Karnatak University Journal of Science 54(3), (2023), 46–57

Karnatak University Journal of Science

ISSN: 0075-5168

Original Article

Study and Characterization of Biosynthesized Silver Nanoparticles and their Biological Applications

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ARTICLE INFO

Article history:
Received 08.06.2023
Accepted 07.07.2023
Published 15.11.2023

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https://doi.org/10.61649/kujos/v54i3.santosh

ABSTRACT

Nanotech field is a rapidly growing, which has led to promising revolutionary applications in medical and engineering in terms of their efficacy, safety and economy. The synthesis and obtained nanomaterial where, the advancement of green synthesis over chemical and physical methods is environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles. In the present work we were focused on the synthesis of Silver nanoparticle, it was obtained by green method by using *Camellia sinensis* tea powder extract. Ag NP's were characterized by XRD, FTIR, UV-spectral and SEM measurements analysis. Antibacterial activity of Ag NP's are studied by Diffuse disc method. The activity of AgNPs is dependent on the size and capping agents used. Since the particles are in range as proven by characterization studies.

Keywords: Nanomaterial; Antibactirial activity; Camelle Sinensis; Diffuse disc method

1 INTRODUCTION

Nanotechnology is a rapidly growing field which has led to promising revolutionary applications in medical and engineering in terms of their efficacy, safety and economy. Nanobiotechnology is an offspring of nanotechnology that has emerged at the interface of nanotechnology and biology. Nanotechnology deals with the production and stabilization of various type of nanoparticles. In order to obtain nanoparticles in large quantities within a short period, physical and chemical procedures are used. At present, there exists a need to develop eco-friendly processes for the synthesis of nanoparticles.

Nanoparticles, microscopic objects with at least one dimension less than 100nm [1] have attracted intensive scientific attention. Distinctive size-dependent properties of nanoparticles often exist, which are mainly due to their relatively large surface area [2] Moreover, when the size of a particle approaches nanoscale with the characteristic length scale close to or smaller than the de Broglie wavelength of the charge carrier (electrons and holes) or the wavelength of light, the periodic boundary conditions of the crystalline particle are destroyed, or the atomic density on the amorphous particle surface is changed [3]. Due to these, a lot of the physical properties of nanoparticles are quite different from bulk materials, yielding a wide variety of new applications.

Particle structure ranging from approximately 1 to 100nm in size, within this range all the properties (like, chemical, physical and biological) changes in fundamental ways of both individual atoms/molecules and their corresponding bulk. Novel applications of nanoparticles and nanomaterials are growing rapidly on various fronts due to their completely new or enhanced properties based on size, their distribution and morphology. It is swiftly gaining renovation in a large number of fields such as health care, cosmetics, biomedical, food and feed, drug-gene delivery, environment, health, mechanics, optics, chemical industries, electronics, space industries, energy science, catalysis, light emitters, single electron transistors, nonlinear optical devices and photo-electrochemical applications.

Unique features of nanoparticles result from the three major physical properties that are interrelated. First, nanoparticles have very high specific surface area (area per unit volume), rendering the electronic activity and their interaction with outside influences significant [4]. Second, they have high mobility not only in free state, but also in porous media. Finally, nanoparticles exhibit quantum effect due to their comparable dimension with the wavelength of the electron wave function [5]. e.g., Currently, modified or fabricated of NPs is widely utilized in industrially manufactured cosmetics, electronics, and textiles. Furthermore, the rapid increase in the number of microbes resistant to existing antibiotic drugs that has led to the requirement of novel medicines in the form of bare NPs or in conjunction with existing antibiotics to exert a favourable synergistic effect resulted in the wide spread use of NPs in several medical fields [6]. Nowadays, NPs have been utilized for molecular imaging to achieve profoundly resolved pictures for diagnosis. In addition, contrast agents are impregnated onto NPs for the tumour and atherosclerosis diagnosis. Furthermore, nanotherapeutic has been promoted everywhere throughout the world after the first FDA affirmed nanotherapeutics in 1990, to build up different nano-based drugs [7]. In the present work we focus on the silver nanomaterials.

They have numerous applications in biomedical devices, medicines, highly conductive composites, cosmetics, textile industries, and so on because of their interesting physical properties. They exhibit effective SPR, strong absorption peaking near 400nm, and tunable scattering property at larger wavelength, all of which are suitable for bio-imaging, molecular labeling, and enhanced optical spectroscopy. For a long time, nano scaled silver is considered a very popular biomaterial with anti-microbial activity described briefly [8]. Their antibacterial behavior is popularly used for minimizing the bio-fouling. They have recently performed well against HIV virus and for apoptosis of cancer cells. Moreover, their anti-inflammatory activity is suitable to heal wounds. Toxicity of silver nanoparticles is majorly dependent on their morphological status and surface charge. Negatively charged silver nanoparticles are reported to be less toxic compared to positively charged particles.

Synthesis of silver nanoparticles is of much interest to the scientific community because of their wide range of applications. These silver nanoparticles are being successfully used in the cancer diagnosis and treatment as well [9, 10]. Generally, nanoparticles are prepared by a variety of chemical and physical methods which are quite expensive and potentially hazardous to the environment which involve use of toxic and perilous chemicals that are responsible for various biological risks. The development of biologically-inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. Generally, there are two approaches which are involved in the synthesis of silver nanoparticles, either from "top to bottom" approach or a "bottom to up" approach (Figure 1). In bottom to up approach, nanoparticles can be synthesized using chemical and biological methods by self-assemble of atoms to new nuclei which grow into a particle of nanoscale as shown in Figure 2 while in top to bottom approach, suitable bulk material breaks down into fine particles by size reduction with various lithographic techniques e.g., grinding, milling, sputtering and thermal/laser ablation.

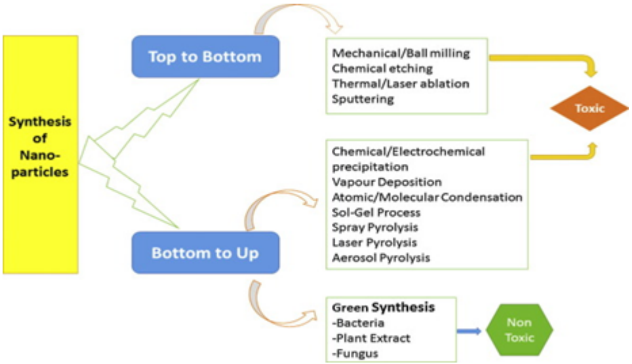

Figure 1: Different approaches of synthesis of nanoparticles

In bottom to top approach, chemical reduction is the most common scheme for synthesis of silver nanoparticles [11, 12]. Different organic and inorganic reducing agents, such as sodium borohydride ($NaBH_4$), sodium citrate, ascorbate, elemental hydrogen, Tollens reagent, N,N-dimethyl formamide (DMF) and poly (ethylene glycol) block co polymers are used for reduction of silver ions(Ag^+) in aqueous or non-aqueous solutions [13, 14]. Capping agents are also used for size stabilization of the nanoparticles. One of the biggest advantages of this method is that a large quantity of nanoparticles can be synthesized in a short span of time. During this type of synthesis; chemicals used are toxic and led to non-eco-friendly by-products. This may be the reason which leads to the biosynthesis of nanoparticles via green route that does not employ toxic chemicals and hence proving to become a growing wanton want to develop environment friendly processes. Thus, the advancement of green synthesis of nanoparticles is progressing as a key branch of nanotechnology; where the use of biological entities like microorganisms, plant extract or plant biomass for the production of nanoparticles could be an alternative to chemical and physical methods in an eco-friendly manner [15].

In case of top to bottom approach; nanoparticles are generally synthesized by evaporation–condensation using a tube furnace at atmospheric pressure. In this method the foundation material; within a boat; place centred at the furnace is vaporized into a carrier gas. Ag, Au, Pbs and

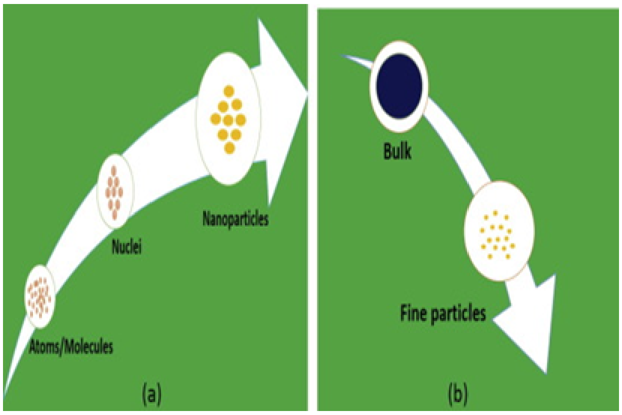

Figure 2: The different route employed for synthesis of nanoparticles (a) bottom to top approach and (b) top to bottom approach

fullerene nanoparticles have previously been produced using the evaporation/condensation technique. The generation of silver nanoparticles using a tube furnace has numerous drawbacks as it occupies a large space and munches a great deal of energy while raising the environmental temperature around the source material, and it also entails a lot of time to succeed thermal stability [7, 16–19]. In addition; a typical tube furnace requires power using up of more than several kilowatts and a pre-heating time of several tens of minutes to attain a stable operating temperature. One of the biggest limitations in this method is the imperfections in the surface structure of the product and the other physical properties of nanoparticles are highly dependent on the surface structure in reference to surface chemistry.

In general, whatever the method is followed, it is generally concluded that the chemical methods have certain limitations with them either in the form of chemical contamination during their synthesis procedures or in later applications. Yet; one cannot deny their ever-growing applications in daily life. For instances; "The Noble Silver Nanoparticles" are striving towards the edge-level utilities in every aspect of science and technology including the medical fields; thus, cannot be neglected just because of their source of generation. Due to their medicinal and antimicrobial properties, silver nanoparticles have been incorporated into more than 200 consumer products, including clothing, medicines and cosmetics. Their expanding applications are putting together chemists, physicist, material scientist, biologists and the doctors/pharmacologists to continue their latest establishments. Hence, it is becoming a responsibility of every researcher to emphasize on an alternate as the synthetic route which is not only cost effective but should be environment friendly in parallel. Keeping in view of the aesthetic sense, the green synthesis is rendering itself as a key procedure and proving its potential at the top.

The advancement of green synthesis over chemical and physical methods is environment friendly, cost effective and easily scaled up for large scale synthesis of nanoparticles, furthermore there is no need to use high temperature, pressure, energy and toxic chemicals [20]. A lot of literature has been reported to till date on biological synthesis of silver nanoparticles using microorganisms including bacteria, fungi and plants; because of their antioxidant or reducing properties typically responsible for the reduction of metal compounds in their respective nanoparticles. Although; among the various biological methods of silver nanoparticle synthesis, microbe mediated synthesis is not of industrial feasibility due to the requirements of highly aseptic conditions and their maintenance. Therefore; the use of plant extracts for this purpose is potentially advantageous over microorganisms due to the ease of improvement, the less biohazard and elaborate process of maintaining cell cultures [21]. It is the best platform for synthesis of nanoparticles; being free from toxic chemicals as well as providing natural capping agents for the stabilization of silver nanoparticles. Moreover, use of plant extracts also reduces the cost of micro-organisms isolation and their culture media which enhance the cost competitive feasibility over nanoparticles synthesis by microorganisms. Hence, a review is compiled describing the bio-inspired synthesis of silver nanoparticles that provide advancement over physical and chemical methods which are eco-friendly, cost effective and more effective in a variety of applications especially in bactericidal activities.

The use of plants as the production assembly of silver nanoparticles has drawn attention, because of its rapid, eco-friendly, non-pathogenic, economical protocol and providing a single step technique for the biosynthetic processes. The reduction and stabilization of silver ions by combination of biomolecules such as proteins, amino acids, enzymes, polysaccharides, alkaloids, tannins, phenolics, saponins, terpenoids and vitamins which are already established in the plant extracts having medicinal values and are environmental benign, yet chemically complex structures [22]. A large number of plants are reported to facilitate silver nanoparticles synthesis are mentioned (Table 1) and are discussed briefly in the presented review. The protocol for then a no particle synthesis Involves: the collection of the part of plant of interest from the available sites was done and then it was washed thoroughly twice/thrice with tap water to remove both epiphytes and necrotic plants; followed with sterile distilled water to remove associated debris if any. These; clean and fresh sources are shade-dried for 10–15days and then powdered using domestic blender. For the plant broth preparation, around 10 of the dried powder is boiled with 100mL of deionized distilled water (hot percolation method). The resulting infusion is then filtered thoroughly until no insoluble material appeared in the broth. To 10^{-3} M $AgNO_3$ solution, on addition of few mL of plant extract follow the reduction of pure Ag(I) ions to Ag(0) which can be monitored by measuring the UV–visible spectra of the solution at regular intervals [23].

Figure 3: Green synthesis of Silver Nanoparticle using plant extracts

Due to their anti-bacterial properties, silver nanoparticles have been used most widely in the health industry, food storage, textile coatings and a number of environmental applications. In spite of decades of its use, it is important to note that the evidences of toxicity of silver are still not clear. Products prepared with silver nanoparticles have been approved by a range of accredited bodies including the US FDA, US EPA, Korea's Testing, SIAA of Japan and Research Institute for Chemical Industry and FITI Testing and Research Institute [62]. The antimicrobial properties of silver nanoparticles have also been exploited both in the medicine and at home. Silver sulfadiazine creams sometimes use to prevent infection at the burn site and at least one appliance company has incorporated silver into their washing machines. Currently silver is used in the expanding field of nanotechnology and appears in many consumer products that include baby pacifiers, acne creams, and computer's keyboard, clothing (e.g., socks and athletic wear) that protects from emitting body odour in addition to deodorizing sprays.

It is a well-known fact that silver nanoparticles and their composites show greater catalytic activities in the area of dye reduction and their removal. Kundu et al. studied the reduction of methylene blue byarsinein the presence of silver nanoparticle [63]. Mallick et al. studied the catalytic activity of these nanoparticles on the reduction of pheon safranine dye [64]. In this study, the application of silver nanoparticles as an antimicrobial agent was also investigated by growing *E. coli* on agar plates and in liquid LB medium, both supplemented with silver nanoparticles [65]. Single silver nanoparticles were applied to investigate membrane transport in living microbial cells (*P. aeruginosa*) in real times [66]. The triangular silver nanoparticles fabricated by nanosphere lithography indeed function as sensitive and selective nanoscale affinity biosensors. These nanosensors retain all of the other desirable features of Surface Plasmon Resonance (SPR) spectroscopy which is the fundamental principle behind many colour based biosensor applications and by changing nanoparticles size and shape, these nano sensors possess at least two unique characteristics: (I) modest refractive sensitivity and (II) a short-range, sensing length scale determined by the characteristic decay length of the local electromagnetic field. These two factors combine to yield an area of mass sensitivity of $\sim$100–1000Pg/mm^2, which is only a factor of 100 poorer than the best propagating SPR sensitivities [67].

Silver nanoparticles synthesized through green method have been reported to have biomedical applications as well as in controlling the pathogenic microbes. In a study, silver nanoparticles were synthesized using aqueous *piper longum* fruit extract. The aqueous *P. longum* fruit extract and the green synthesized silver nanoparticles showed powerful antioxidant properties *in vitro* antioxidant assays [68]. The toxicity of starch-coated silver nanoparticles was studied using normal human lung fibroblast cells (IMR-90) and human glioblastoma cells (U251). The toxicity was evaluated using changes in cell morphology, cell viability, metabolic activity, and oxidative stress. These nanoparticles produced ATP content of the cell causing damage to mitochondria and increased production of reactive oxygen species (ROS) in a dose-dependent manner. DNA damage, as measured by single cell gel electrophoresis (SCGE) and cytokinesis blocked micronucleus assay (CBMN), was also dose-dependent and more prominent in the cancer cells [69]. The high frequency electrical behaviour of nano-silver based conductors is up to 220GHz. [70]. Silver nanoparticles have proven to exert antiviral activity against HIV-1 at non-cyto-toxic concentrations, but the mechanism underlying their HIV-inhibitory activity has been not fully elucidated. These silver nanoparticles were evaluated to elucidate their mode of antiviral action against HIV-1 using a panel of different in vitro assays [76]. Special interest has been directed at providing enhanced bio-molecular diagnostics, including SNP detection gene expression profiles and biomarker characterization. These strategies have been focused on the development of nanoscale devices and platforms that can be used for single molecule characterization of nucleic acid, DNA or RNA, and protein at an increased rate when compared to traditional techniques [71].

In this work we focus on the preparation of the silver nanoparticles from plant (Tea- *Camellia sinensis*) extract, characterized by XRD, FTIR, UV-spectral measurements and SEM.

2 MATERIALS AND METHODS

- **Tea (Camellia sinensis)**

Tea polyphenols are the major active compounds present in tea. The Catechins are the major polyphenolic compound which includes Epigallocatechin-3-gallate (EGCG),

Table 1: Synthesisof silver nanoparticles by different plant extracts

Plants	Size (nm)	Plant's part	Shape	Ref.
Alternanthera dentate	50–100	Leaves	Spherical	[24]
Acorus calamus	31.83	Rhizome	Spherical	[7]
Boerhaavia diffusa	25	Whole plant	Spherical	[25]
Tea extract	20–90	Leaves	Spherical	[26]
Tribulus terrestris	16–28	Fruit	Spherical	[27]
Cocous nucifera	22	Inflorescence	Spherical	[27]
Abutilon indicum	7–17	Leaves	Spherical	[28]
Pistacia atlantica	10–50	Seeds	Spherical	[29]
Ziziphora tenuior	8–40	Leaves	Spherical	[30]
Ficus carica	13	Leaves	–	[31]
Cymbopogan citratus	32	Leaves	–	[32]
Acalypha indica	0.5	Leaves	–	[33]
Premna herbacea	10–30	Leaves	Spherical	[34]
Calotropis procera	19–45	Plant	Spherical	[35]
Centella asiatica	30–50	Leaves	Spherical	[36]
Argyreia nervosa	20–50	Seeds	–	[37]
Psoralea corylifolia	100–110	Seeds	–	[38]
Brassica rapa	16.4	Leaves	–	[39]
Coccinia indica	10–20	Leaves	–	[40]
Vitex negundo	5 & 10–30	Leaves	Spherical & fcc	[41]
Melia dubia	35	Leaves	Spherical	[42]
Portulaca oleracea	<60	Leaves	–	[43]
Thevetia peruviana	10–30	Latex	Spherical	[44]
Pogostemon benghalensis	>80	Leaves	–	[45]
Trachyspermum ammi	87, 99.8	Seeds		[46]
Swietenia mahogany	50	Leaves		[47]
Musa paradisiacal	20	Peel		[48]
Moringa oleifera	57	Leaves		[49]
Garcinia mangostana	35	Leaves		[50]
Eclipta prostrate	35–60	Leaves	Triangles, pentagons, hexagons	[51]
Nelumbo nucifera	25–80	Leaves	Spherical, triangular	[52]
Acalypha indica	20–30	Leaves	Spherical	[53]
Allium sativum	4–22	Leaves	Spherical	[54]
Aloe vera	50–350	Leaves	Spherical, triangular	[55]
Citrus sinensis	10–35	Peel	Spherical	[56]
Eucalyptus hybrid	50–150	Peel		[57]
Memecylon edule	20–50	Leaves	Triangular, circular, hexagonal	[58]
Nelumbo nucifera	25–80	Leaves	Spherical, triangular	[52]
Datura metel	16–40	Leaves	Quasilinear superstructures	[59]
Carica papaya	25–50	Leaves		[60]
Vitis vinifera	30–40	Fruit		[61]

Epigallocatechin, Epicatechins-3-gallate and Epicatechin, gallcatechins and gallcatechin gallate.

- **Chemistry of Tea**

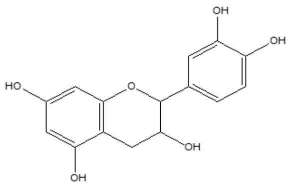

Figure 4: Structure of Catechin

The compounds in tea derived from catechins can have antioxidant effects on the body beneficial effect on cardiovascular health. It's suggested that the casein proteins in milk could bind to polyphenols and as a result prevent their antioxidant effect, but research on this subject remains conflicted.

- **Composition of Tea powder**

There are approximation 600 traces of aroma compounds in tea leaves, some of which are lost and some that are released during the manufacturing process. Once plucked, tea leaves begin to wither, their all walls begin to break down and chemical compounds begin to form new chemical compounds, not all of which are water-soluble. The main constituents of tea leaves belong to the polyphenols group accounting for 25 to 35% a dry weight basis. The polyphenols in tea mainly include the following six groups of compounds; flavanols, hydroxyl-4-flavanols, anthocyanins, flavones, flavanols and phenolic acid.

All the chemicals were used are of analytical grade, $AgNO_3$ (SDFine). Throughout the experiment double distilled water was used in the preparation of the solutions. The Tea powder (Red label from Tata (India) Ltd.99%), which was purchased from store.

2.1 Synthesis of silver nanoparticles from plant extract

- **Step 1: Tea extract**

1g of tea powder (Red label from Tata (India) Ltd.99%) was boiled (15 min) in 50mLof water and same was repeated for 5 times using fresh distilled water, the extract was filtered through filter paper.

- **Step 2: Nanoparticle synthesis by top-down method:**

The 50 mL of 10^{-3}N $AgNO_3$of was mixed with 1 mL of above tea plant extract. The solution was then shaken to ensure through mixing. The reaction mixture was allowed to settle at room temperature. The color of the mixture was observed changes from colorless to brown, that indicates nanoparticles are formed.

Figure 5: Formation of silver nanoparticlesby varying concentration of the of tea extractat different interval of time in presence of sun light, (a) initial (b) 1 min (c) 2 min (d) 3min (e) 4 min (f) 5 min.

The above formed silver nanoparticles are collected by centrifugation at rate of 8000 rpm, and dried at room temperature 24 hrs. The grinded fine silver nanoparticles were further used for characterization which was done by the UV-Visible, XRD, FTIR.

2.2 Antibacterial activity of Ag NPs

The antibacterial activities of AgNP's were carried out by disc diffusion method. Nutrient agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution (10 mg/mL) and placed in the nutrient agar plate and kept for incubation at 37°C for 24 hours. Zone of inhibition for control, AgNP's was measured. The experiments were repeated thrice and mean values of zone diameter were recorded. Activity was done in the Department of Biotechnology of KLE's Institute of P.C. Jabin Science college Hubballi.

3 RESULTS AND DISCUSSIONS

3.1 UV-Visible absorption studies

The absorption spectra of the synthesized silver nanoparticles were recorded against water in order to monitor the formation and stability of silver nanoparticles. The colour change of the mixture solution plant extract and silver ion is first recorded through visual observation. Colours of silver nitrate, the evolution of surface plasmon absorbance bands during the synthesis of silver nanoparticles using 50mL of $AgNO_3$ 10^{-3}M with 1mL of extract concentration during the first minute. The solution colour change within seconds to pale yellow, and then to yellowish brown, due to

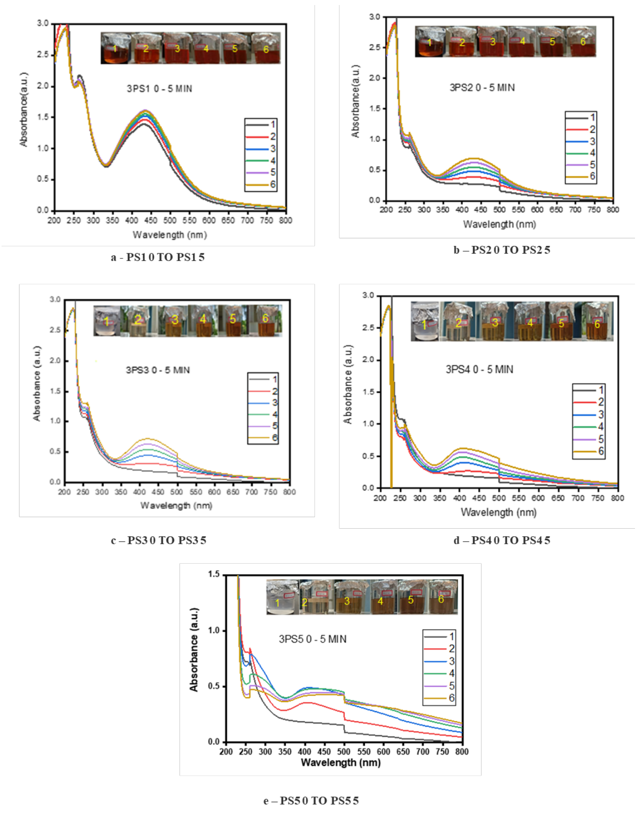

Figure 6: Absorption spectra of AgNP's., (a) initial (b) 1 min (c) 2 min (d) 3min (e) 4 min (f) 5 min.

formation of plasmons at the colloid surface indicating the formation of silver nanoparticles. The same sharp surface plasmon resonance absorbance band has been obtain with different extract concentrations of 10^{-3}M $AgNO_3$ with different time intervals. Then, 1mL of extract is enough to reduce completely 50mL(10^{-3}M) of silver ions. The conduction electrons undergo oscillation due to the strong interaction of light with the silver nanoparticles. As the concentration of the *Camellia sinensis* extract increases, the absorption peak gets more intensity but tend to be polydisperse (Figure 6). Sharp narrow shape SPR band indicating the formation of spherical and homogeneous distribution of silver nanoparticles was generally observed. The UV–vis spectra also revealed that the formation of Ag-nanoparticles occurred rapidly within few minutes indicating that Camellia sinensis speeds up the biosynthesis of silver nanoparticles. Silver nanoparticles have free electrons, which give surface Plasmon resonance (SPR) absorption band, due to the combined vibration of electrons of silver nanoparticles in resonance with light wave. A broad absorption peak was observed at 400 to 450 nm, which is a characteristic band for the Ag, it is similar range was reported in [72, 73]. The obtained peaks were observed in the spectrum which confirms that the synthesized products are Ag only.

3.2 2X-ray diffraction (XRD

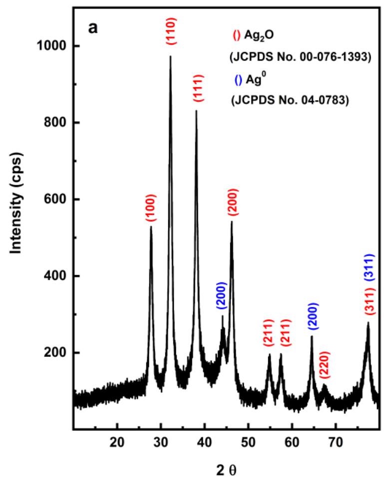

Figure 7: Powder X-ray diffraction of AgNPs

The XRD pattern of the synthesized AgNPs is shown in Figure 7. The diffracted intensities were recorded from 20° to 80 using Cu Kα radiation. Four strong Bragg reflections at 2θ values of 38 .05°, 46.35°, 64.75° and 78.05° corresponds to the planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively which can be indexed according to the facets of face cente red cubic crystal structure of silver [80]. The inter planar spacing ($d_{calculated}$) values are 2.336, 1.955, 1.436 and 1.224Å for (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes respectively and matched with standard silver values. The average crystalline size is calculated using Debye-Scherrer formula.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the average crystalline size of the nanoparticles, k is geometric factor (0.9), λ is the wavelength of X-ray radiation source and β is the angular FWHM (full-width at half maximum) of the XRD peak at the diffraction angle θ [74]. The calculated average crystallite of the AgNP's is ∼25nm.

3.3 Fourier transforms infrared (FTIR spectroscopy)

FTIR measurements were carried out in order to identify the presence of various functional groups in biomolecules responsible for the bio-reduction of $Ag^+$and capping/stabilization of silver nanoparticles. The observed intense bands were compared with standard values to identify the functional groups. FTIR spectrum shows absorption bands at 3422, 1631, 1450, 1240, 1043 and 596cm^{-1} indicating the presence of capping agent with the nanoparticles.

The bands at 3422cm^{-1} in the spectra corresponds to O–H stretching vibration indicating the presence of alcohol and phenol. The band at 1631cm^{-1} in the spectra corresponds to C–N and C–C stretching indicating the presence of

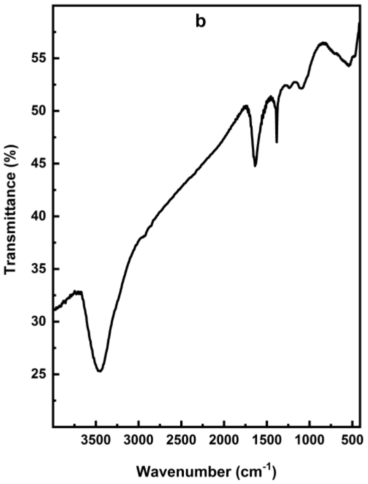

Figure 8: FTIR analysis of AgNPs

proteins [75]. The band at 1450cm^{-1} was assigned for N–H stretch vibration present in the amide linkages of the proteins. These functional groups have role in stability/capping of AgNPs as reported in many studies. The bands at 1450cm^{-1} and 1043cm^{-1} were assigned for N–H and C–N (amines) stretch vibration of the proteins respectively. The band at 1240cm^{-1} corresponds to C–N stretching of amines. The band at 588 cm–^{1}is attributed to the Ag-O (i.e., metal oxide) [76]. The band at 596cm^{-1}region could be attributed to C–Br stretching, which is characteristic of alkylhalides. It may be concluded from the FTIR spectroscopic study that the secondary structure of proteins in the *Camellia sinensis* are not affected because of their interaction with Ag^{+}ions or nanoparticles.

3.4 SEM - EDAX And TEM Study of AgNP's

To assess the morphology of as synthesised silver nanoparticles (AgNPs) Scanning Electron Microscope (SEM) images were taken. In Figure 9A & B are two images at different resolution. In these images relatively spherical nanoparticles are observed with average diameter of approximately 40 nm with some deviations. In addition to that, the High Resolution Transmission Electron Microscope (HRTEM) images were taken to identify micro structure at high resolution. As shown in Figure 9 C, it was observed that the as synthesised silver nanoparticles using tea-leaf are surrounded by a thin layer of some capping material and were stable in solution for many days. In literature, it was reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from plant leaf broth [84]. Thus, the possible reason for longer stability was due to the capping material on the surface of nanoparticles. The Fig. C inset shows selected

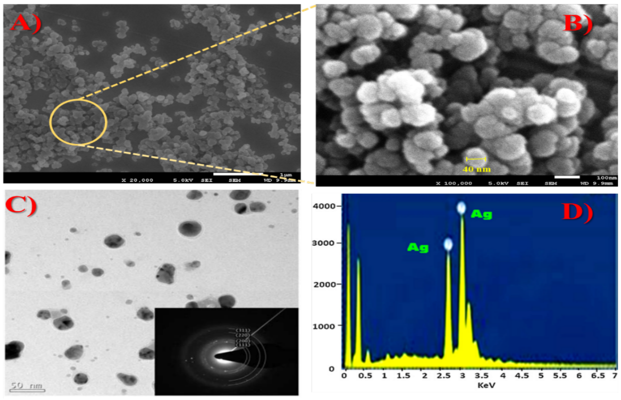

Figure 9: A and B) SEM images of silver nanoparticles at 1 μm and 100 nm resolution respectively, C) TEM image. Inset electron diffraction pattern recorded from the particles shown in b with lattice planes of fcc silver. D) EDAX spectrum of silver nanoparticles

area electron diffraction (SAED) pattern recorded from the AgNP's. The ring-like diffraction pattern was observed and it shows that the particles are crystalline in nature. The diffraction rings could be indexed on the basis of the fcc structure of AgNP's. Four rings observed due to the reflections recorded from lattice planes of fcc silver such as (111), (200), (220), and (311), respectively. The Energy Dispersive Spectroscopy (EDAX) spectrum of as synthesised AgNP's were performed to find the elements present in the sample. AgNPs generally show typical absorption peak approximately at 3 KeV due to Surface Plasmon Resonance [77]. In EDAX graph, the presence of the elemental silver can be observed (Figure D). This indicates the reduction of silver ions to elemental silver during the analysis.

3.5 BIOLOGICAL APPLICATION OF THE SILVER NANOPARTICLES

3.5.1. Antibacterial activity

There are several evidence suggest that silver ions are important in the antimicrobial activity of silver nanoparticles [78–81]. One important parameter of the antimicrobial toxicity of silver nanoparticles is the surface area of the nanomaterial.

The highest concentration of released silver ions was observed in case of highest surface area of silver nanoparticles. The lowest concentration of released silver ions was noted for silver nanoparticles with lowest surface area in case of highest surface area of silver nanoparticles. resulting in weak antimicrobial properties [82]. Some authors [83, 84] sustained that the mechanism of antibacterial action of silver nanoparticles is due to release of silver ions whereas the particles -specific activity of silver nanoparticles is negligible (Figure 10) show the mechanism of bacterial activity). In our study we found the value of bacterial activity increases from PS1 to PS5 (i.e., 24 to 55 mm), which results overlaps with standard drug (Ciproflaxin 55 mm). Figures 11 and 12, shows the photographic image of zones of inhibition at 24 and 48 hrs. Activity measurements were continued for 48 h, is due there are probabilities of back growth of bacterial zone. However, in our case time factor increases dense or intense bacterial growth zone occurs, and also small increase in activity was observed. In below Tables 2 and 3, mention the activity of different sample and microorganisms. As per our literature knowledge, in our case activity for *Bacillus subtilis* and *E. coli* have more than the previous reported values. This may due to the increase in surface area of the Ag NP's from PS1 to PS5.

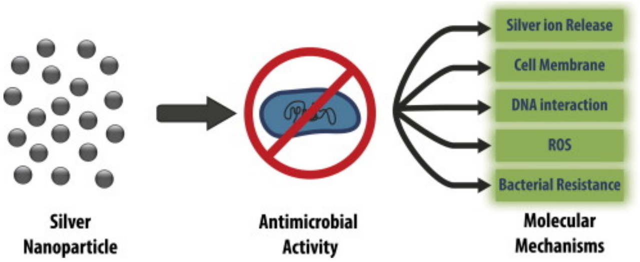

Figure 10: Mechanism of bacterial activity

- **Microorganisms**

Pure culture of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtills*, *Salmonella typhi* species of bacteria were cultured and experiments of antimicrobial activity were carried out in The Department of Biotechnology of K L E's Institute of P.C.Jabin Science college Hubballi.

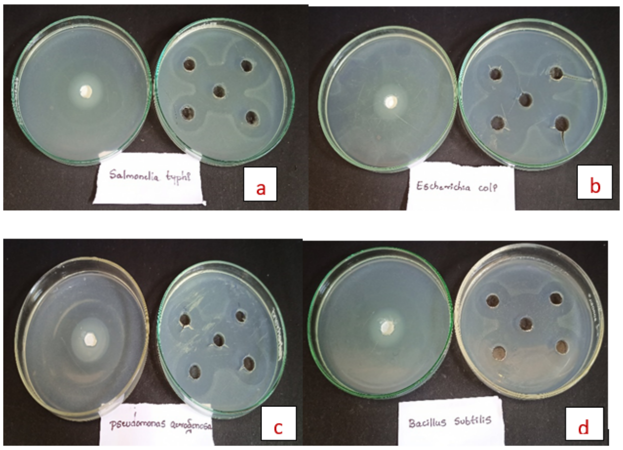

Figure 11: (a) Photographic images of antibacterial activities of synthesized silver nanoparticles of *Camellia sinensis* @ 24hrs

4 CONCLUSIONS

In this study, silver nanoparticles were synthesized using a tea source. The method is considered to be green because the

Table 2: Percentage activity of zone of inhibition

Organisms	PS1	PS2	PS3	PS4	PS5	Standard drug (Ciprofloxacin)	Percentage activity
	In mm						
Salmonella typhi	24	30	29	32	32	55	58.18%
Bacillus subtilis	22	25	24	30	40	52	84.61%
E. coli	26	26	29	39	40	53	75.47%
Pseudomonas aeruginosa	24	27	25	27	33	55	60%

Table 3: Percentage activity of zone of inhibition

Organisms	PS1	PS2	PS3	PS4	PS5	Standard drug (Ciprofloxacin)	Percentage activity	Literature data in mm
	in mm							
Salmonella typhi	24	30	30	33	35	55	63.63%	
Bacillus subtilis	25	25	30	32	40	52	76.9%	12
E. coli	26	27	29	39	44	53	83.01%	11 [93]]
Pseudomonas Aeruginosa	25	27	27	27	55	55	100%	

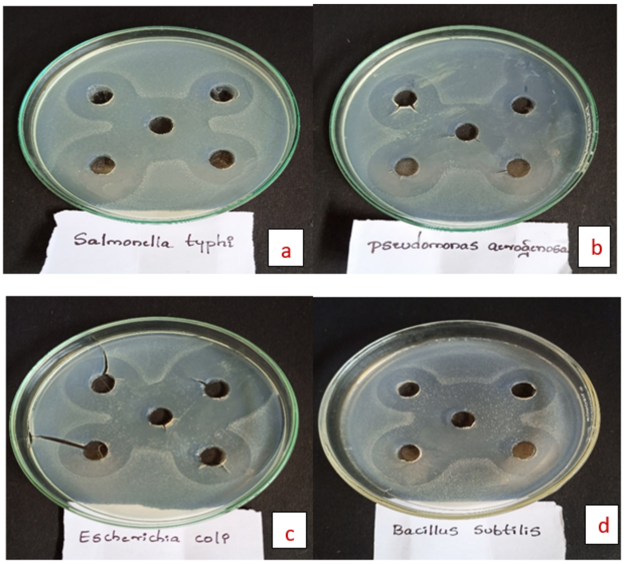

Figure 12: Photographic images of antibacterial activity of silver nanoparticles of Camellia sinensis @ 48 hrs

synthesis is carried out at ambient temperature, and without the addition of any chemical reductant, therefore it does not generate any environmental pollution. Characterization results obtained from UV-Spectroscopic, FT-IR SEM with EDEX TEM and XRD 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 $AgNO_3$, their effectiveness as an antibacterial agent is further established by the antibacterial assay performed. Hence the biosynthesized AgNPs are also suitable for developing antibacterial bandages and dressings. The nanoparticles showed good percentage of zone of inhibition thus they can be used in various bioremediation processes.

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