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Green Synthesis of Nanoparticles and their Antibacterial Activity Against Pathogenic Bacteria

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Abstract:
This work examined the phytochemical characterization of the primary bioactive ingredients of Punica granatum peels in its aqueous extract, green production of silver nanoparticles, and their antibacterial efficacy. Flavonoids, phenol, tannins, carbohydrates, glycosides, etc. were abundant in peel extract. UV-Visible spectroscopy verified silver nanoparticle production and characterization. UV-Visible spectroscopy of silver nanoparticle-containing reaction media shows maximal absorbance peaks at 430nm (1%), 373nm (3%), and 379nm (5%). The disc diffusion technique was used to assess the green production of silver nanoparticles against bacterial cultures. The silver nanoparticles killed E. coli (MTCC-40), Staphylococcus aureus (MTCC-7443), and Proteus vulgaris (MTCC-*1771). Punica granatum peel extract rapidly lowers Ag+ to Ago and aids silver nanoparticle formation with antimicrobial properties.

Introduction

Nanotechnology is a fast-growing discipline used in science and technology to make tiny materials [1]. Currently, synthetic silver and other new metal particles have various key biolabelling sensuous uses. These nanoparticles have novel physico-chemical and biological characteristics not absorbed by the bulk. [2]. Bacteria, yeast, fungi, actinomycetes, and viruses may be used to synthesize metal particles.

Plant-mediated green synthesis of noble nanoparticles is becoming more popular owing to its simplicity and eco-friendliness. [3]. Punica granatum may cure and prevent cancer, cardiovascular disease, diabetes, dental issues, and UV radiation. Infant brain ischemia, Alzheimer's, male infertility, arthritis, and obesity are further possible uses. [4]. Trianthema decandra [5], Mulberry [6], B. ovalifolitata S. tumbuggaia, S. hyderobadensis [7], Ocimum sanctum [8], Allium cepa [9], Elaeagnus latifolia [10], Cassia auriculata [11] have been reported to biosynthesize silver nanoparticles, but their potential as biological materials for nanoparticle synthesis is still unexplored. Nanoparticles fall into two categories: Due to their structure, size, and benefits over biological imaging drug agents and medications, inorganic particles have been investigated as medical imaging and disease treatment tools. The Indian Institute of Technology, Bombay found metal nanoparticles in homeopathic tablets and ayurvedic bhasmas [7].

Silver nanoparticles are widely used in biological tests. Silver nanoparticles have several uses. Silver has disinfecting properties and has been used in traditional medicine and cooking. Silver nanoparticles are non-toxic and most efficient against bacteria, viruses, and eukaryotic microorganisms at low concentrations without adverse effects. Due to bacterial resistance to traditional antibiotics, silver nanoparticle antibacterial research has grown [12]. Since ancient times, silver species have been recognized to have antibacterial properties and to be nontoxic to human cells in low concentrations. [13].

Researchers believe silver species emit Ag+ ions that interact with thiol groups in bacterium proteins, inhibiting DNA replication. Size and shape determine how silver nanoparticles interact with bacteria. Due to their higher surface area per volume, silver nanoparticles are more antimicrobial. Silver nanoparticles kill bacteria by disrupting their cell membrane permeability and respiratory function [14]. The plant includes phytochemical chemicals such phenols, tannins, flavonoids, amino acids & proteins, carbohydrates, alkaloids, and others, which affect metal ion reduction form.

The current work examined the antibacterial activity of silver nanoparticles generated from Punica granatum peels of aqueous extract by preliminary disc diffusion test screening. We tested the extracts against Staphylococcus aureus, E. coli, and Proteus vulgaris.

Material and Methods:

Preparation of Punica granatum peels extract:

The alwar local market provided fresh punica granatum peels (Fig.1). After washing several times with distilled water to remove dust, the peels were air-dried at room temperature for seven days. Boiling dried peels powder in distilled water for 10-15 minutes turns the watery solution yellow. 1%, 3%, and 5% aqueous extract were produced. Filtered, room-temperature extract was used. The extract was kept at room temperature for future experiments.11]

Figure 1: Punica Granatum (A) Fruit (B) Peels

figure 2: Aqueous Extract

Preliminary phytochemical screening:

The stock solution was used for preliminary screening of phytochemicals such as carbohydrates (Fehling's test), amino acid & proteins (Millon's test), alkaloid (Mayer's test), flavonoids (NH3 & HCl test), phenol and tannin's (Ferric chloride test), saponin's (Froth & foam test), glycosides (Killer kilani test), steroids, phytosterols, phlobatannin's [15]

Synthesis of silver nanoparticles:

Typical reaction: 10ml of aqueous peels extract was added to 100ml of 0.01mM AgNO3 in a conical flask at room temperature. Solution color changes within 15 min. from yellow to greenish brown (1%), dark yellowish to brownish black (3%), and dark yellowish to dark brown (5%), suggesting silver nanoparticle production (Fig.3). It is widely known that silver nanoparticles change color in aqueous solution owing to surface Plasmon vibration. Most herbal-mediated silver nanoparticle solutions became black following incubation.

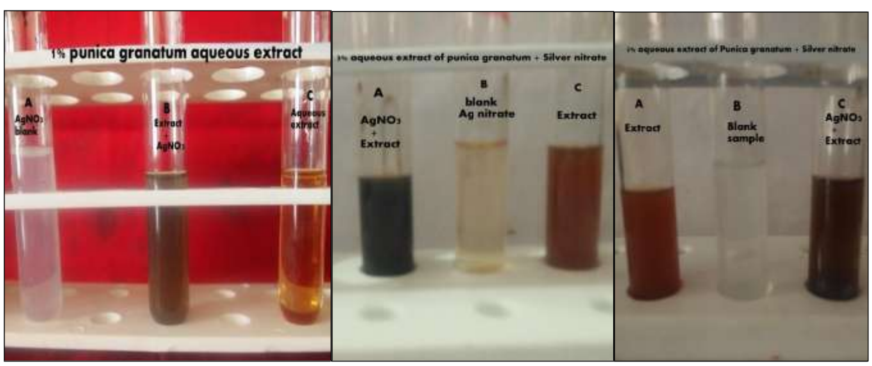

Figure 3: Color Change Seen in Plant Extract Taken for Silver Nanoparticle Synthesis (A) 1% Punica Granatum Peels Aqueous Extract (B) 3% Punica Granatum Peels Aqueous Extract (C) 5% Punica Granatum Peels Aqueous Extract

Synthesized nanoparticles have UV-Vis spectroscopy between 300 and 800nm (Fig.4). The Shimadzu UV-1800 spectrophotometer was used for UV-Vis spectrum analysis. The final nanoparticle solution was centrifuged at 8,000rpm for 25 minutes. Pellets were kept at -4˚C and the supernatant was discarded. This study identified promising weeds for silver nanoparticle synthesis, which was intriguing.

Screening of green synthesized nanoparticles by Disc diffusion method:

Soil cultures of E. coli (MTCC-40), Staphylococcus aureus (MTCC-7443), and Proteus vulgaris (MTCC-*1771) was purchased from the Microbial Type Culture Collection Centre (MTCC) located at the Institute of Microbial Technology Chandigarh, India. A green synthesized silver nanoparticle was screened for their antibacterial activity against E. coli, Staphylococcus aureus, and Proteus vulgaris by disc diffusion method.

Antibacterial activity was measured using 6mm No.1 filter paper discs sterilized by dry heat at 80˚C in an oven for 1 hour. Prepared nutrient agar for disc diffusion test. It solidified on sterilized petri plates after sterilization. The bacterial culture suspensions were made from 1-2 day-old cultures separately. Fresh cultures of chosen pathogenic bacteria were swabbed on duplicate plates. Synthesized nanoparticles were diluted (100%) and immersed on sterilized filter paper discs. Nanoparticles discs (100µg/ml) were put on an agar plate with bacterial cultures. Standardized antibiotic (Streptomycine sulfate) solution (100µg/ml per disc) was impregnated in filter-paper discs for antibacterial activity. These discs were put over microorganism-preceded plates. The plates were incubated at 37˚C for 24 hours. Averages were determined from two replicates each instance.

Inhibition zone diameter was measured in mm and used to construct activity index. The following formula tested synthesized nanoparticle activity: [16]

Activity index: Inhibition zone of sample/ Inhibition zone of standard.

Results And Discussion:

Qualitative phytochemical analysis:

Secondary metabolites may explain therapeutic plant cures [17]. The qualitative phytochemical examination of Punica granatum peel aqueous extract showed steroid, glycosides, carbohydrates, proteins, amino acids, phytosterol, phenols, tannins, flavonoids, alkaloids, phlobatannins, and more (Table 1).

Table 1: Phytochemical Screening of Punica Granatum Peels Aqueous Extract

S.No.	Name of the test	Aqueous extract
1	Carbohydrates	+
2	Proteins and amino acid	+
3	Alkaloids	+
4	Flavonoids	+
5	Steroids	+
6	Phlobatannins	+
7	Glycosides	+
8	Phenols and tannins	+
9	**Saponins**	+

The early phytochemical screening assays may help identify bioactive principles and lead to medication discovery and development. Additionally, these tests qualitatively separate pharmacologically active chemical substances [18]. The peel extract included phenols, tannins, carbohydrates, glycosides, proteins, amino acids, flavonoids, saponins, phytosterol, phlobatanins, alkaloids, and steroids, according to phytochemical screening. These extracts may make Punica granatum therapeutic. The main constituents are phenols, saponins, and tannins. Glycosides alleviate congestive heart failure and cardiac arrhythmia naturally. Saponins are hypotensive and cardiodepressant. UV-Vis spectrophotometers from 300 to 800nm confirm silver nanoparticle stability and production in sterile distilled water. After mixing Punica granatum peels extract in aqueous silver ions complex solution, UV-Vis spectra of the reaction medium were measured regularly to monitor the reduction of pure Ag+ ions to Ago. **(Fig 4)**.

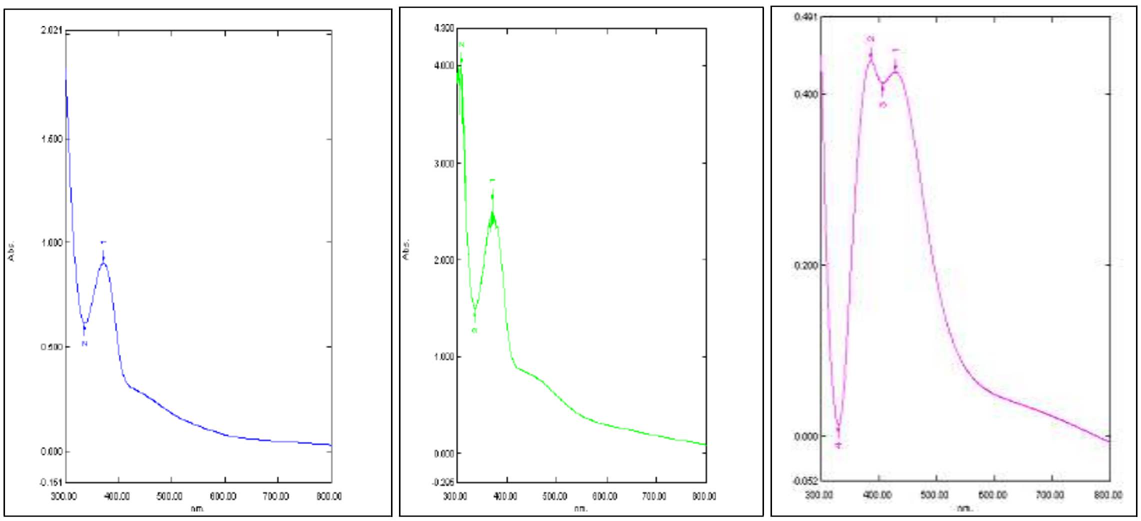

Figure 4: UV-Vis Spectra of Silver Nanoparticles at the Following Concentration of Peels Extract (A) 3% (B) 5% (C) 1%

We observe there is highest peak showing sign for the synthesis of silver nanoparticles the surface Plasmon resonance of silver occur at 430nm (1%), 373nm (3%), 379nm (5%) **(Table.2)** is clearly visible and is attributed to electronic excitation in tryptophan and tyrosine residues in proteins. This indicates the release of extracellular proteins in the colloidal solution and their possible mechanism in bioreduction process.

The reduction of green synthesized silver nanoparticles during exposure to the plant extracts could be followed by color change. Silver nanoparticles exhibit dark colour in aqueous solution due to the surface Plasmon resonance absorption band, due to the combined vibration of electrons of metals nanoparticles in resonance with light wave. The sharp bands of silver colloids were observed best peaks. These UV-Visible spectroscopy characteristics color variation is due to the excitation of the surface Plasmon resonance in the synthesized nanoparticles. The mechanism behind the activity of nano silver on bacteria are not yet fully elucidated the most common mechanism of toxicity proposed up to now are: (a) Direct damage to cell membrane (b) Formation of Reactive Oxygen Species (ROS) (c) Uptake to free silver ions followed by disruption of ATP production and DNA replication. The bactericidal activity of nanoparticles depends on the stability in the cultured medium too.

Table 2: UV-Vis Spectroscopy of the Synthesized Nanoparticles at 1%, 3% And 5% Concemtration of Aqueous Extract from Peels of Punica Granatum

S.No.	Wavelength (5%)	Observation	Wavelength (3%)	Observation	Wavelength (1%)	Observation
1	379nm	2.186	373nm	0.902	430nm	0.426
2	315nm	4.000	336nm	0.613	386nm	0.440
3	339nm	1.486			406nm	0.413
4					351nm	0.014

Antibacterial assay: Antibacterial activity of the green synthesized silver nanoparticles was examined against selected bacterial strains was investigated and represented in **(Table. 3 and Fig 5 & 5a).** It reveals that the green synthesized silver nanoparticles antibacterial activity against Staphylococcus aureus (MTCC-7443), Escherichia coli (MTCC-40), Proteus vulgaris (MTCC-*1771) showed the maximum inhibition of bacterial zone. Silver nnoparticles exerted highest toxicity against Proteus vulgaris and lowest effect on Staphylococcus aureus (1%, 3%, 5% green synthesized AgNPs). Silver nanoparticles green synthesized at 5% concentration were found better on all the bacteria tested followed by 3%, 1%.

The maximum toxicity was observed in silver nanoparticles synthesized from 3% and 5% of leaf extract. The reason could be that the smaller size of the particles which leads to increased membrane permeability and cell destruction. Our results are in agreement with those of found in Cassia auriculata. Finally, these reports suggest that silver nanoparticles synthesized from even green plant sources. Some report shows that many microorganism-like algae, bacteria, and fungi.

Table 3: Antibacterial Activity of Silver Nanoparticles Synthesized Using Punica Granatum Peels Extract

S.No.	Bacterial name	Zone of inhibition			
		Silver nanoparticles (100µg/ml)			Streptomycine sulphate (Standard Antibiotics) (100µg/ml)
		1%	3%	5%	
1	Staphylococcus aureus	22mm	23mm	26mm	35mm
2	Escherichia coli	30mm	31mm	33mm	30mm
3	Proteus vulgaris	35mm	39mm	43mm	45mm

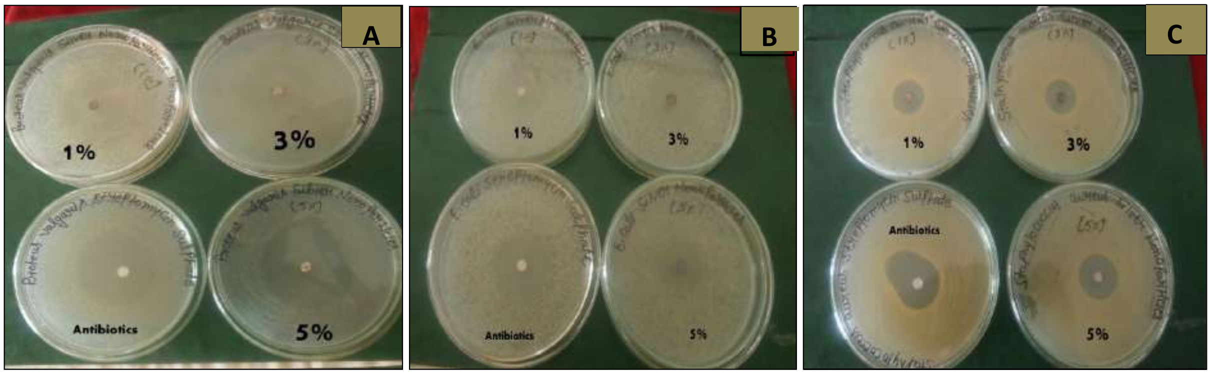

Figure 5: Antibacterial Activity of Silver Nanoparticles (1) 1%, (2) 3%, (3) 5%, (4) Antiboitic (A) Proteus Vulgaris (B) Escherichia Coli (C) Staphylococcus Aureus.

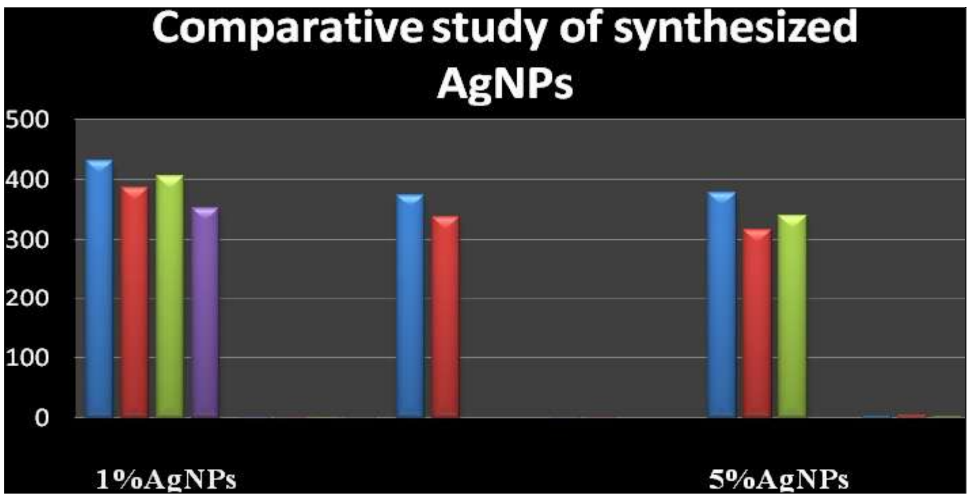

Figure 5 A: Graphical Representation of Synthesized Silver Nanoparticles at the Following Concentration of Peels Aqueous Extract

SEM Analysis: Scanning electron microscopy image has shown individual silver nanoparticles as well as a number of aggregates. The surface deposited silver nanoparticles are clearly seen at high magnification (X 10,000) in the micrograph. The morphology of the silver nanoparticles was equally spherical (1%) with interparticles distance, spherical shape (3%) and predominately spherical and aggregated in to smaller irregular (5%) shape with no well-defined morphology observed in the micrograph. It was clear from the SEM pictures that control silver nanoparticles were more than 1000nm size, whereas silver nanoparticles in the bioreduced colloidal suspensions measured 100- 200nm (1%), 50-200nm (3%) and 50-100nm (5%) in size (Fig.6) is the SEM of bioreduced silver nitrate.

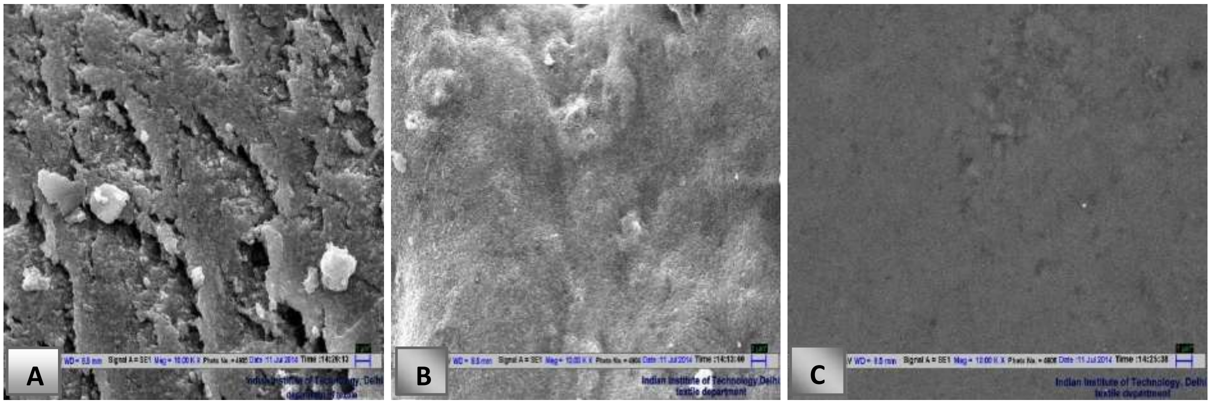

Fig. 6: Sem Anlaysis Image of Silver Nanoparticles Formed by Punica Granatum Leaves Aqueous Extract (A) 1%, (B) 3% And (C) 5%.

The silver nanoparticles exhibit a high antibacterial effect due to their well-developed surface which provides tha maximum contact with the environment. Furthermore, toxicity is presumed to be size and shape dependent because small size of nanoparticles may pass through cell membranes. Insides a bacterium, nanoparticles can interact with DNA, thus losing its ability to replicate which may lead to the cell death. Green synthesized nanoparticles also have the more effective antimicrobial zone inhibition of the pathogens.

The main problem is that bacteria have developed resistance towards many antibacterial agents. The silver nanoparticles produced here shows significant inhibitory activity against E. coli (MTCC-40), Proteus vulgaris (MTCC-*1771) and Staphylococcus aureus (MTCC-7443).

Opportunistic bacterial infections are frightening. The expanded range of bacterial pathogens includes opportunistic diseases. Silver nanoparticles may have significant antibacterial action via affecting cell membrane integrity. This study shows silver nanoparticles have significant antibacterial action, warranting additional chemical application research.

Conclusion: The aqueous extract of Punica granatum peels contained the majority of physiologically active phytochemicals. The aqueous extract, including a greater number of elements, may be deemed advantageous for future analysis. We have synthesized nanoparticles using an extract from Punica granatum peels, which serve as an exceptional reducing agent. The principal confirmation for the silver nanoparticles was seen by color shifts and UV- Visible spectroscopy of silver nanoparticles exhibited the largest peaks at 430 nm (1%), 373 nm (3%), and 379 nm (5%). The green produced nanoparticles have enhanced antibacterial efficacy against microorganisms. Synthesized nanoparticles were evaluated against bacterial strains Staphylococcus aureus, E. coli, and Proteus vulgaris, demonstrating that silver nanoparticles have a heightened antibacterial activity with increasing concentration. The green production of nanoparticles may be environmentally friendly and is applicable in many healthcare electronics and other fields.

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