnetic stirrer in darkness at room temperature. The changes in colour of the solution to brownish were monitored periodically. "The details of time and colour change were recorded along with periodic sampling and scanning by UV-visible spectrophotometry" [15]. Then the colour of the solution changed into reddish brown, which confirmed the reduction of $AgNO_3$ to Ag+ ions. The whole process of silver nanoparticle synthesis mediated by plant extract is shown in Fig. 4.The formation of AgNPs was again confirmed by spectrophotometric scanning in the range of 300 to 800 nm wavelength using a UVvisible spectrophotometer. Then AgNPs colloidal mixture was properly sealed and stored at 4oC for future use and the sterilization was maintained [16]. Synthesis of AgNPs was also carried using the mixture of all two leaf extracts together at equal ratio for evaluate the combined antimicrobial effect of the prepared leaf extract-AgNPs

3.7 Synthesis of AgNPs with varying $AgNO_3$ and leaf extract concentration:

Synthesis of AgNPs was done with different concentrations of silver nitrate and leaf extract such as 1:9 (i.e. 5 ml leaf extract added to 45 ml $AgNO_3$), 1:4 (i.e. 10 ml leaf extract added to 40 ml $AgNO_3$ and 3:7 (i.e. 15 ml leaf extract added to 35 ml $AgNO_3$).

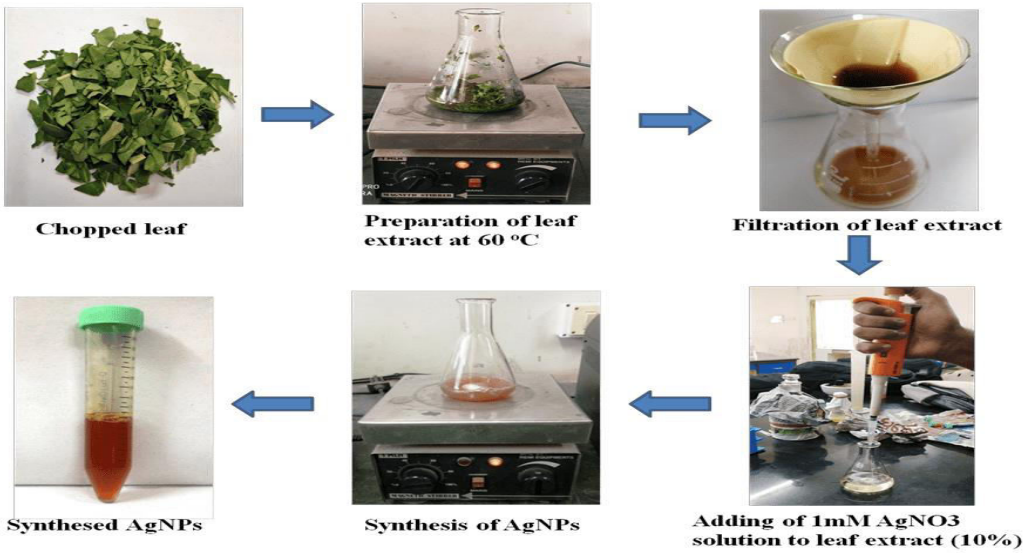

Fig. 3: The whole process involved in synthesis of AgNPs from leaf extracts.

3. Characterization of silver nanoparticles:

The physical characterization of silver nanoparticles was done at SAIF, IIT Madras.

3.1 Ultraviolet-visible spectra analysis:

The UV-Vis spectrum of the synthesized nano-silver particles was studied with Cary 5E UV-Vis spectrophotometer from 300-800 nm. The below Fig 4

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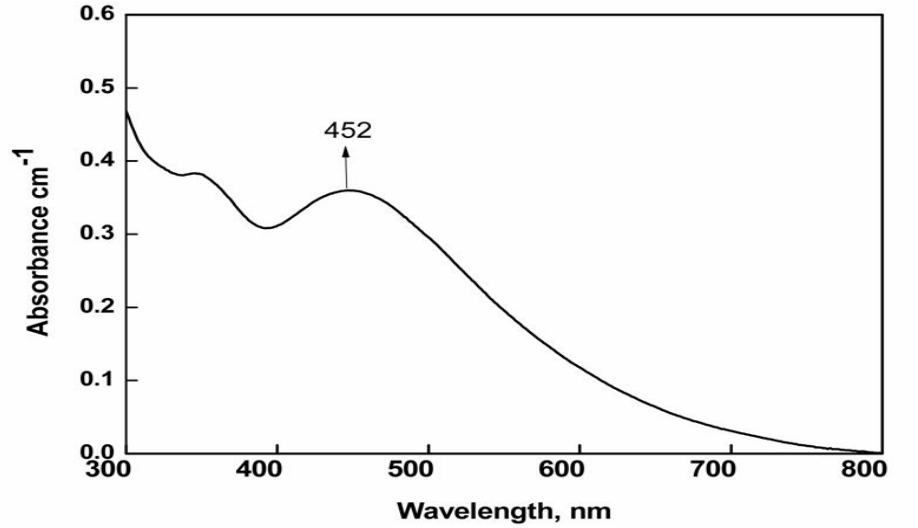

Fig. 4: UV-Vis spectral analysis of *Tridax procumbens* leaf extract mediated AgNPs

3.2 X-ray diffraction analysis:

The X-Ray Diffraction (XRD) pattern of the silver nanoparticles synthesized was studied by Bruker Kappa AXE XII with Cu K" (l = 1.54 Å) radiation, scanning range from 10^0-90^0. X-ray diffraction study confirmed the presence of silver nanoparticles. The Bragg reflections at 2 h = 38.18, 44.37, 64.48 and 77.63 can be indexed to the (111), (200), (220) and (311) orientations, respectively, confirmed the presence of silver nanoparticles. Spurious diffractions due to crystallographic impurities were absent Fig. 5

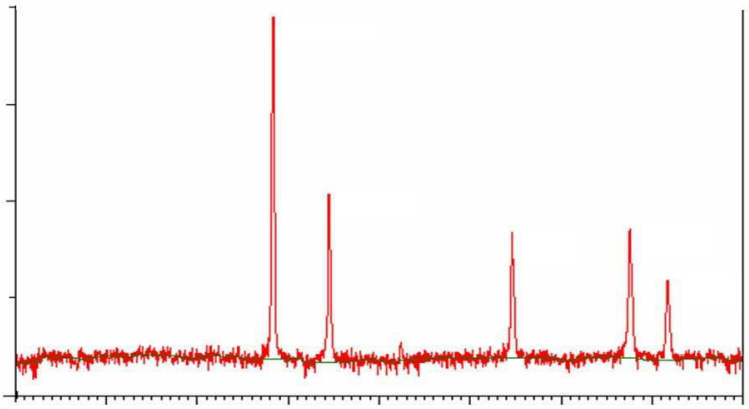

Fig. 5: X-ray diffraction patterns of silver nanoparticles synthesized using T. procumbens leaf extract

3.3 Fourier transform infra-red spectroscopy analysis:

The Fourier Transform Infra-Red Spectroscopy (FTIR) of the dried nanoparticles was studied using Perkin-Elmer Spectrum-One instrument. The 256 scans of silver nanoparticles were taken in the range of 450-4000 cm^{-1} and the resolution was kept as 4 cm^{-1}.

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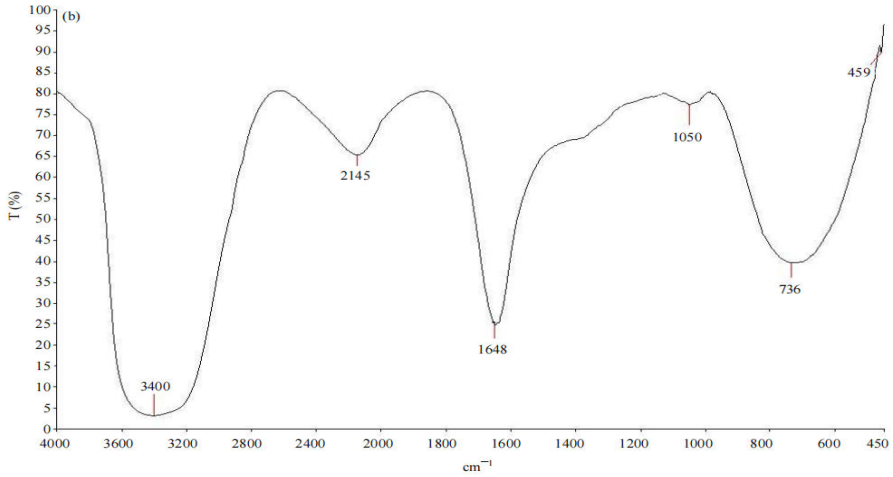

Fig.6 : FTIR spectrum analysis Silver nanoparticles synthesized using T. procumbens leaf extract.

3.4 Scanning Electron Microscope:

The solution with leaf extract was subjected SEM analysis after preparing the samples for SEM study. The SEM images are shown in the figure3. The SEM images show clearly the presence of nanoparticles in the nanoscale range 91 – 170 nanometer. The majority portion of nanoparticle show cubical shape. But some of the nanoparticles are in spherical shapes, but not well defined. The SEM images show approximately 30 % of particles are in nanoscale while the other exceeds in size and some of the nanoparticles are in various shapes than cubical one. From the above results, it may be concluded that the nanoparticles formed are at various shapes, but cubical nanoparticles are in majority. This morphology could be due to the bio-active agents of the extract mixture. The bicomponent nature of extract may also be a reason for the formation of multi shaped nanoparticle formation.

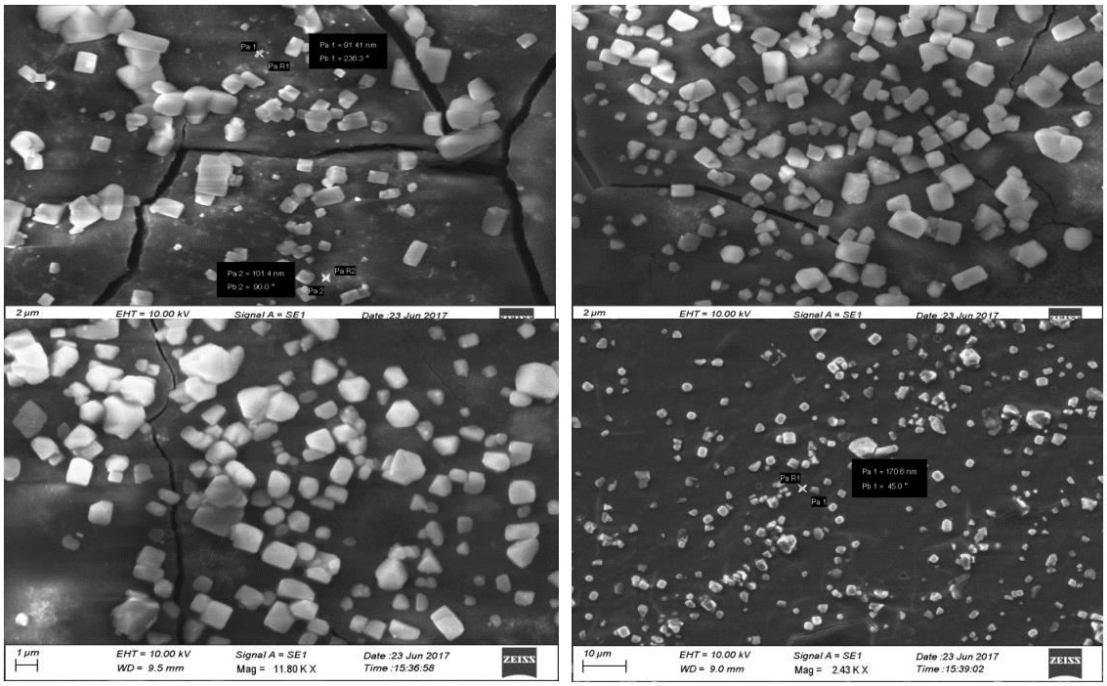

Fig.7.SEM image of silver nanoparticle synthesized from 0.001M silvernitrate solution with leaf extract

4. Analytical study of antibacterial activity:

With many advanced medicinal properties, the AgNPs thus prepared also indicated some high level

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specific antibacterial activities that were shown in Table 2. The obtained inhibition zone size (in mm) indicates maximum antibacterial activity was found higher for prepared AgNPs than the raw leaf extracts. As compared to all the AgNPs, it was found that Jatropha, Marigold, Gaint milk weed are showing more antimicrobial activities than Neem, Tridax daisy, black Tulsi. From the results obtained in the earlier studies, we came to a confirmation of the antibacterial potential of AgNPs prepared from these two above plant extracts [17,18]. The zone of bacterial inhibition by AgNPs were prepared from the two leaves extracts which indicated maximum inhibition for Gram positive *S. aureus* and *K. Pnemoniae* and Gram negative *E. coli* as well as *A. niger*.From the antimicrobial results of AgNPs prepared in different ratios of leaf extracts and $AgNO_3$ solution, it was found that ratio 2:8 and 3:7 of leaf extract and silver nitrate solution was showing slightly higher antimicrobial activities than the 1:9 ratios. In case of the antimicrobial properties of the mixture of all leaf extract AgNPs, all the ratio of AgNPs composition are showing well but ratio 1:9 ratio has little more antimicrobial effect.

Table 2: Antimicrobial Activities of Silver Nanoparticles By Using Tridax Procumbens Leaf Extract with AgNPs:

	B.subtilis	*S.typhiium*	*S.aureus*	*K.pneumoniae*	*E.coli*	*A.niger*
	Diameter of inhibition zone presented in mm					
TE-O	0	3.0	2.0	1.0	0	1.0
TE-AgNPs	1.0	2.0	4.0	3.0	9.0	3.0
TP-O	0	0	0	0	0	0
TP-AgNP	0	0	4.0	4.0	0	5.0

Conclusion:

The green synthesis of silver nanoparticles has various advantages over conventional synthesis. In this study, green synthesis of nanoparticles of silver was achieved using the extract of T. procumbens leaves. The synthesized nanoparticles were characterized and analysed for blood coagulation activity. The studies ascertained the presence of plant secondary metabolites on the surface of nanoparticles. Thus these silver nanoparticles can be used to promote blood clotting.

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