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RESEARCH ARTICLE

Green Synthesis of Silver Nanoparticles Using Waste Tea Leaves

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ABSTRACT

Green synthesis of silver nanoparticles has gained momentum since the demand to synthesize nanoparticles in an eco-friendly way has increased significantly. Here we report, economic and cost-effective biosynthesis of silver nanoparticles using waste of tea leaves (*Camellia sinensis*). The aim of the study was to biosynthesize silver nanoparticles and to assess its potential applications such as antibacterial activity, plant growth induction and dye degradation. Standardization studies were done using UV- Spectroscopy to determine the optimum synthesis condition for synthesis of silver nanoparticles. The optimum conditions were found to be pH 6.0, ambient temperature condition and 5mM $AgNO_3$ concentration. Characterization studies using UV-Visible Spectroscopy, TEM and AFM analysis show nanoscale range of the particles. The silver nanoparticles showed maximum antibacterial activity against *K. pneumonia* followed by *E. coli* and minimum activity against *C. diptheriae*. The nanoparticles showed significant effect on the growth of *Vigna radiata* seeds at 50% concentration of nanoparticles. The particles immobilized on cotton cloth showed antibacterial activity against Gram positive organisms. Dye degradation studies showed that the nanoparticles are able to degrade phenol red and blue textile dye effectively.

Keywords: tea leaves, biosynthesis, silver nanoparticles, antibacterial activity, PTC, antibacterial cloth, dye degradation

1 Introduction

The field of nanotechnology is one of the most active areas of research in modern materials science [1]. Nanoparticles exhibit novel properties depending upon their size, shape, and morphology, enabling them to interact with plants, animals and microbes effectively [2]. Their unique physical and optical properties such as surface plasmon resonance (SPR), high surface to volume ratio, and surface enhance raman scattering (SERS) have resulted in the recent development of metal nanoparticles. These unique features have led to the increased applications of metal nanoparticles in the field of cosmetics, medicine, agriculture, sensing & bio imaging, water purification & treatment and textile waste treatment [3]–[10]. Apart from all the nanoparticles developed so far, AgNPs hold a significant position owing to their inherent characteristic of acting as an antimicrobial agent [2]. The green synthesis of nanoparticles is an emerging branch of nanotechnology as it has many advantages over chemical and physical methods of nanoparticle synthesis. It is safe, simple, cost-effective, relatively reproducible, eco-friendly, easily scaled up for mass-scale synthesis, no need of high temperature, high pressure, energy, toxic chemicals, and often results in more stable materials. The integration of the principles of green chemistry with nanotechnology has become a key area in nanoscience and has received great attention in recent years. Biological methods are being used in the synthesis of metal and metal oxide nanoparticles since the particles obtained are of desirable size and morphology and the properties of the particles are enhanced in a greener way. Due to the rich biodiversity of plants and their potential secondary metabolites, plants and plant parts have been well exploited in recent times in the synthesis of a variety of nanoparticles. Plant extracts can act as both reducing and stabilizing agents for the formation of nanoparticles, hence the use of chemical reductants and stabilizers can be avoided. They help to produce metal nanoparticles that are much stable as compared to the other organisms and can reduce the metal ions faster than that of bacteria and fungi [11]. They also reduce the cost of isolation and culturing bacteria and fungi thereby increasing their cost-competitive feasibility of nanoparticle synthesis [12].

A variety of plant sources have been used to synthesize silver nanoparticles such as fruits, vegetables, spices, fruit and vegetable peels, and agricultural waste products [13]–[19]. A. Yashin, *et al.* in their study summarized the chemical composition of tea leaves which includes catechins, oxyaromatic acids, flavonols, theaflavins, teagallins, thearubigins, pigments, alkaloids, sugars, amino acids, vitamins, dibasic acids, cations, metals, lignans and triterpenoid saponins [20]. More attention has been paid to catechins, theaflavins, tannins and flavonoids since these compounds affect the flavor of tea and show potential health benefits arising from their antioxidant activity. Because of the chemical structure of polyphenols which allows electron delocalization, high reactivity to quench free radicals is exhibited [21].

Recently, green tea extract was used as a biogenic source for the synthesis of silver nanoparticles to check their cytotoxic and antibacterial effect. Polyphenolic compounds like catechins present in the *C. sinensis* extract which act as reducing and capping agents seemed to be responsible for the formation and stabilization of silver nanoparticles (AgNPs) [22]. One study reported that the AgNPs synthesized from red tea leaves were monodispersed and the size distribution of the synthesized AgNPs was in the range of 25 to 85nm with the average particle size of 53nm. Moreover, the number of particles with a diameter of around 45 nm was found to be greater rather than the amount of bigger particles [23]. Both waste green and black tea extracts were used to synthesize AuNPs and AgNPs. The nanoparticles formed were stable and were in the nanoscale range i.e. AuNPs of ~10nm and AgNPs of ~30nm [24].

In this study, silver nanoparticles have been successfully synthesized by using a waste source i.e. used tea leaves extract as a reducing and stabilizing agent. The formed AgNPs were characterized using UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), and Atomic Force Microscopy (AFM) analysis. These AgNPs were further used for various antibacterial and decolorization applications.

2 Materials and Methods

Silver nitrate ($AgNO_3$) was obtained from SRL Laboratories Pvt. Ltd., India and used without further purification. The purity was at least 99.5%. The solutions for the metal salts were prepared in deionized water. The media for antibacterial testing was obtained from HiMedia Laboratories Pvt. Ltd., India.

2.1 Synthesis of AgNPs

For this study, waste tea leaves (*Camellia sinensis*) were collected from daily kitchen waste. The tea leaves were washed properly with water, air dried and then stored in an air tight container for further use. The tea extract was prepared by two methods-

1. **Method I:** 5g of sample was boiled in a conical flask with 50ml of deionized water with an inverted funnel over it for 20min, cooled and filtered using muslin cloth for further use.
2. **Method II:** 5g of sample was soaked in 50ml of deionized water for over 3 days. The extract was then centrifuged and further filtration was carried out using muslin cloth.

5mM $AgNO_3$ solution was added to 50ml of waste extract under sonication conditions at room temperature. A prominent colour change of the extract of tea leaves waste from light brown to dark brown was observed indicating bioreduction process for the formation of AgNPs. The optimization of AgNPs synthesis was carried out using variation in pH (pH 3.0, pH 5.0, pH 7.0, pH 8.0, pH 9.0 and inherent pH at 6.0), temperature (10°C, 37°C, 55°C and Control at 28±2°C) and concentration of the $AgNO_3$ solution (2mM, 4mM, 5mM, 8mM, 10mM). The confirmation of AgNPs was carried out by measuring their absorbance by UV-Visible Spectrophotometer.

2.2 Characterization of the synthesized AgNPs

UV-Visible Spectroscopy analysis was carried out on a dual beam spectroscopy (JASCO Corporation, Tokyo, Japan). Equal amounts of the suspension (0.5 mL) were taken and analyzed at room temperature. The progress of the reaction between metal ions and the leaf extract was monitored by UV-Visible spectra of silver

nanoparticles in aqueous solution with different wavelength in nanometers from 340nm to 800nm. Control was maintained by using de-ionized water. The samples from the maximum time point of production of silver nanoparticles were sonicated at 30°C for 20min. A thin film of the colloidal solution was formed on a clean grease free quartz chip and kept for air drying. The film was characterized by Atomic Force Microscopy (Nanosurf, Naio AFM, NTRC, B. K. Birla College, Kalyan) for its detailed size, morphology and agglomeration of silver. Transmission electron microscopy of the AgNPs were carried out using JEOL Electron Microscope (Make JEE- 2100) operating voltages: 20-200kv. The analysis was carried out at SAIF–NEHU, Shillong.

2.3 Antibacterial activity of the AgNPs

The biosynthesized AgNPs were checked for their antibacterial activity using well diffusion method [25] against *Staphylococcus aureus*, *Corynebacterium diphtheriae, Proteus vulgaris*, *Bacillus sp.*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*. 24h old culture suspensions were inoculated into molten Mueller Hinton agar butt, and poured into sterile petriplates. Wells were made in each plate using the sterile cork borer of 6mm diameter. The solutions were added to the plate according to figure 1. The plates were then incubated at 37°C for 24h. After 24h, the zones of inhibition were measured and interpreted.

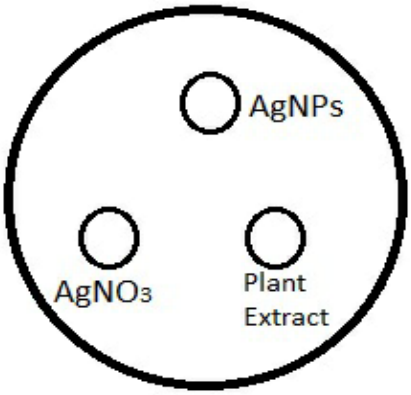

Figure 1: *Schematic representation of setup for testing antibacterial effect of AgNPs*

2.4 Effect of AgNPs on *Vigna radiata* (moong) seeds *in-vitro*

Effect of different concentrations of AgNPs (25%, 50% & 75%) in comparison to that of Ag^+ ions on the growth of *Vigna radiata* (moong) seeds was checked *in-vitro* using PTC technique. Surface sterilization of the seeds was carried out before inoculating them in Murashige & Skoog (MS) media fortified with 25%, 50% and 75% concentration of the synthesized AgNPs. A set with 5mM concentration of $AgNO_3$ solution was also made to assess the effect of Ag^+ ions. The PTC tubes inoculated with surface sterilized seeds were incubated under proper illumination and observed for growth. A control tube with the normal composition of MS media was also maintained. Fully grown plantlets were removed from the tubes. Residual media on the roots was washed. Fresh weight of the plantlets was noted, the shoot and root length were measured, and the plantlets were further used to quantify the carbohydrate and protein content in them by using Hedge and Hofreiter Method [26] and Folin-Lowry Method respectively [27].

2.5 Immobilization of nanoparticles on cloth

AgNPs were immobilized on a cotton cloth using immersion technique and its antibacterial activity was tested against Gram-positive and Gram-negative cultures of *Corynebacterium diphtheriae, Proteus vulgaris*, *Bacillus sp.* and *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, respectively. The cloth (10cm x 10cm) was washed thoroughly with deionized water and immersed in the aqueous extracts of tea leaves wastes and $AgNO_3$ solution was added to the extract. The extract was left overnight for visual colour development indicating the synthesis of AgNPs. After visual confirmation of synthesis of AgNPs, the cloth was removed from the now AgNPs solution and dried in a hot air oven till dry, autoclaved and then placed on Mueller and Hinton agar plates to

check its antibacterial activity. 24h old cultures were streaked across the cloth (Perpendicular manner). The plates were incubated at 37°C for 24h and observed for inhibition.

2.6 Photocatalytic degradation of biological and textile dyes

The photocatalytic activity of the freshly synthesized was checked for biological dye (phenol red dye) and blue textile dye. For this purpose, different concentrations of the dyes like 10ppm, 20ppm, 30ppm and 40ppm were prepared and their absorbance values were measured colorimetrically. The biosynthesized AgNP solution was added to these different concentrations of both the dye solutions. The tubes were then incubated for a period of 5 days under continuous illumination. A control was also maintained for all the concentrations of dyes without the addition of AgNPs. After the incubation period the dye solutions treated with the AgNPs solution were filtered and their absorbance was measured colorimetrically at 470nm for phenol red dye and 670nm for blue textile dye. The % decolorization was calculated using the following formula:

$$\%\, Decolourization = \frac{(Ao-A)}{Ao}\, x\, 100 \qquad (1)$$

where A_0 is the initial concentration of dye solution and A is the concentration of dye solution after photocatalytic degradation [28].

3 Results And Discussion

Silver nanoparticles were successfully biosynthesized using used tea leaves by two methods. Out of the two methods used, Method I was found to give better results. The aqueous silver ions were reduced to silver nanoparticles when added to tea waste extract without the use of any additional reductant or stabilizer. It was observed that the color of the solution turned from pale red to reddish brown and then to dark brown after 1h, 24h and 48h of the reaction, which indicated the formation of silver nanoparticles (shown in figure 2). The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by UV–Visible spectrophotometer analysis. The visible spectral peak of AgNPs was found between 390-440nm which is due to the small size of the nanoparticles formed.

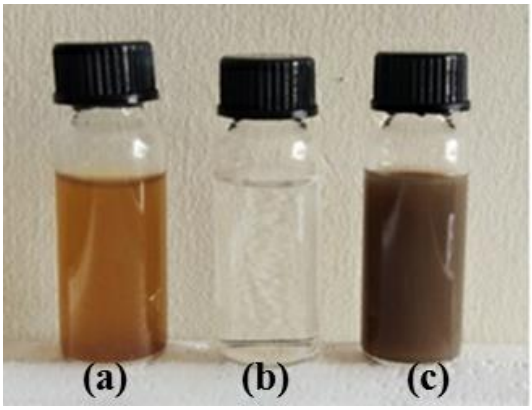

Figure 2: *Synthesis of Silver nanoparticles.*
(a) Tea leaves waste extract, (b) 5mM $AgNO_3$ solution, (c) AgNPs solution

3.1 Optimization of the AgNPs synthesis process

3.1.1 pH

The extract was adjusted to different pH values from 3.0 – 9.0 and inherent pH of the extract (i.e. 6.0). After 12 hours the extract showed visible colour change. From the results (shown in figure 3), it can be noted that the optimum pH of tea extract was found to be the inherent pH i.e. 6.0. It shows acceptable level of synthesis under natural pH conditions of the extract. Thus indicating that this biogenic process of synthesizing AgNPs is cost effective, easy and does not require any special temperature conditions.

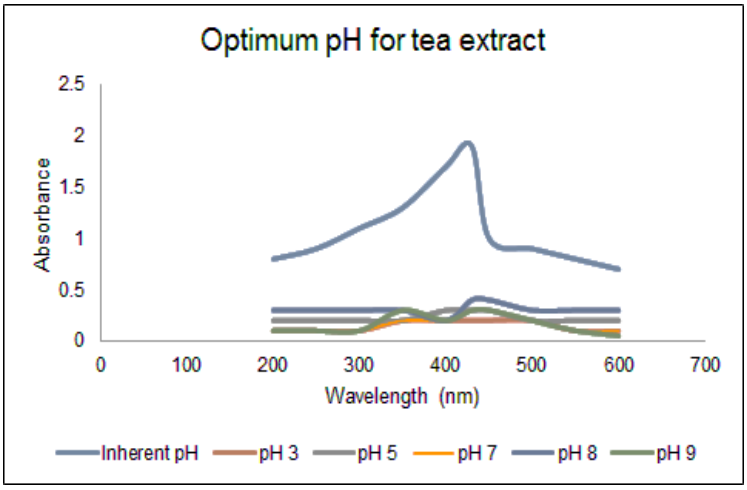

Figure 3: *Optimum pH for tea extract*

3.1.2 Temperature

The extract was adjusted to its optimum pH value and was incubated at different temperatures like 10°C, 37°C and 55°C. After 12 hours the extract showed visible colour change. The optimum temperature of tea extract was found to be control i.e. ambient room temperature (28±2° C) as shown in figure 4. The initial extraction process which involved boiling at high temperature might have played a crucial role in releasing and activating certain enzymes present in the plant material. Similar results were reported where smaller AgNPs were obtained at higher temperatures. It was suggested that the enzymes activated at higher temperature might responsible for reducing and capping of these nanoparticles [29]. Later on during the synthesis process this extract was capable of synthesizing AgNPs at normal room temperature conditions which indicates the feasibility and effectiveness of this biological process. A remarkable property of biologically synthesized metal nanoparticles is to prevent agglomeration of the particles and stabilizing them in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the AgNPs in aqueous medium. It is well known that proteins can bind to AgNPs either through free amine groups in the proteins and therefore, stabilization of the SNP by surface-bound proteins is a possibility [29].

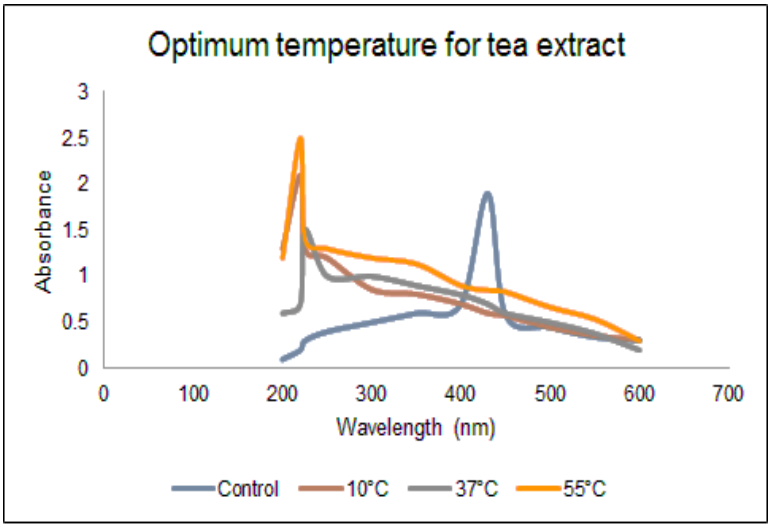

Figure 4: *Optimum temperature for tea extract*

3.1.3 $AgNO_3$ solution concentration

Optimum $AgNO_3$ concentration was determined using 2, 4, 5, 8, and 10mM concentrations of $AgNO_3$ solution. After 12 hours the extract showed visible colour change. The optimum concentration for tea extract was found to be 5mM (shown in figure 5). The biosynthesized AgNPs have different crystallite shapes which are dependent on the concentration of the Ag+ ion in solution, the enzymes released by the plant extract, and pH of the solution. It should be noted that the crystallite shape is not the only important factor influencing the properties of metal nanoparticles, but the particle size is also a crucial factor for optoelectronics and other applications of the nanomaterial [29]. Hence, silver ion concentration was varied in order to get desired sized AgNPs. As observed in figure 5, 5mM concentration of $AgNO_3$ was found to be the most influential concentration which gave a SPR band at 430nm for AgNPs synthesized from tea extract. Other concentrations viz. 2mM, 4mM and 8mM gave SPR bands at 370nm and below indicating absence of AgNPs and 10mM gave SRP band above 450nm indicating formation of larger sized of AgNPs.

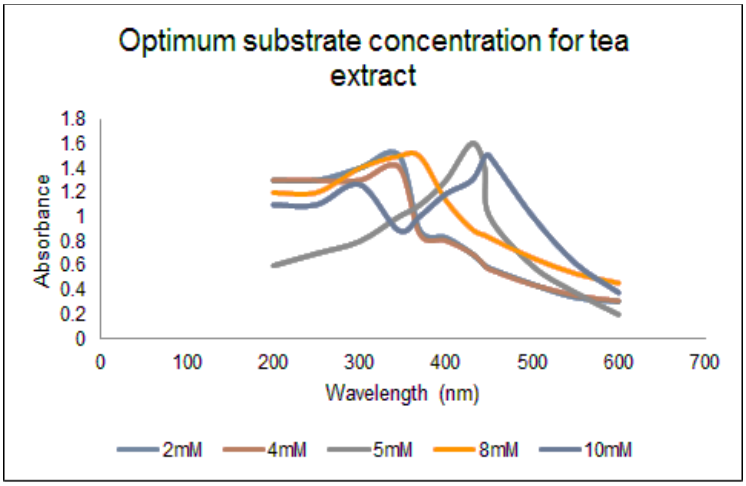

Figure 5: *Optimum substrate ($AgNO_3$) concentration for tea extract*

3.2 Characterization of AgNPs

The detailed characterization was carried out using UV-Visible Spectroscopy and TEM analysis. The visible spectral peak of AgNPs was found between 390-440nm which is due to the small size of the nanoparticles formed. The capping agents in this case might be the bio-organic compounds from the tea leaves extract. The particle size of the silver nanoparticles was found to be 40 to 60nm. Transmission electron microscopy (TEM) was employed to characterize the size, shape and morphology of the AgNPs prepared by boiling method. From the TEM images (shown in figure 6) it is evident that the particles are in the nano scale range of 40-50nm. The images also show a mixed population of AgNPs like circular, hexagonal, triangular nanoparticles. The SAED pattern for the AgNPs shows concentric circles with intermittent dots indicating the crystalline form of the nanoparticles. The silver nanoparticles were characterized by Atomic Force Microscopy (AFM) for its detailed size and morphology of silver. The topographical images of irregular silver nanoparticles synthesized by tea waste extract are shown in figure 7. It shows size and shape of the nanoparticles which are obtained directly from tip-corrected AFM measurements which comes to be around 40nm to 60nm, and the shape of the nanoparticles is estimated on the basis of AFM images and line scans which appear to be spherical.

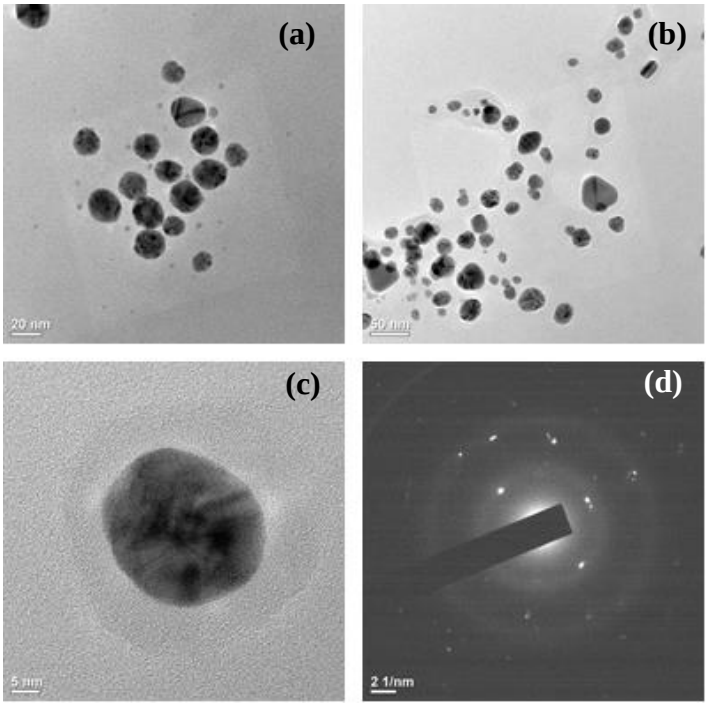

Figure 6: *TEM image of AgNPs prepared from used tea leaves extract. (a) Spherical nanoparticles; (b) mixed populations (hexagonal, octagonal, rods); (c) single particle of 40nm diameter; (d) SAED Pattern*

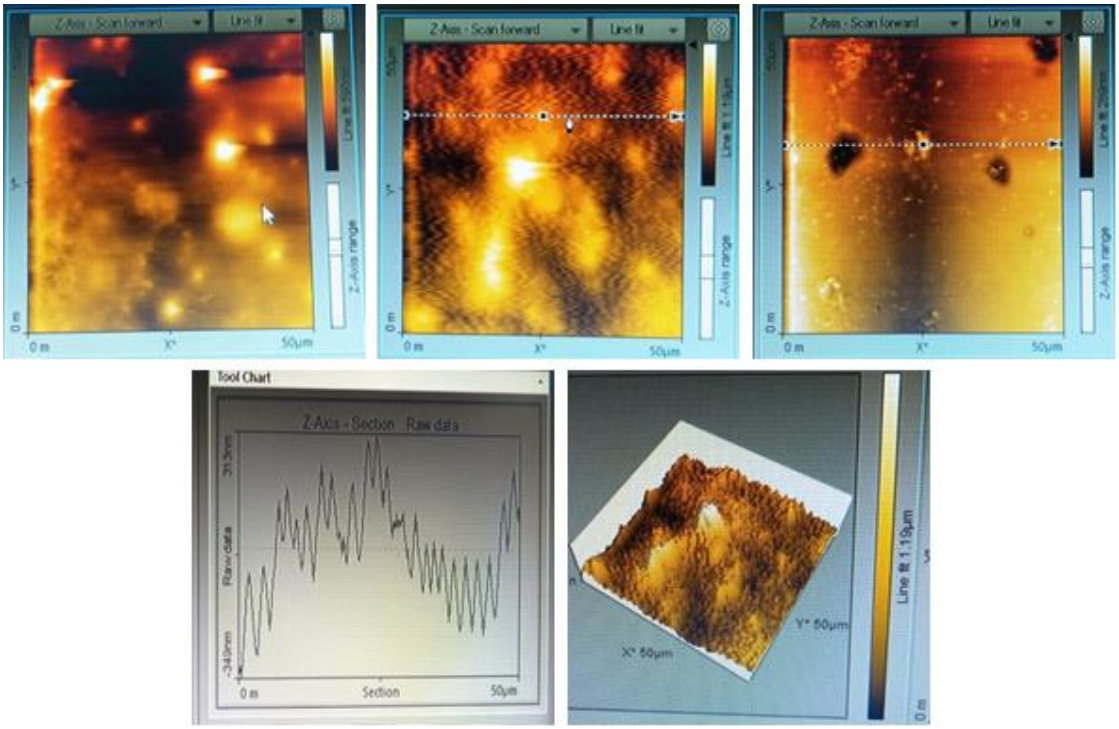

Figure 7: *AFM images of AgNPs*

3.3 Study of antibacterial activity of the AgNPs

The AgNPs were checked for their antibacterial effect using well diffusion assay and were found to be most effective against *K. pnuemoniae* and *E. coli* with zone of inhibition of 23mm and 20mm, respectively and least effective against *C. diptheriae* with zone of inhibition of 13mm (shown in figure 8). These results are in agreement with the fact that Gram-negative cultures are more susceptible to the antibacterial effect of Ag^{+} than Gram-positive cultures [30]. The zone of inhibition shown by the test organisms against AgNPs is documented in

Table 1. Since the tea extract has no anti-microbial activity which is evident with no zone of inhibition, the bactericidal activity might be due to synergistic activity of extract stabilized AgNPs and unreduced Ag^+ ions [31]. The exact cause of the antibacterial action of AgNPs against the pathogenic bacteria is still under investigation. Few studies suggest that the electrostatic attraction between negatively charged bacterial cells and the positively charged nanoparticles could be responsible for its bactericidal effects [32]. The possible antibacterial activity of Ag NPs might be due to interactions between silver ions with bacterial wall sulfhydryl groups that interfere with and disrupt bacterial cell membrane, enzyme, respiratory, and cell proliferation [4]. It has also been reported that the crystallographic structure and high surface-to-volume ratio increases the contact area of metallic nanoparticles with the microorganism which influences the antibacterial activity of nanosized silver particle [32]. These properties of AgNPs make it a potential candidate for development of antimicrobial products, polymer materials for packaging of food items to help preserve the freshness of food products and other durable materials. [33]

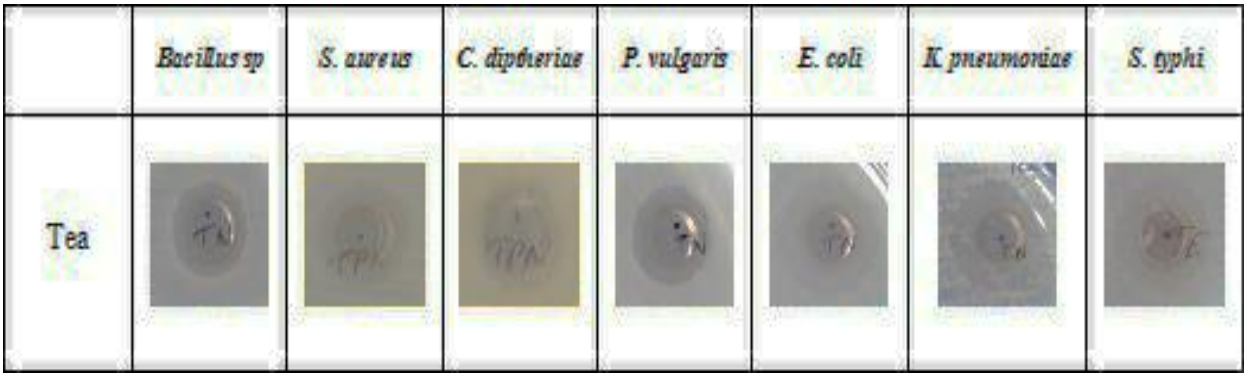

Figure 8: *Antibacterial activity of AgNPs against Gram-positive and Gram-negative organism*

Table 1: *Zone of inhibition shown by various pathogenic bacteria against AgNPs*

Test organisms	*Bacillus sp.*	*S. aureus*	*C. diptheriae*	*P. vulgaris*	*E. coli*	*K. pneumoniae*	*S. typhi*
Diameter of zone of inhibition (mm)	15	17	13	17	20	22	15

3.4 AgNPs Effects on *Vigna radiata* (moong) seeds *in-vitro*

3.4.1 Growth on MS media

Vigna radiata (moong) seeds were grown on MS media fortified with 5mM $AgNO_3$ solution and AgNPs prepared from tea extract. One tube was set as control. The medium with $AgNO_3$ solution showed fully grown plantlet within 8 days. The control medium and medium with AgNPs developed plantlets within 21 days as shown in figure 9. After recording the fresh weigh, the colour of the leaves, number of leaves, and length of the induced plantlets, they were used to estimate the carbohydrate and protein content present in them using Hedge and Hofreiter Method and Folin-Lowry Method respectively. Table 2 shows effect of different concentrations AgNPs on physical morphology of *Vigna radiata* plantlets.

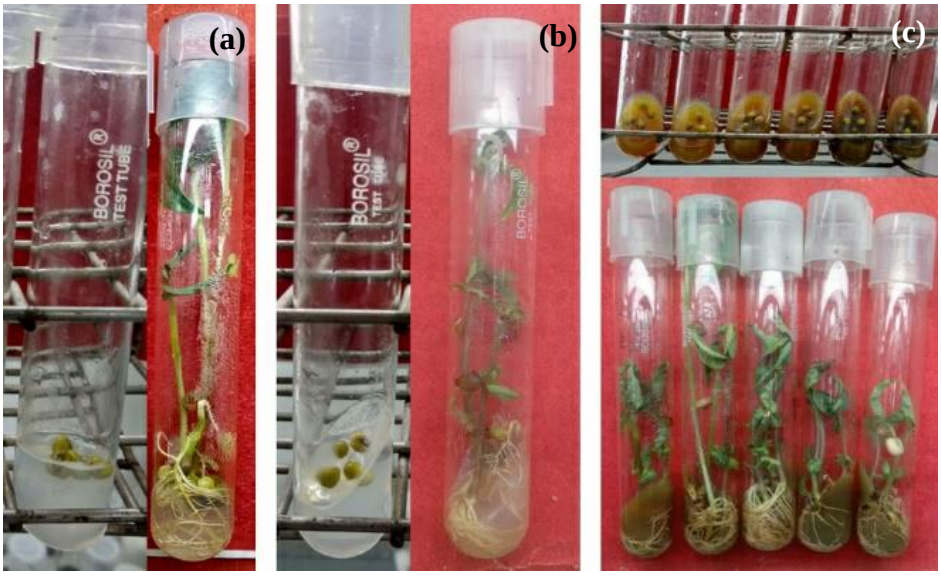

Figure 9: *Effect of AgNPs on Vigna radiata (moong) seeds in-vitro.*
(a) Growth of moong seeds on MS media fortified with 5mM $AgNO_3$ solution within 8 days; (b) Growth of moong seeds on normal MS media after 21 days; (c) Growth of moong seeds on MS media fortified with AgNPs prepared from tea extract in varying concentrations (25%, 50% and 75%)

3.4.2 Physical growth characteristics of the plantlets

Addition of $AgNO_3$ showed significant faster growth compared to all the other tubes, within 8 days. No contamination was observed in any of the tubes. The maximum plantlet length and fresh weight was obtained from the medium containing 50% of AgNPs concentration. The lowest length was obtained from the control tube and the lowest fresh weight was obtained from the medium containing $AgNO_3$ (Table 2). AgNPs synthesized from tea extract added to MS medium resulted in better plant length and highest fresh weight of *V. radiata* seeds at 50% concentration whereas the plant growth at 75% concentration was found to be significantly low. This observation is in agreement with studies made by J. Spinoso-Castillo *et al.*, which showed that lower concentrations of AgNPs (25 and 50 mg/L) resulted in better shoot number, shoot length, and fresh and dry weight of *V. plantifolia* grown in-vitro [34]. Similar results were reported by Seif *et al.* wherein they reported better height of borage plant, dry weight, inflorescence dry weight, seed yield at 60ppm AgNPs concentration [35]

Table 2: *Plant morphology grown at different concentrations of AgNPs*

Tube	Colour of the leaves	Number of leaves	Fresh Weight (g)	Length of the plantlet (cm)
Control	Green	2	1.79	11
25%	Green	2	1.83	11.75
50%	Green	3	2.67	16.54
75%	Green	2	1.55	10.13
$AgNO_3$	Green	3	1.75	13.5

3.4.3 Carbohydrate content

Carbohydrate content estimated using Hedge and Hofreiter Method showed significant difference among the samples. From the graph (shown in figure 10), it can be seen that the plantlets grown in medium containing 25% of the AgNPs concentration showed the highest carbohydrate content (930mg/ml) whereas the medium containing $AgNO_3$ showed the lowest carbohydrate content (180mg/ml).

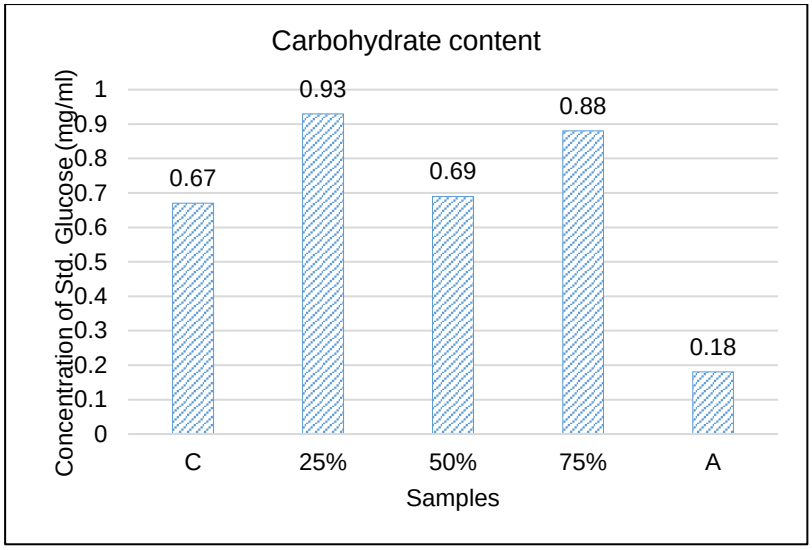

Figure 10: *Carbohydrate content estimated using Hedge and Hofreiter Method*

3.4.4 Protein content

The protein content estimated using Folin-Lowry's method showed significant difference. From the graph (shown in figure 11), the maximum protein content was observed in plantlets grown medium containing 50% concentration of AgNPs (0.096mg/ml) followed by control tube (0.084mg/ml). The plantlets grown on medium with 5mM $AgNO_3$ solution showed the lowest protein content (0.01mg/ml). These results are similar to the findings of J. Spinoso-Castillo *et al.* [34]. They reported highest total phenolic content in vanilla shoots treated with 50mg/L concentration of AgNPs and reported this to be inversely proportional to ROS generation levels. ROS generation was observed in increasing order from 20 to 100mg/L concentration of AgNPs. They attributed their results to the fact that silver at high concentrations inhibit the production of phenolic compounds and antioxidant capacity, thereby favoring increased ROS production. Thus the further analysis of this work include evaluating the effect of AgNPs synthesized from tea leaves extract on the TPC levels and ROS generation in *V. radiata* seeds and the possible correlation between them.

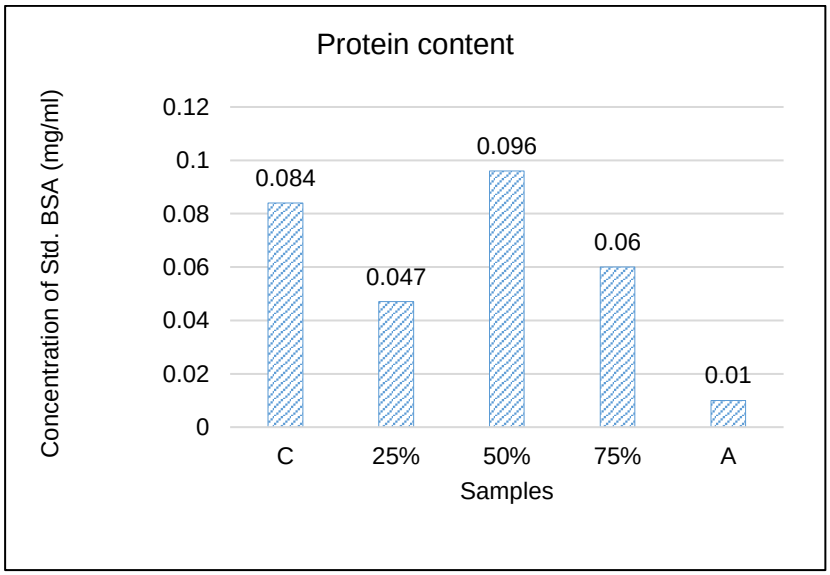

Figure 11: *Protein content estimated using Folin-Lowry's Method*

3.5 Immobilization of nanoparticles on cloth

Clean cotton cloth was impregnated of AgNPs prepared from tea leaves extract without the use of any binders or stabilizers (shown in figure 12). Their antibacterial activity was checked against 3 Gram-positive and 3 Gram-negative organisms (shown in figure 13). The cloth showed antibacterial activity against Gram-positive organisms (*C. diptheriae, P. vulgaris,* and *Bacillus sp.*). Similar results were reported by Xu *et al.* in their studies where they used carboxymethyl chitosan (CMCTS) binder to link AgNPs to the cotton cloth and found better result of their antibacterial cotton cloth against Gram-positive *S. aureus* as compared to Gram-negative *E. coli.* [36]. As compared to this, we have immobilized AgNPs on the cotton cloth without the use of any linkers or binders showing significant antibacterial activity. Successful immobilization and antibacterial activity of the AgNPs against both Gram-positive and Gram-negative bacterial cultures makes the AgNPs suitable for developing antibacterial layering on fabrics. This work can be further improvised by the inclusion of various physico-mechanical parameters e.g., tensile strength, rheological stress, flame retardant ability, acid and alkali resistance etc. Microscopic investigations like TEM, SEM can be done on the cotton cloth to check the extent surface distribution of the AgNPs. Various eco-friendly binding agents can be assessed to increase the pressure handling ability of the fiber so that it possesses its activity even after numerous of washing cycles. The antibacterial activity of the nanoparticle impregnated clothes even after repeated washing opened up a new horizon for devising a wound healing strategy especially for the defense personnel and others who work in various remote areas or harsh environments. [37]. The wound healing properties of silver are reported in many *in vivo* studies where AgNPs promoted the healing process directly by reducing the cytokine- modulated inflammation [38]. The chance of infection can be decreased by covering the wounded area with these nanocoated bandages as reported by B. Pannerselvam *et al.* in their study wherein they found increased wound recovery with increasing concentration of AgNPs [39].

Figure 12: *Optical images of (a) untreated cotton cloth and (b) cloth treated with AgNPs made from tea extract*

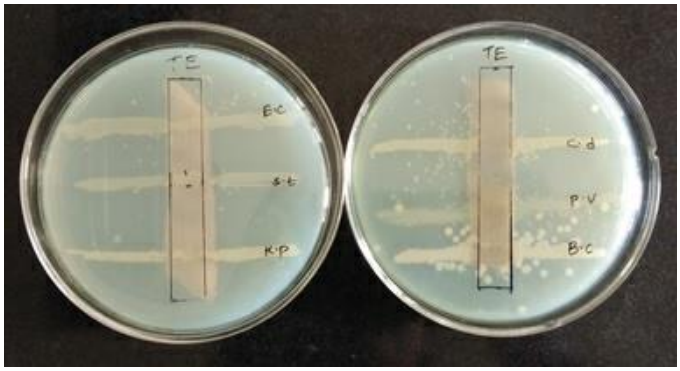

Figure 13: *Antibacterial activity of the AgNPs impregnated cloth.*

3.6 Photocatalytic degradation of biological and textile dyes

Biosynthesized AgNPs were tested for their potential to degrade dyes. A biological stain - phenol red and a blue textile dye were used in different concentrations i.e. 10ppm, 20ppm, 30ppm, & 40ppm. As shown in figure 14 the AgNPs showed high dye degradation efficiency of 97% and 91% against both dyes. Dyes are recalcitrant

molecules which are difficult to degrade biologically. In this study, the AgNPs synthesized from waste tea leaves were able to degrade dye without the use of any supplementary reducing agents such as sodium borohydride ($NaBH_4$). The enzymes present in the tea extract itself might play a role in degrading these dyes at ambient temperature conditions, hence making them relevant in eco-friendly environmental applications. Thus this method of reduces the cost of the degradation process and results in overall low environmental impact [28]. The possible underlying mechanism of dye degradation was reported by a photodegradation study of methyl orange and Congo red dyes by Ag/TiO_2 nanocomposite. It was concluded that the dyes may react with hydroxyl radicals and superoxide ions and undergo degradation [40]. It is also documented that the hydroxyl radical obtained from the oxidation of oxygen acts a oxidant and degrades methylene blue dye [41]. E. Leon *et al.* proposed in their study that zeolites absorb oxygen in their pores and excited electrons on the surface of Au nanoparticles which leads to the formation of ROS causing methylene blue degradation. Further work involves the establishment of the potential mechanism of the degradation of dye by carrying out studies of hydroxyl ions and ROS formation.

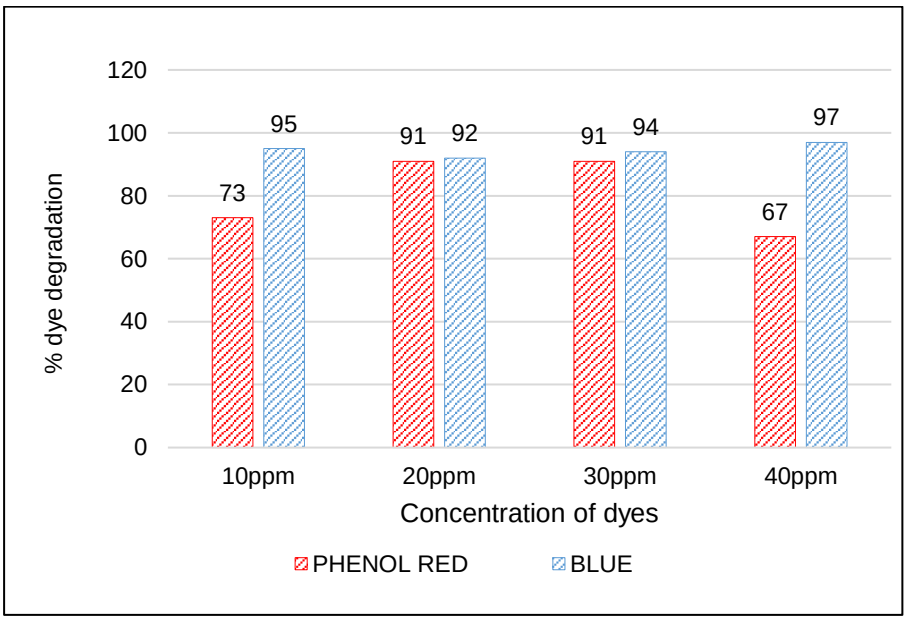

Figure 14: *Percentage degradation of dyes after treatment with AgNPs for 5 days*

4 Conclusion

In this study, silver nanoparticles were synthesized using a waste source i.e. used tea leaves. The method is considered to be green because the synthesis is carried out at ambient temperature, using a leftover waste material and without the addition of any chemical reductant, therefore it does not generate any environmental pollution. Characterization results obtained from UV-Spectroscopic, TEM and AFM analysis prove that the particles synthesized are in nanoscale range and crystalline in nature. The small size and stability of the particles can be attributed to heat applied during preparation of the extract and the concentration of $AgNO_3$. The antibacterial activity of the AgNPs is dependent on the size and capping agents used. Since the particles are in nanoscale range as proven by characterization studies, their effectiveness as an antibacterial agent is further established by the antibacterial assay performed. The particles have also shown enhanced the growth of *Vigna radiata* plantlets grown *in-vitro*, thus indicating their usefulness to control microbial contamination and to induce shooting and rooting in the plantlets. The cloth impregnated with AgNPs shows inhibition of various bacterial strains, hence the biosynthesized AgNPs are also suitable for developing antibacterial bandages and dressings. The nanoparticles showed good percentage degradation of both biological and textile dyes, thus they can be used in various bioremediation processes to clear the waste water from the contamination of dyes.

5 Declarations

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5.2 Competing Interests

All the authors declare no conflict of interest in this research work.

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