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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING THE LEAF EXTRACT OF *ARGYRIA NERVOSA* AND ANTIMICROBIAL STUDIES

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ABSTRACT: The green synthesis of silver nanoparticles (AgNPs) gained interest and attracted to many researchers due to physical, optical, chemical and biological properties, enhancing a range of activities such as antibacterial, antifungal, anti-inflammatory and anticancer us activity. The purpose of this study is to synthesize and characterize silver nanoparticles from fully expanded leaves of *Argyria nervosa* as well as to test their effectiveness in antibacterial activity. In this study, at 0.1 mM concentration of silver nitrate (AgNO3), stable AgNPs were synthesized and authenticated by monitoring the colour change of the solution from yellow to brown, which was confirmed with spectrophotometric detection of optical density. The crystalline nature of these AgNPs was detected through an SEM, EDX and FTIR pattern. AgNPs were characterized through a Scanning Electron Microscopy to study the morphology of the nanoparticles. AgNPs obtained showed significantly higher antimicrobial activities against *Escherichia coli* (E. coli) and *Bacillus sp*. in comparison raw plant extracts.

INTRODUCTION: Silver nanoparticles (SNPs) have gained considerable attention in the different fields of science. They are recently applied in various products including sensors, filters, image contrast agents, nanoelectronics devices and antimicrobial agents due to their good optical properties, stability, electric conductivity and antimicrobial activity [1]. SNPs have the properties of very small size (< 100 nm), high surface area and extraordinary dispersion rate [2]. In textile industries, SNPs are most widely used as an antimicrobial agent for the water treatment.

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They are safe and effective antibacterial agents as they are highly toxic to bacteria but non-toxic to animal cell [3]. They are extensively used in several biomedical applications like efficient antimicrobial agents, *in-vivo* imaging, drug delivery, anticancer agents, biosensors in present days the most common application of AgNPs is their use as an antimicrobial agent and in antiseptic wound dressings.

So, scientists working in the nanotechnology area are searching for an alternative method for the synthesis of metal nanoparticles with the help of biological sources which is the basis of many technological innovations in the twenty-first century with simple, fast, eco-friendly, and environmentally safe methods popularly known as "Green synthesis" [4]. In the present investigation we have selected medicinal plant namely "*Argyria*

nervosa" which was reported to have several medicinal properties and is widely used in Ayurveda. A. nervosa attracted the attention of several researchers worldwide for its pharmacological activities. *A. nervosa* is mostly spread throughout India, belongs to the family Convolvulaceae is a climbing shrub with a woody tomentose stem. The various plant parts of *A. nervosa* prove to have a broad spectrum of activity on quite a large number of ailments *A. nervosa* plant parts are reported to contain flavonoids, sterol glycoside, flavonoids, and essential oil which are responsible for several pharmacological properties [5].

Keeping this several abundant pharmacological properties, the current work was focused on biosynthesis silver nanoparticles (AgNPs) using leaf extract of *A. nervosa*. The An-AgNPs were characterised by different FTIR, SEM and EDAX methods and also investigate the antibacterial studies.

MATERIAL AND METHOD:

Collection of Plant Material: Fresh leaves of A. nervosa were collected from the Vaijapur hills of Aurangabad and they are brought to the laboratory and washed with running tap water several times followed by distilled water. Then the leaves were air dried on filter paper to remove the water content then they are shaded for seven days after that the leaves are made into powder with the help of an electric blender.

Green synthesis and Characterisation of An-AgNPs: 10 grams of fresh leaf powder was added to 100 ml of sterile Milli Q water and it is left overnight at room temperature. The next day morning the sample was heated at 70°C for one hour in a hot water bath. The aqueous leaf extract of *A. nervosa* was filtered through cheesecloth followed by Whatman no 1 filter paper. This filtered extract was stored at 4°C in the refrigerator for further studies and analysis.

The initial time of colour change of reaction mixture was recorded by visual observation, soon after the green synthesis the An-AgNPs were analysed in Nanodrop-8000, UV-visible spectrometer, and recorded its surface plasmon resonance (SPR) peak. Fourier transform infra-red spectroscopy (FTIR) analysis of *A. nervosa* leaf extract and green synthesised An-AgNPs was carried out (Bruker Tensor 27) to find study the morphology in the leaf extract which are actively involved in bio-reduction and green synthesis of An- AgNPs. Scanning Electron microscopy (SEM) and EDAX analysis was done by using (SU8010, Hitachi) machine, used to characterize the shape of AgNPs. On the carbon-coated copper grid, a thin film of the dried samples was made by simply placing a little sample followed by drying for 5 min under the mercury lamp. A Field-Emission Scanning Electron Microscope (FESEM) images were used to study the size and morphology of AgNPs.

Estimation of Antibacterial Activity: The comparative antibacterial activities of the plant leaf extracts and of the Ag NPs synthesized from the respective extracts were effectively accessed against one Gram(+) ve (Bacillus) bacteria and one Gram (-) ve (*Escheri chiacoli* (*E. coli*)) bacteria and disc diffusion method was followed for testing of plant leaf extract and their respective Ag NPs containing solution. The discs were soaked with double distilled water, plant leaf extracts, silver nitrate solution and solution containing silver nanoparticles of each type separately. Then the discs were air dried in sterile condition. The plates containing nutrient agar media were prepared by swabbing them with the microbial cultures. Plates containing media as well as culture were divided in to four equal parts and previously prepared discs were placed on each part of the plate. Disc soaked with solution containing plant leaves mediated synthesized silver nanoparticles. The plates were incubated at 37°C for 24 to 48 h. Then, the maximum zone of inhibition was observed and measured for analysis against each type of test microorganism.

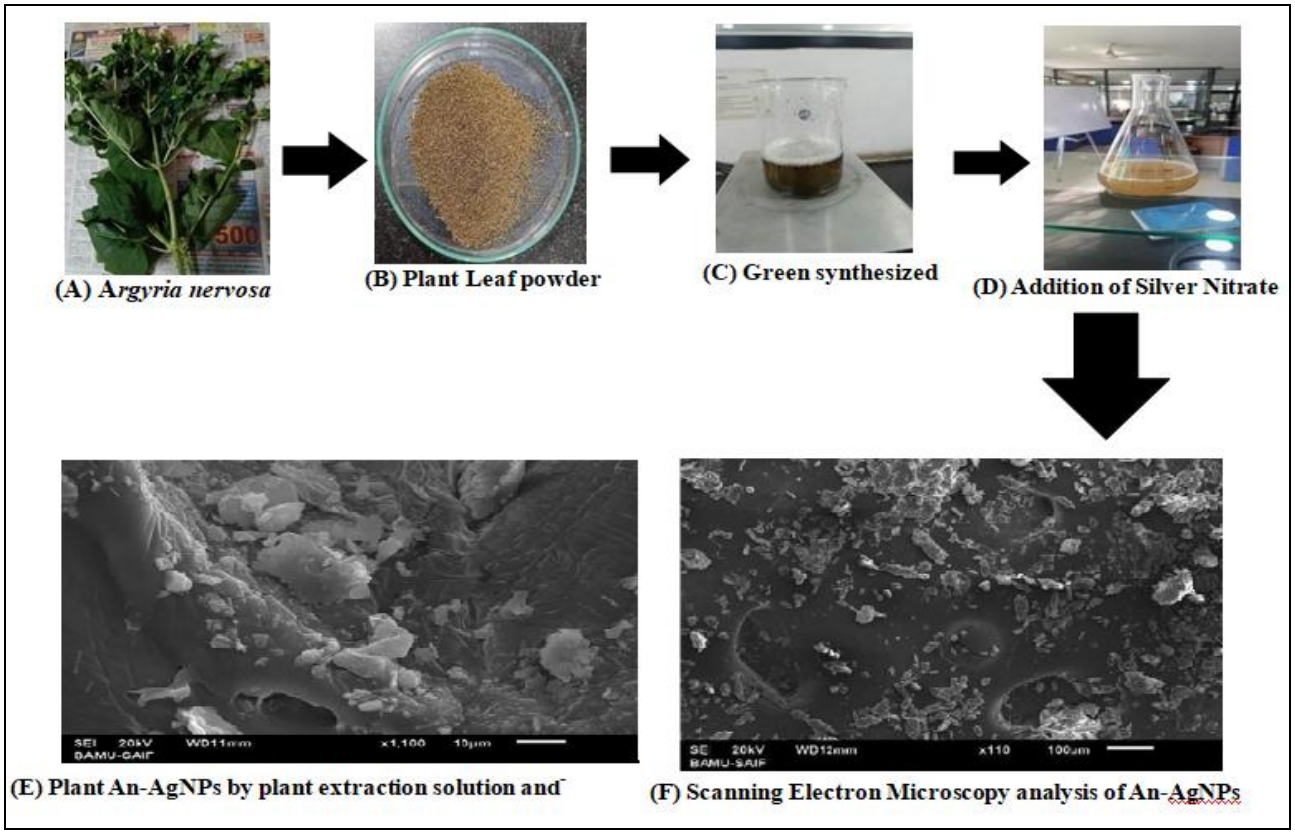
RESULTS AND DISCUSSION: Addition of plant extracts to the aqueous solution of $AgNO_3$ resulted in a colour change from light brown to dark brown in case of aqueous extract **Fig. C**. The plant extract/$AgNO_3$colour deepens with the time indicating the chemical formation of SNPs. The colour change was further accomplished by taking a UV–visible spectrum of a brown-coloured solution after 24 h of reaction.

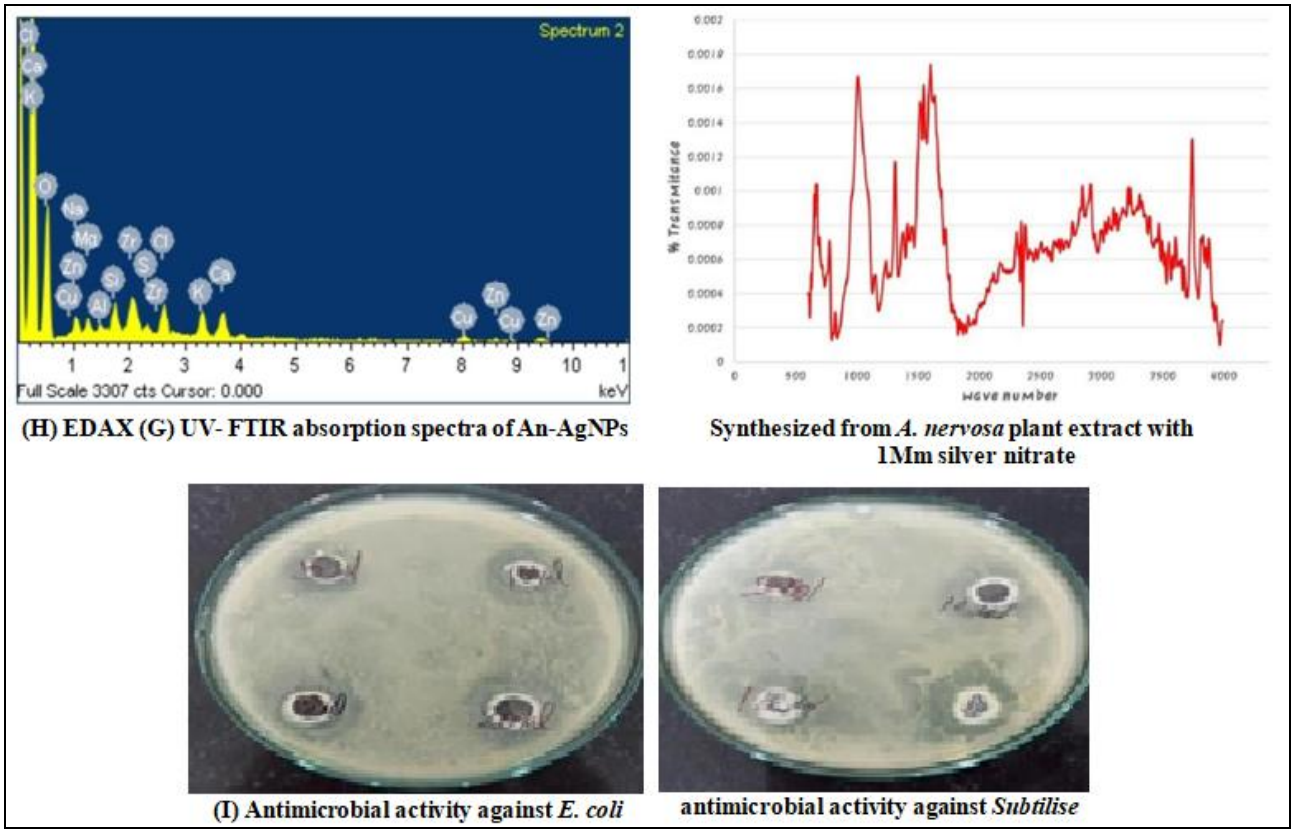
FTIR Analysis of Green Synthesized An-AgNPs: FTIR analysis of *A. nervosa* leaf extract and green synthesized An-AgNPs is shown in **Fig. G**. The peaks for *A. nervosa* leaf extract were found at 3395, 1629, 999, 819, 505.67 cm^{-1}.

The peak at 3395 corresponds to the O-H stretch of free hydroxyl alcohol and phenols, The IR band at 1629 cm^{-1} is due to the N-H bond of primary amines. The IR band at 999 cm^{-1} and 819 cm^{-1} corresponds to the C-N stretch of aromatic amines, aliphatic amine groups. The bio synthesized.

An-AgNPs showed some prominent peaks in FTIR analysis such as 3388, 1629, and 496.49 cm^{-1}. The peaks at 3388 cm^{-1} was due to O-H bonds of alcohol and phenols the intensity of the peak is reduced when compared with the extract, the peak at 1629 cm^{-1} corresponds to the stretch of N-H bending of primary amines and 496.49 cm^{-1} due to aliphatic Odo compounds of C-I stretch. The results revealed that the different phytoconstituents like flavonoids, polyphenols tannins, of the leaf extract have actively participated in the reduction of silver nitrate to An-AgNPs and in capping and stabilization of the nanoparticles. The results were similar to earlier reports on silver nanoparticles synthesized by green methods.

SEM and EDAX Analysis: SEM technique was employed to visualize to size and shape of silver nanoparticles, in **Fig. E** SEM images were obtained with leaf extract of A. nervosa shown their mean size distribution histograms in **Fig. 2**. An SEM-EDX analysis of the surface morphology of the AgNPs synthesized using the Leaf extract of *A. nervosa* **Fig. E** revealed the presence of nanoparticles with different morphologies, although a predominance of spherical- elliptical NPs of different sizes was observed. The particles are irregularly distributed and have an average size of 25.77, 20.53 and 17.27 nm for aqueous-SNPs and distinctly separated from each other some aggregation was also detected, and most of our analysis results showed separate particles with spherical shapes. Few particles attached to each other, because the sample preparation resulted in NPs with rough surface areas, or the region selected for analysis had NPs with rough surfaces. [7]. The elemental composition analysed by EDX **Fig. H** spectroscopy indicated the presence of C, O, K, Cl, Cu, and Ag, and their mass percentages were 17.52, 29.17, 4.16, 5.93 and 45.89, respectively. Of the elements, silver exhibited the highest perfect peak, indicating that the synthesized silver nanoparticles were pure.





(H) EDAX (G) UV- FTIR absorption spectra of An-AgNPs Synthesized from *A. nervosa* plant extract with 1Mm silver nitrate

(I) Antimicrobial activity against *E. coli* antimicrobial activity against *Subtilise*

The colour transformation to dark brown from the original yellow confirmed the formation of silver NPs from *A. nervosa* plant extract. Prasad and Elumalai noticed that silver nanoparticles come in a variety of colours, ranging from light yellow to brown. Furthermore, because of surface plasmon excitation fluctuations in silver nanoparticles, [11] observed that these nanoparticles, in aqueous solutions, gave out a yellowish-brown colour. The maximum absorbance peak for silver nanoparticles synthesized from the *Acer oblong folium* plant extract was observed at 450 nm using the UV visible spectrum. According to [11] a 430–450 nm peak of absorption is observed in the silver NPs' spectra present in the reaction media, whereas [12] narrowed the gap and reported an absorbance peak of 438 nm. Similarly, previously reported green synthesisations showed similar results with *A. nervosa* [8] like leaf extract *Artemisia annua*, [10] and *Boerhavia erecta*, [9] extract mediated silver nanocomposites. Plant extracts' reduction by stabilizing agents plays a role in the reduction of Ag+ ions to Ag nanoparticles, according to FTIR study [13, 14] observed that silver nanoparticles bind to the carboxyl or amino groups of extracted proteins. The amine (–NH), hydroxyl (–OH), and carboxyl (–C=O) groups of leaf extracts are primarily engaged to fabricate silver nanoparticles, according to studies conducted by (Tuama and Mohammed 2019) [15] Scanning electron microscopy (SEM) was utilized to determine particle size as well as morphology of biosynthesized AgNPs. The SEM micrograph predicted the morphology, i.e., rod-like, ellipsoidal shape [16]. *A. nervosa* derived silver nanoparticles have a potent inhibitory effect one. Coli and Bacillus subtilis. Silver nanoparticles synthesized from. nervosa leaves are very small size, due to which they have unique physical and chemical properties. Nanoparticles have a more germicidal effect than the mass of silver metal because of the reduction of size, increase of the ratio of surface to volume of nanoparticles, and increase of the contact area with microorganisms. Similarly, [17] published a human pathogen antibacterial assay using silver nanoparticles synthesized from Papaya fruit extract, which was revealed to be highly toxic against bacteria that are resistant to multiple drugs [12] also found that AgNPs were moderately toxic to *E. coli*, *Pseudomonas* species, including putida, aeruginosa, vulgarism *B. subtilis*.

CONCLUSION: The present study demonstrated the eco-friendly, inexpensive, facile and fast

synthesis of AgNPs using aqueous leaf *Argyria nervosa* plant species used in traditional medicine, Various molecular characterization techniques confirmed successful formation of spherical and crystalline AgNPs and evidenced the role of phytochemicals from the extracts as reducing and capping agents in the green synthesis of AgNPs. Moreover, the biological evaluation of AgNPs revealed good bactericidal properties against *Bacillus subtilis*, and Escherichia coli, which are pathogens commonly involved in infectious skin diseases. Hence, this study testifies that bio-functionalized AgNPs obtained by green synthesis using medicinal plants hold the potential to tackle microbial cutaneous disorders (i.e. infections, and burn wounds, etc.).

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CONFLICT OF INTERESTS: The authors declare that there is no conflict of interests regarding the publication of this paper.

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