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GREEN SYNTHESIS OF SILVER NANOPARTICLES USING ROOT EXTRACT OF *AERVA TOMENTOSA* FORSK. AND ANALYSE ANTIBACTERIAL AND ANTIOXIDANT PROPERTY

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ABSTRACT: During the present investigations attempt was made to the green synthesis of silver nanoparticles using root extract of *Aerva tomentosa* Forsk. and to analyze its antibacterial and antioxidant activities. Exposure of the root extract of *Aerva tomentosa* to aqueous $AgNO_3$ resulted in the formation of silver nanoparticles. The synthesis of silver nanoparticles was confirmed by UV-Vis spectrophotometer and was characterized by X-ray diffractometer. The bactericidal activity of silver nanoparticles against *Escherichia coli*, *Bacillus thuringiensis*, *Bacillus subtilis* and *Pseudomonas putida* was determined using bacterial growth inhibition method. The antioxidant study of silver nanopartcles was analyzed by the 2, 2-diphenyl-1-picrylhydrazil (DPPH) radical scavenging method. The synthesis of AgNPs *via* this green approach by *Aerva tomentosa* root extract were average size particles and crystalline in nature. The synthesized silver nanoparticles with two concentrations (0.01g/100µl) and (0.005g/100µl) exhibited high antibacterial activity in terms of maximum zone of inhibition against *Escherichia coli*, *Bacillus thuringiensis*, *Bacillus subtilis*, and *Pseudomonas putida*. Silver nanoparticles also showed significant antioxidant activity. There was a dose-dependent increase in the percent antioxidant activity for all concentrations (80, 160, 240, and 320 µg/ml of AgNPs) tested. Owing to its good antibacterial and antioxidant activity, present study supports that AgNPs might be used as potential antibacterial and antioxidant drug in traditional medicine to treat various diseases.

INTRODUCTION: Nanoparticle research is an area of immense scientific research due to a wide range of potential applications in biomedical, optical, and electronic fields. AgNPs have received enormous attention due to their defense against micro-organisms as well as drug resistance against some frequently used antibiotics [1]. The nano-particles from a medicinally important plant has the upper hand as medicinal properties are added to synthesized particles.

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The green synthesis of silver nanoparticles and their application in biomedical sciences additionally contribute to the antimicrobial, antioxidant, and anti-inflammatory properties [2, 3]. These advan-tageous properties of AgNPs have been integrated into commercially available wound dressings, pharmaceutical preparations, and medical implant coatings [4, 5, 6]. There are several reports on the synthesis of AgNPs using micro-organisms and plants as reducing agents [7, 8]. The green synthetic route holds better chances as it is cost-effective and environment friendly compared to chemical and physical methods [9, 10].

Plant extract-driven green synthesis of silver nanoparticles has been studied in plants like *Brillantaisia patula*, *Crossopteryx febrifuga* and *Senna siamea*, [11] *Lysimachia foenum-graecum*, [12]

Citrus, [13] *Impatiens balsamina* and *Lantana camara*, [14] *Myrtus communis*, [15] *Memecyclon edule*, [16] *Calicarpa maingayi*, [17] *Terminalia chebula*, [18] *Trachyspermum ammi*, and *Papaver somniferum*, [19] *Bahunia variegate*, [20] *Hevea brasiliensis*, [21] *Aloe vera*, [22] Tea leaf [23] and *Cestrum nocturnum*, [24] *Cassia angustifolia*, [25] *Hibiscus rosa-sinensis*,[26] *Sorbus aucuparia*, [27] *Cinnamomum camphora*, [28] *Citrullus colocynthis*, [29] *Catharanthus roseus*, [30] *Aerva lanata*, [31] *Aerva javanica* [32]. To the best of our knowledge, there is no report on the synthesis of AgNPs using *Aerva tomentosa* root.

Aerva tomentosa Forsk. is a common wayside weed-grown mostly on the waste place and found on the plains of the warmer parts of India includes Rajasthan, Gujarat, Haryana, Punjab, and Central India, excluding India it's occur in Burma, Baluchistan, Ceylon and in tropical Africa, commonly known as Bui/Boi/Bur [33]. In Ayurveda medicine, it is used as one of the best remedies for kidney and bladder stone [34] diarrhea and dysentery [35] and toothache [36, 37]. The plant contains a number of galactosides, flavanol glycosides, amyrin, and sitosterol [38, 39] and showed significant antimicrobial activity against gm^{-ve} bacteria [40].

The ethnobotanical study indicated that all parts of *A. tomentosa* have diverse applications in folk medicine to treat various diseases [41, 42]. The root extract has high amounts of alkaloids and saponins, compared to the aerial parts contained triterpenes and flavonoids in abundance [43]. Moreover, root extract qualitative phytochemical analysis *A. tomentosa* showed the presence of carbohydrates, flavonoids, saponins, alkaloids, and tannins. The flavonoids and tannins content have antiulcer properties and can be used for antiulcer drugs [44, 45]. Likewise, *A. lanata* root analysis showed a rich source of natural antioxidants, contains medicinally important bioactive compounds [46]. Therefore, owing to ethanobotanical aspect of *A. tomentosa* root, the present study was undertaken to the synthesis of AgNPs and further analyzed its antibacterial and antioxidant activity.

MATERIALS AND METHODS:

Preparation of the Root Extract: *A. tomentosa* roots were collected from nearby areas and washed with tap and distilled water, respectively, to get rid of all unwanted visible particles. After that, the root was cut into small pieces and oven-dried at room temperature. About 1g of the finely incised root was put into 250 ml beakers containing 100 ml of distilled water and boiled it for about 20 min. The extract was then filtered (Whatman filter paper no. 1) to remove particulate matter and to get clear solutions which was then refrigerated at 4 °C.

Biosynthesis of Silver Nanoparticles (AgNP): Silver nitrate ($AgNO_3$) aqueous solution (1mM) was prepared in 250 ml Erlenmeyer flask, and root extract was added for reduction of $AgNO_3$ into AgNPs. To avoid photoactivation of $AgNO_3$, a reaction was performed in darkness at room temperature. Suitable controls were maintained all throughout the conduction of experiments. Following the centrifugation at 5000 rpm for 20 min, the pellets of AgNP were re-dispersed into de-ionized water. The purified AgNPs recovered after freeze-drying was confirmed using UV-VIS double beam spectrophotometer in UV-VIS spectra between wavelengths of 300 to700 nm.

Antibacterial Activity of Silver Nanoparticles:

Analysis of Bacterial Growth Inhibition Method: The inoculation of different bacterial strains (*Escherichia coli*, *Bacillus thuringensis*, *Bacillus subtillis*, and *Pseudomonas putida*) were swabbed onto a nutrient agar plate and incubated at 37 ºC for 24 h. The testing solution (AgNPs) was applied in two different concentrations [(0.01g/ 100µl) and (0.005g/100µl)]. The disc diffusion method was used for bacterial growth analysis [47, 48]. The maximum zone (in mm) of bacterial growth inhibition was recorded to analyze bacterial growth.

Antioxidant Activity of Silver Nanoparticles:

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Method: The DPPH free radical scavenging method was performed according to previously described by Eshwarappa *et al*., (2014) [49]. Different concentrations (80, 160, 240, and 320 µg/ml) of AgNPs were prepared by adding AgNps to DPPH solutions and incubated in the dark for 25 min at room temperature. The absorbance of AgNPs and ascorbic acid (Standard) were observed at 517nm by UV-VIS double beam spectrophotometer. Using the given equation 1, the percent inhibition of DPPH free radicals was calculated.

Inhibition (%) = Pc – Ps × 100 / Ps …..Eq 1

Where Pc - absorbance of the control, Ps - absorption of AgNPs /Ascorbic acid

RESULTS:

Characterization of AgNPs:

UV-VIS Spectra Analysis: The conversion in mixture color from faint light to yellowish-brown signalized the formation of AgNPs as soon as root extract of *A. tomentosa* was mixed with $Agno_3$ **Fig. 1B**. The synthesis was confirmed by UV-VIS spectrum between wavelengths of 300 to 700 nm in a double beam spectrophotometer. The characteristic absorbance peak was observed at 443 nm **Fig. 1A**. This absorbance peak further confirmed the presence of silver nanoparticles in the samples.

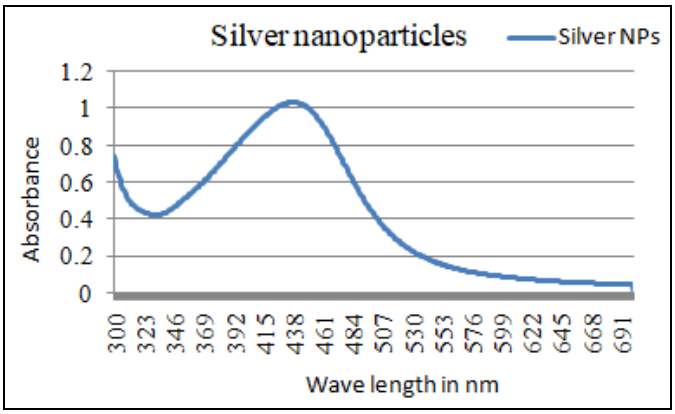

FIG. 1A: UV-VIS SPECTRA OF SILVER NANOPARTICLES

FIG. 1B: SILVER NANOPARTICLES

X-ray Diffraction Analysis: XRD patterns of the synthesized AgNPs exhibited distinguished diffraction peaks of silver metal at $2\theta = 38.45^{o}$, 45.04^{o} and 53.90^{o} **Fig. 2**, which indicated the presence of the fcc (face-centered cubic) crystalline phase of the AgNPs that resembles to the standard JCPDS File No. 00-004-0783 of the metallic silver. The absence of unwanted peaks confirmed the phase purity of the AgNPs.

The hump in the diffractogram is associated with the glass substrate since the sample was dried on a glass substrate before XRD analysis. The results expressed that the Ag^{+} ions were reduced to Ag^{+} by *A. tomentosa* plant extract under suitable reaction conditions.

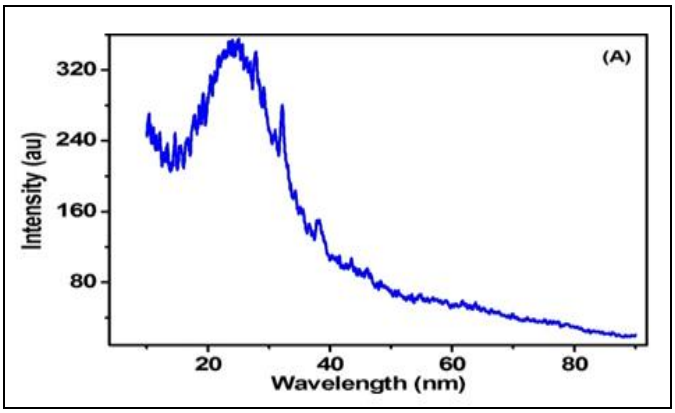

FIG. 2: XRD GRAPH OF SILVER NANOPARTICLES

Analysis of Antibacterial Property: It was observed that the maximum zone of inhibition was obtained against *Escherichia coli* followed by *Bacillus thuringiensis*, *Bacillus subtilis*, and *Pseudomonas putida* in concentration A (0.01g/-100µl).

This pattern of zone of inhibition varied with concentration B (0.005g/100µl) where, the maximum zone of inhibition was found in *Pseudomonas putida* followed by *Bacillus thuringiensis*, *Escherichia coli* and *Bacillus subtilis* **Fig. 3, Table 1**.

TABLE 1: ANALYSIS OF ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SHOWING MAXIMUM ZONE OF INHIBITION

	AgNPs (Maximum Zone of Inhibition)	
	Concentration A (0.01g/100µl)	**Concentration B (0.005g/100µl)**
Bacillus subtilis	15.20±0.07	12.00±0.05
Pseudomonas putida	15.05±0.03	13.10±0.07
Bacillus thuringiensis	18.10±0.02	12.40±0.01
Escherichia coli	18.30 ±0.05	12.20±0.01

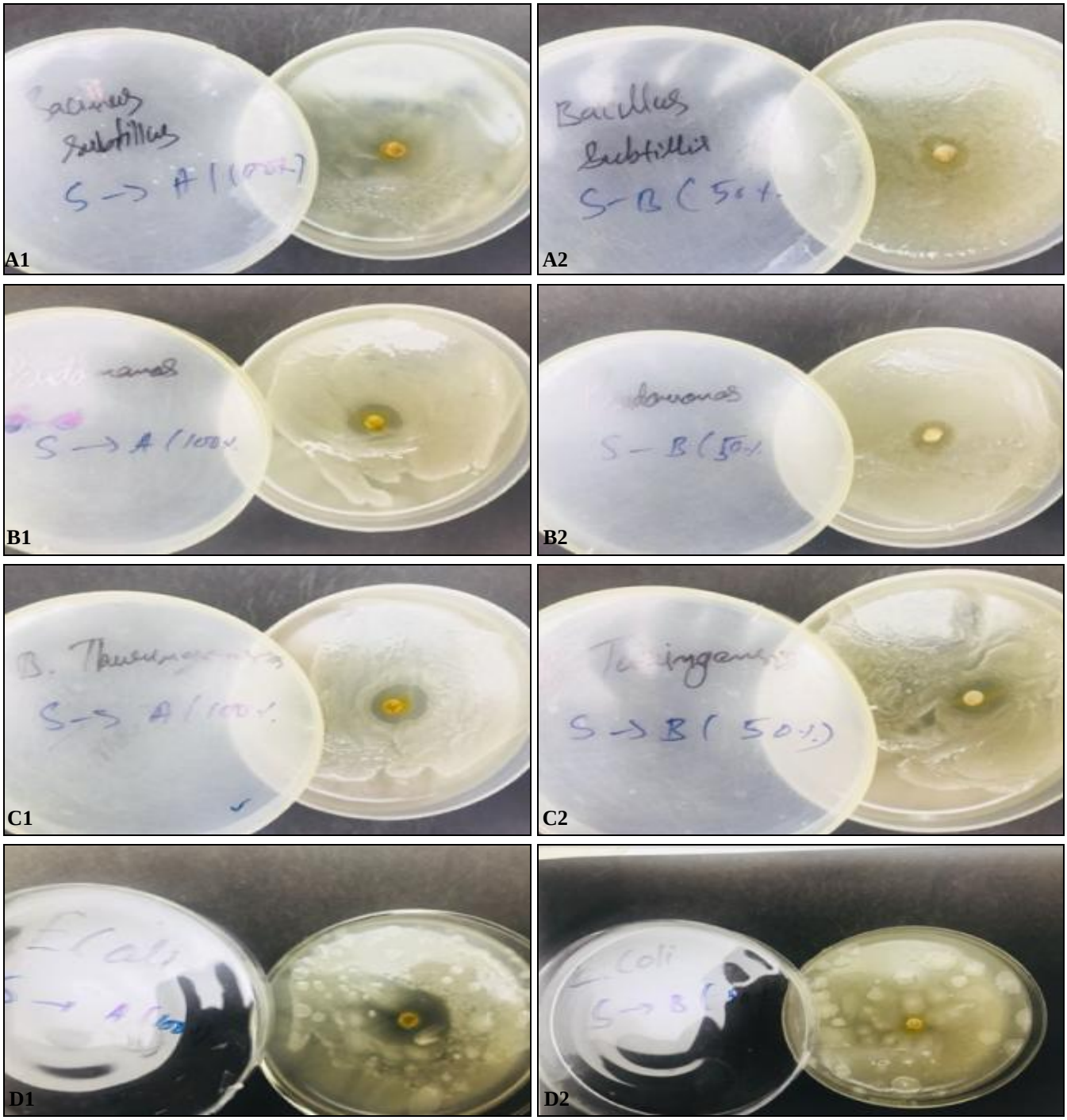

FIG. 3: ANALYSIS OF ANTIBACTERIAL ACTIVITY OF TWO CONCENTRATIONS OF SILVER NANOPARTICLES ON BACTERIA. A1- *Bacillus subtilis* (0.01g/100µl), A2- *Bacillus subtilis* (0.005g/100µl), B1- *Pseudomona putida* (0.01g/100µl), B2- *Pseudomona putida* (0.005g/100µl), C1- *Bacillus thuringiensis* (0.01g/100µl), C2- *Bacillus thuringiensis* (0.005g/100µl), D1- *Escherichia coli* (0.01g/100µl), D2- *Escherichia coli* (0.005g/100µl)

Several concentrations ((80,160,240 &320 µg/ml) of AgNPs were tested for, exhibited a comparable activity with ascorbic acid. Ascorbic acid was used as standard. The percent inhibition in antioxidant activity revealed a dose-dependent increase for all concentrations tested. Results confirmed **Fig. 4, Table 2** that AgNPs showed significant DPPH radical scavenging activity; however, results were more pronounced in the case of ascorbic acid.

TABLE 2: PERCENT INHIBITION OF CONTROL (ASCORBIC ACID) AND TEST DRUG

Concentration (µg/ml)	Ascorbic Acid (% Inhibition)	Silver nanoparticles (% Inhibition)
80	37.32%	29.00%
160	37.14%	32.85%
240	37.23%	33.92%
320	37.41%	34.37%

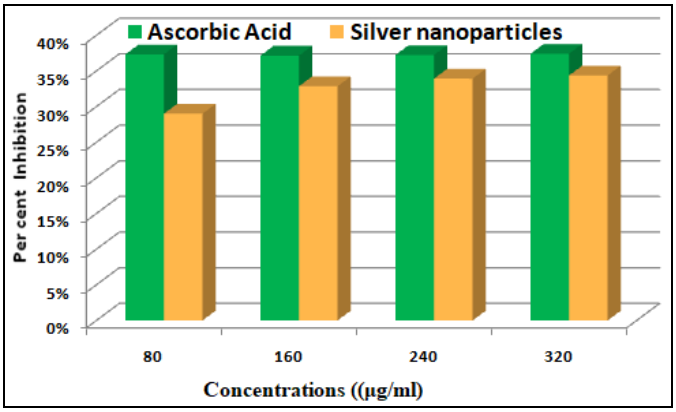

FIG. 4: ANTIOXIDANT ACTIVITIES OF SILVER NANOPARTICLES

DISCUSSION: The present research findings revealed AgNPs synthesis *via* green route utilizing *A. tomentosa* root extracts. Exposure of root extract to aqueous $AgNO_3$ resulted in the formation of silver nanoparticles. The color change from faint light to yellowish-brown reflected the reduction of silver nitrates into the silver nanoparticles **Fig. 1B.** Moreover, UV-VIS spectra between 300-700 nm and characteristic absorbance peak at 443 nm confirmed the AgNPs synthesis **Fig. 1A**. Green synthetic methods have the edge over chemical and physical methods in terms of their cost-effectiveness and ecofriendly nature [9, 10]. The crystalline nature of silver nanoparticles was further confirmed by XRD results **Fig. 2**. The antibacterial activity proved that silver nanoparticles showed significant antibacterial activity. The AgNPs concentrations A (0.01g/100µl) and B ((0.005g/100µl) showed maximum zone of inhibition against the *Escherichia coli* and *Pseudomonas putida*, respectively **Fig. 3**, **Table 1.** The antibacterial activity, also confirmed by findings reported previously by many researchers [35, 36]. Moreover, it has been investigated earlier that *A. tomentosa* root extract showed antibacterial activity up to a certain extent in comparison to aerial parts [37]. The DPPH method proved that silver nanoparticles have antioxidant scavenging activities. It has also been observed that the antioxidant activity was in a concentration-dependent manner[37] **Fig. 4, Table 2**. The antioxidant activity was due to the presence of photochemical in the form of flavonoids and phenolic content [50, 51, 52].

CONCLUSION: Current study reveals the successful synthesis of AgNPs *via* the biological green method using root extract of *Aerva tomentosa*. The synthesized AgNPs have strong antibacterial activity against the selected bacteria. Moreover, AgNPs also exhibit significant DPPH radical scavenging activity. These antibacterial and antioxidant activities with other known traditional medicinal values of root extract of *A. tomentosa* when blended in green synthesized AgNPs, might be used as a potent drug to treat various diseases.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding this paper's publication.

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