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Tectona grandis Seed Mediated Green Synthesis of Silver Nanoparticles and Their Antibacterial Activity

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Abstract

Nanobiotechnology is a fast expanding scientific field with potential applications in life sciences and human health care. Silver is a precious metal that occurs naturally, most commonly as a mineral or in combination with other elements. Aqueous *T. grandis* plant seed extract was utilized in the current investigation as a reducing and capping agent in the biogenesis of silver nanoparticles. Nanoparticles were examined using different techniques like UV-visible spectral analysis, Fourier transform infrared spectra technique, X-ray diffraction technique, and transmission electron microscopy technique. The peak at 429 nm of the surface plasmon resonance by using UV-visible spectra, confirmed the synthesis of nanoparticles. Utilizing Fourier transforms infrared spectral analysis, the explicit functional groups that reduced silver nitrate to create silver nanoparticles in seed extract were found. X-ray diffraction analytical technique was utilized to found the crystalline nature and Face-centered cubic (FCC) configuration of green synthesized silver nanoparticles. Silver nanoparticles with sizes ranging from 32 to 62 nm are visible in images taken using transmission electron microscopy. Using disc diffusion technique, the generated silver nanoparticles showed remarkable antibacterial activity against selected organism, *Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. AgNPs demonstrated broad spectrum antibacterial action as a result at lower concentrations, and they may provide an effective therapeutic alternative in the future like, development of new antibacterial drugs. This research identified a quick and eco-friendly green synthetic method for producing stable silver nanoparticles.

Keywords: *Tectona grandis*, UV-visible, XRD, AgNPs, Antibacterial activity, FTIR, TEM, Seed extract

Introduction

Nanotechnology is a regulation of science which deals with mechanized, exploitation and usage of materials ranging among 1-100 nm. The topic of nanotechnology is one of the most dynamic researches currently in modern material science and technology [1-3]. In recent times, metal nanoparticles have garnered momentous interest in numerous domains spanning from materials science to biotechnology [4-6]. Metal nanoparticles which have a high detailed surface area and high fraction of surface atoms been considered extensively because of their distinctive physic chemical characteristics including optical properties, catalytic activity, magnetic properties and antibacterial properties [7,8]. An enormous array of physical, chemical and organic approaches existing to generate nanoparticles [9,10]. When using nanoparticles for biomedical applications, the problem with the majority of physical and chemical techniques is the use of toxic substances and high pressure, which may result in potential biological and environmental dangers. When creating nanoparticles, researchers usually have difficulties controlling size/shape fluctuation and achieving mono dispersity in addition to the toxicity issues. Most promising approaches for the manufacture of nanoparticles have emerged to defeat these problems: The utilization of plant extracts. For the diminution of metal ions to their equivalent nanoparticles and to prevent them from aggregating by capping, plant ingredients function as reducing and protective agents [11]. The maintenance of microbial cell cultures and lack of intricate purification processes are this method's main benefits [12]. As the importance of nanotechnology and biotechnology has grown over the past few years, it has become increasingly necessary to create new technologies for the production of nanoparticles [13].

Silver nanoparticles have been extensively used as an antibacterial agent in biological applications. Consequently, there is a need for an environmentally friendly and practical method of producing silver

nanoparticles. Microorganisms and plant extracts have been planned as impending environmentally friendly alternatives to chemical and physical processes for the green synthesis of nanoparticles [14]. Unavoidably, human handling of the generated silver nanoparticles is required, and they must be accessible at lower costs for successful use. In this study, we reported employing *T. grandis* seed extract as a reducing agent to synthesize silver nanoparticles using a green method.

These investigations have used natural items as reducing agents, such as mono saccharides or plant extracts. According to numerous studies, the creation of nanoparticles via microbiological methods is substantially slower than using plant extracts and other chemical reducing agents. When the nanoparticles could be produced more quickly and biologically in the reaction vessels, their commercial viability would increase. In general, all biological systems are rich in reductase enzymes, and olive plants are no exception. This mechanism has been exploited in the past, and the current attempt is to use olive seeds to reduce silver nitrate salts to metallic silver and olives, which have an advantage due to their innate antimicrobial properties. Sastry and colleagues [15] were the first to report producing nanoparticles at rates comparable to those of chemical reagents. They also pioneered the use of plant extracts in the production of nanoparticles. Utilizing biological systems, different metallic nanoparticles of silver, gold, zinc, palladium, *etc*. are being created [16].

T. grandis, sometimes known as timber, teak, or sagun, may be useful medically. Furniture, cabinets, and musical instruments are all frequently made using it [17]. Quinones such lapachol, tectoquinone, deoxylapachol and its isomer, anthraquinone, tectoleafoquinone, steroidal compounds like polyisoprene-a-tolyl methyl ether betulinic acid, squalene, monoterpene, tectograndone, apocarotenoids like tectoinols-A Its bark contains the antibacterial chemical 5-hydroxy-1,4-naphthalenedione, which has been expose to be effectual against MRSA and *Listeria monocytogenes* [18]. It also has anti-hemolytic anemia action and is used to treat anemia. Its seeds are revered as a hair tonic, and it has been suggested that they enhance the quantity of hair follicles in the antigenic phase [19]. It has antioxidant capabilities in its leaves, bark, and wood, with wood exhibiting the highest (98.6 %) inhibition of DPPH [20].

In this study, silver nitrate was converted to silver nanoparticles using *T. grandis* seed extract. The created nanoparticles were then characterized using XRD, TEM, and FT-IR analysis. The generated nanoparticles antimicrobial efficacy was examined against representative human pathogenic bacteria (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*). We investigated the synthesis of stable silver nanoparticles with the bioreduction method using aqueous seed extract and evaluated their antibacterial activity against drug resistant bacterial isolates.

Materials and methods

Materials

Silver nitrate, $AgNO_3$ (99.8 %) purity was purchased from Sigma-Aldrich chemical Pvt. Ltd. Filtration was established using Whatman No. 1 filter papers. The glassware was washed with dilute nitric acid, thoroughly washed with double-distilled water and dried in hot air oven. Fresh seeds of *Tectona grandis* L. were collected from the Botanical garden of Sheth M.N. Science College situated in Patan, Gujarat, India (Location: 23°86'12" N 72°13'03" E). Identification and authentification of *Tectona grandis* L were done threw Flora of Gujarat by Dr N.K. Patel, HOD of Botany Department, Sheth M.N. Science College, Patan, Gujarat, India. Antibacterial assay was carried out (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*) using well diffusion technique. The microbial culture was maintained by Department of Biotechnology, IDMC, Patan, Gujarat.

Preparation of aqueous seed extract

The *T. grandis* seed aqueous extract was made by combining 5 g of the seeds with 100 mL of deionised water in a beaker. After that, the seed suspension was gradually heated until it boiled. The suspension was then given another 10-15 min to boil while being constantly stirred. The resulting *T. grandis* seed extract was then stored at 4 °C for later use after being cooled at room temperature and filtered with Whatman No. 1 filter paper to remove any plant material.

Synthesis of silver nanoparticles (AgNPs)

By reducing $AgNO_3$, AgNPs were created in a typical one step synthesis. 9 mL of 1 mM aqueous $AgNO_3$ solution and 1 mL of *T. grandis* seed extract were combined to reduce Ag^+ ions. The reaction mixture was kept at room warmth until the colourless solution became brown; signifying the production of nanoparticles. UV-visible spectra technique was used to monitor the reduction of pure Ag^+ ions, and high-rpm centrifugation was used to split the formed nanoparticles.

Characterizations of AgNPs

UV-vis spectra analysis

Bioreduction of silver nanoparticles is responsible for the colour change and it was monitored using UV-vis Double beam Spectrophotometer UV-1,800 SHIMADZU by scanning the sample solution between the wavelength 190-1,100 nm. It gives the highest absorbance value to prove the presence of silver nanoparticles.

XRD analysis

Powder X-Ray Diffraction pattern were recorded on a X-Ray Diffractometer (Model: X'pert Pro, PANalytical Netherlands) equipped with 5 to 140 ° 2q with Nickel beta filter CuKα radiation source on 1.54 A° wavelength and 15 position sample changer. The data was collected in the 2θ range to examine the crystallographic structure of the purified AgNPs. The mean particle length of silver nanoparticles was calculated from the XRD pattern according to the line width of the plane, reflection peak using Scherrer's formula.

FTIR analysis

By using FTIR, the sediment layers were identified (Model: Perkin Elmer instrument). The mid-IR range of 400–4,000 cm^{-1} is where the FTIR spectrum was collected. ATR was used to record the spectrum (Attenuated Reflectance Technique). The dried experimental material was placed directly over the potassium bromide crystals, and the transmittance mode spectrum was recorded.

TEM evaluation

TEM was used to analyze the particle's morphology. A drop of aqueous AgNPs sample was put onto a copper grid coated with carbon, and the solvent was allowed to evaporate for an hour at room temperature. The H-7,500 Hitachi instrument was used to capture the TEM micrograph pictures, which had a resolution of 0.36 nm and an accelerating voltage of 40-120 kV on carbon-coated copper grids. Different ranges of magnifications were used to see and record the clear microscopic pictures.

Antibacterial assay

The 4 most effective human pathogenic bacteria *Escherichia coli* (ATCC 8739)*, Bacillus subtilis* (ATCC 6633)*, Staphylococcus aureus* (ATCC 29737), and *Pseudomonas aeruginosa* (ATCC 25619) were standardized using McFarland standard for the synthesized AgNPs antibacterial activity utilizing the agar well diffusion process. By using the spread plate method, each bacterial culture was grown in nutrient broth for 24 h before the grown culture (100 µl from stock solution (3 mg/mL)) was distributed onto a sterilized plate containing autoclaved nutrient agar (Hi Media Laboratories Private Limited, Mumbai, India). Using a micropipette, wells were created on the nutrient agar plates. The dilutions of biosynthesized Ag NPs varying from 5, 10 and 15 mg^3/mL were prepared with 2 fold symmetry. The tested organisms were inoculated in 4 discs, which are dipped in different dilutions of AgNPs (5, 10 and 15 mg^3/mL) solutions. The circular wells of 6 mm diameter were made using a sterile cork-borer. The wells received 50, 60 and 70 µl of the diluted AgNPs solution and Chloramphenicol (50 µl) as a positive control as well as distilled water as a negative control, which was then added, incubated for 24 h at 37 °C, and the zone of clearance was noticed the following day. The diameters of inhibition zones were measured and the mean value for each organism was recorded.

Results and discussion

UV-vis spectra analysis

The reaction mixture's colour changed from yellow to brown when exposed to sunlight, which is evidence that AgNPs were formed by the reduction of $AgNO_3$ during treatment with the *T. grandis* seed extract (**Figure 1**). The surface plasmon ambiance of the seed extracts were excited and their absorption spectrum at various wavelengths from 350 to 700 nm showed a peak at 429 nm, which is what gave the brown colour its look. In *T. grandis*, Uv-vis spectral analysis was captured after intervals of 30 min, 3 and 24 h following the onset of reaction. The produced nanoparticles shape, size, composition, and morphology all have an impact on the surface plasmon resonance (SPR) band. Due to their surface plasmon resonance (SPR), silver nanoparticles have extraordinary and uneven visual properties that depend on the shape and size distribution of the molecules that stabilize them, the surface-adsorbed particles, and the medium's dielectric constant [21]. The spherical silver nanoparticles contribute to the 400 nm absorption bands in the UV-vis spectra, according to earlier research [22,23]. Around 429 nm,

AgNPs' SPR band characteristics were discovered (**Figure 3**). This strongly suggests that the AgNPs were spherical, and the TEM findings of this work support this. As the time passed, the peak narrowed. The mono dispersed nature of the particles in suspension is demonstrated by narrow peaks. The nanoparticles don't clump together over time, as shown by the narrow peak at 24 h, demonstrating both the stability and mono dispersity of the biosynthesized AgNPs.

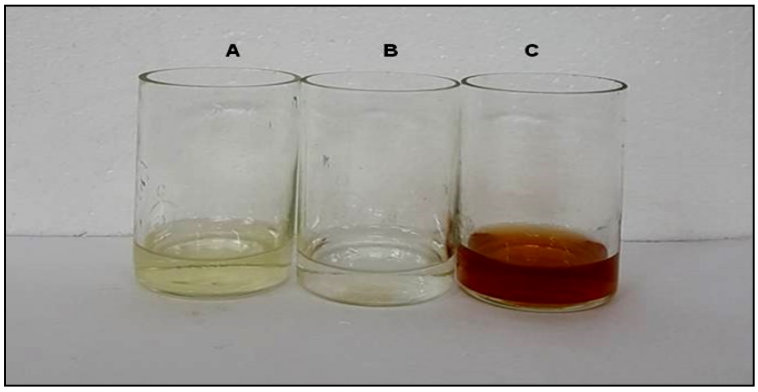

Figure 1 (A) *T. grandis* seed extracts (B) $AgNo_3$ solution (C) Synthesized AgNPs.

Figure 2 Showing different time interval colour intensity of *T. grandis* seed extract AgNPs.

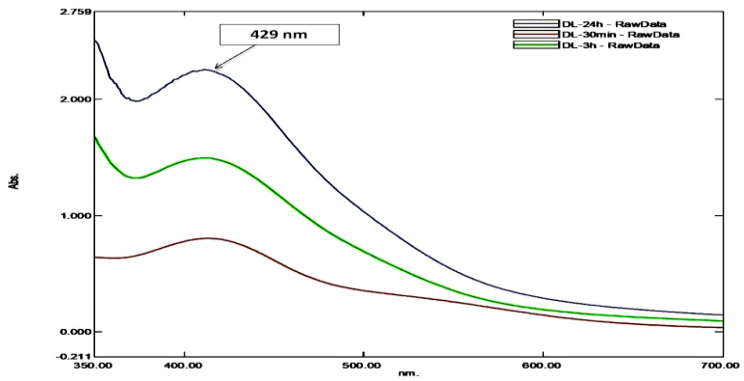

Figure 3 UV-visible spectra of *T. grandis* seed AgNPs at different time intervals.

X-ray diffraction spectroscopy (XRD)

X-ray diffraction was used to determine the phase of the produced nanoparticles, and the relevant XRD patterns are displayed in **Figure 4**. Additionally, the resulting particles in the silver nanoparticles

have distinct cubic face peaks (JCPDS File No. 04-0783). The diffracted intensities are recorded using the 2θ range. The range is between 30 and 80 °. **Table 1** shows that Bragg reflections may be connected to the plane of the face-centered cubic phase of silver at a strong peak of 38.5 ° matching to (111), (200), (220) and (311). Using the Debye-formula, Scherrer's d = 0.89/Cos, where d is the particle size, is the X-ray wavelength (1.5406), is the full-width at half maxima (FWHM) of the most significant peak (in radians) of the diffraction pattern, and 2 is the Bragg angle, one can determine the size of the particulate's crystals. [1, 24] It implies that the 2 phases of the produced silver nanoparticles are crystalline and amorphous organic phases. The decrease in Ag^{+} ions and modest changes in the peak levels in our experiment point to the production of nano crystals. Similar outcomes utilizing *Artemisia annua* extract were noted in silver nanoparticles [25]. By comparing the produced pattern with the reference library and designating its nano crystalline nature, the XRD technique is employed to determine the crystalline nature [26].

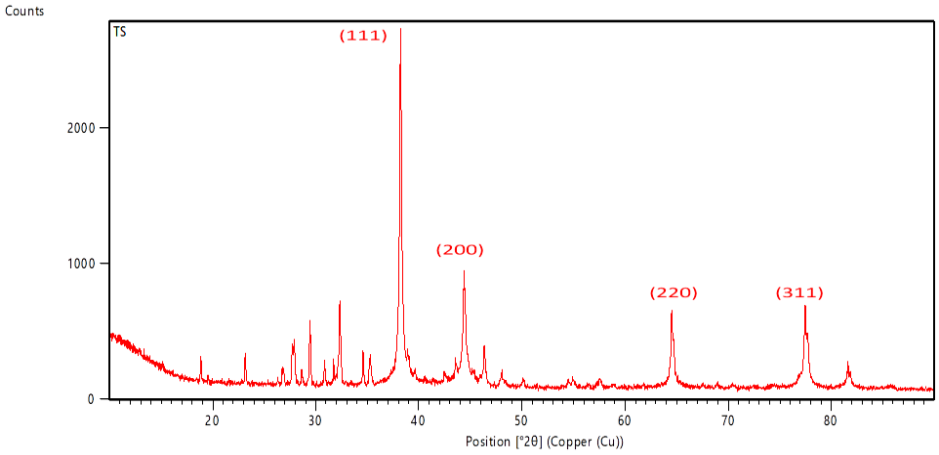

Figure 4 XRD pattern of AgNPs synthesized using the *T. grandis* seed extract.

Table 1 Different parameters of the XRD analysis of AgNPs of *T. grandis* seed extract.

2Θ (Degree)	FWHM	Diffraction plane (hk1)	Interplanar spacing "d"	Crystallite size (nm)
38.87 °	0.75	111	2.35	15.24
44.38 °	0.19	200	2.03	61.39
64.52 °	0.18	220	1.44	70.78
77.47 °	0.23	311	1.23	60.05

Fourier transform infrared microscopy (FTIR)

The biomolecules specifically bound to the synthesized AgNPs were characterized and named using FTIR analysis. In order to create a pellet, the Potassium bromide was combined with the biologically synthesized silver nanoparticles and the powdered seeds. **Figure 5** displays the FTIR spectra of *T. grandis* synthesized seed extract AgNPs following their reaction with $AgNO_3$. According to the FTIR results, there are absorption bands at 3,454.38 (O-H stretching, H-bonded of alcohols, phenols, and N-H stretching of primary, secondary, and protein amides), 2,086.26 (Silicon compounds), 1,631.30 (-C=C-stretching and N-H bend of alkenes and primary amines), 1,383.18 (C-O stretching of alcohols, carboxylic acids, esters, and ether (C-Br stretching of halide) [27-29]. The FTIR analysis revealed that the synthesized AgNPs contained amides, alkanes, carboxyl, alcohols, and phenols groups. Similar results are observed in the biological synthesis of AgNPs using seed extract from *Artocarpus heterophyllus* Lam. [30] and *Abelmoschus esculentus* [31].

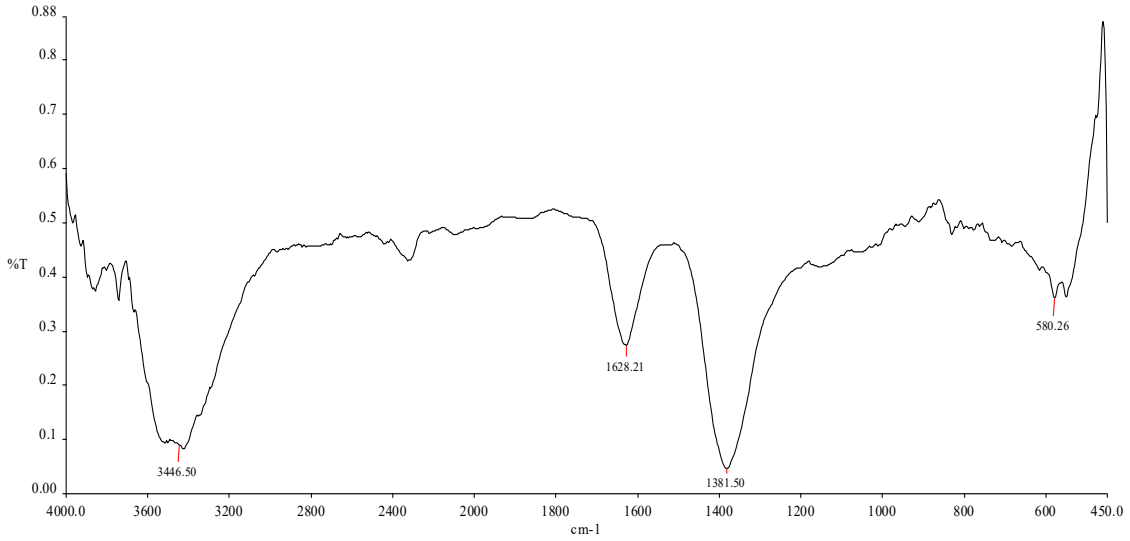

Figure 5 FTIR spectra of synthesized AgNPs of *T. grandis* seed extract.

Transmission electron microscope (TEM)

This method was used to examine the size and shape of the synthetic *T. grandis* silver nanoparticles (AgNPs). The TEM picture of AgNPs is shown in **Figure 6**. The estimated particle size from the XRD analysis is well-aligned with the picture, which shows spherical AgNPs with an average size of 20 - 50 nm. AgNPs were synthesized from *T. grandis* extract and were spherical in nature. The AgNPs were 50 nm in size. Without aggregation, the nanoparticles were monodispersed. According to related investigations, the synthesis of gold and silver nanoparticles from the extract of the *Erigeron annus* (L.) pers flower extract resulted in spheres with diameters between 15 and 60 nm and 20 to 100 nm [32]. The common length of silver nanoparticles produced from *Cynodon dactylon* and subjected to sunlight was 8 to 10 nm in a different study [33].

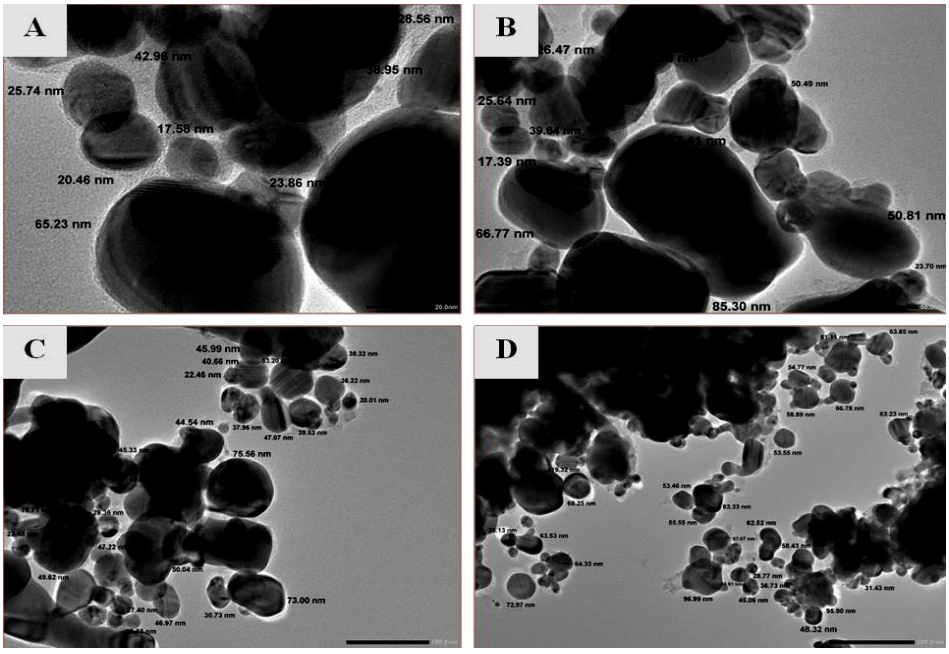

Figure 6 Transmission electron microscopy (TEM) images of AgNPs by *T. grandis* (A) 20 nm (B) 50 nm (C) 100 nm (D) 200 nm.

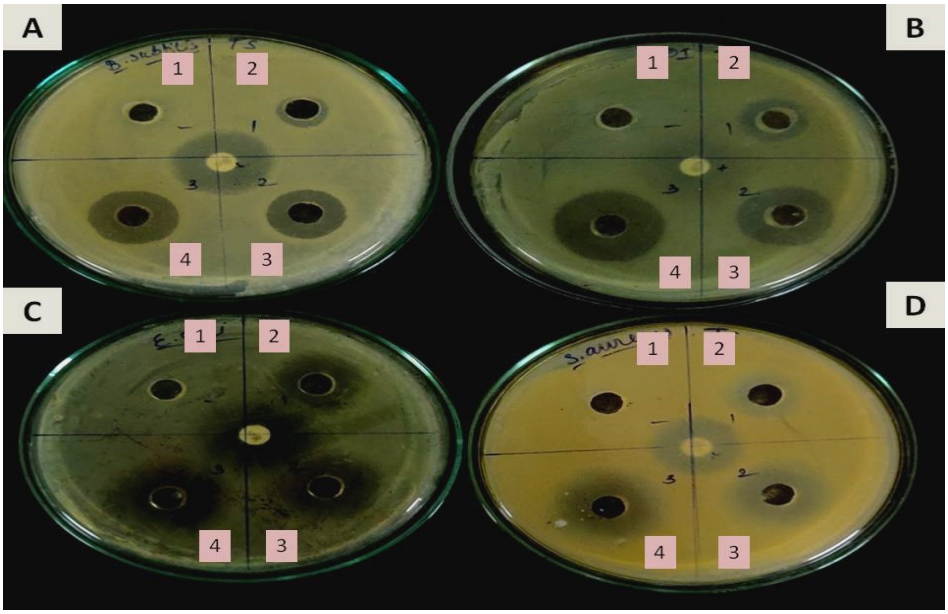

Figure 7 TEM histogram of *T. grandis* AgNPs- Average particle size- 49 nm.

Antibacterial activity

The antibacterial activity of biosynthesized AgNPs is highest against *Pseudomonas aeruginosa* (24.00 ± 0.26 mm) at 70 µg/mL and lowest against *Bacillus subtilis* (16.66 ± 0.15 mm) at 50 µg/mL (**Figure 7**). As well as *Escherichia coli* shown highest antibacterial activity (22.00 ± 0.50 mm) at 70 µg/mL and *Staphylococcus aureus* (21.21 ± 0.11 mm) at 70 µg/mL. The antibacterial activity of silver nitrate solution is highest against *Escherichia coli* (20.61 ± 0.50 mm) at 70 µg/mL and lowest against *Bacillus subtilis* (14.66 ± 0.15 mm) at 50 µg/mL. As well as *Pseudomonas aeruginosa* shown highest antibacterial activity (20.00 ± 0.26 mm) and *Staphylococcus aureus* (18.60 ± 0.11 mm) at 70 µg/mL. In plant extract, *Escherichia coli* shown highest antibacterial activity (18.00 ± 0.50 mm), *Bacillus subtilis* (11.50 ± 0.18 mm), *Pseudomonas aeruginosa* (13.00 ± 0.26 mm) and *Staphylococcus aureus* (14.54 ± 0.11 mm) at 70 µg/mL. Similar to silver nitrate, AgNPs have reportedly been shown to exhibit greater antibacterial activity [34]. Silver nanoparticles high surface area to volume ratio increases their contact with microorganisms, facilitating the dissolution of Ag^+ ions and enhancing biocidal efficacy. When the AgNPs come into contact with bacteria and free radicals, they can damage the cell membrane and make it porous, which will ultimately cause cell death [35]. In this case, a weak acid reacts with a weak base because silver is a weak acid and there is a natural tendency for acids to react with bases. Deoxyribonucleic acid (DNA) contains phosphorus and sulphur as its main building blocks; AgNPs can act on these soft bases to destroy the DNA, which would cause cell death. Additionally, it was demonstrated that the size and shape of AgNPs affected their antibacterial activity. AgNPs (1-10 nm) bind to the cell membrane's surface and severely impair functions like respiration and permeability [36].

Table 2 Antibacterial activity of AgNPs synthesized by using *T. grandis* seed extract.

Bacteria class	Name of the organism	Concentrations (µl)	Zone of inhibition(mm)		
			Plant extract	Silver nitrate ($AgNO_3$)	Silver nanoparticles TS
Gram negative	*Escherichia coli*	50 µl	15.33 ± 0.34	15.33 ± 0.34	17.33 ± 0.34
		60 µl	16.33 ± 0.10	18.33 ± 0.10	19.33 ± 0.10
		70 µl	18.00 ± 0.50	20.61 ± 0.50	22.00 ± 0.50
	Pseudomonas aeruginosa	50 µl	13.83 ± 0.54	14.83 ± 0.54	17.83 ± 0.54
		60 µl	14.87 ± 0.30	16.87 ± 0.30	19.87 ± 0.30
		70 µl	13.00 ± 0.26	20.00 ± 0.26	24.00 ± 0.26
Gram positive	*Bacillus subtilis*	50 µl	10.66 ± 0.15	14.66 ± 0.15	16.66 ± 0.15
		60 µl	12.27 ± 0.67	16.27 ± 0.67	18.27±0.67
		70 µl	11.50 ± 0.18	17.50 ± 0.18	19.50 ± 0.18
	Staphylococcus aureus	50 µl	14.63 ± 0.31	16.63 ± 0.31	18.63 ± 0.31
		60 µl	15.09 ± 0.06	17.29 ± 0.06	19.11 ± 0.06
		70 µl	14.54 ± 0.11	18.60 ± 0.11	21.21 ± 0.11

Note: 1 = Negative control, 2 = Plant extract, 3 = $AgNO_3$, 4 = AgNPs

Conclusions

In the current study, a one pot, straightforward, energy-efficient, economically viable, and environmentally friendly method for the synthesis of AgNPs was established using water as a solvent and non-toxic, renewable aqueous extract of *T. grandis* seed as reducing, capping, and stabilising agents instead of harsh, synthetic reducing capping agents. *T. grandis* seed aqueous extract mediated AgNPs were characterized using UV-vis spectrometry, XRD, and TEM, and the results confirmed the formation of spherical shaped AgNPs with an approximate average size of 32 - 62 nm. The bioactive compounds such as phenolics, flavonoids and antioxidants present in teak seed extract were found to be utilized during biosynthesis process as reducing and capping agents. The synthesized AgNPs demonstrated remarkable antibacterial activity with ZOI (24.00 ± 0.26 mm) against the pathogenic bacteria *Pseudomonas aeruginosa* and also *E. coli*, *B. subtilis*, *S. aureus* making it ideal for medical and therapeutic applications. The biosynthesized AgNPs have potential application in water purifiers, antibacterial fabrics, sportswear and in cosmetics as antibacterial agent and the process used for its synthesis being greener is highly beneficial from environmental, energy consumption and economic perspectives.

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