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Bio-Based Synthesis of *Salvadora Persica* Stem Extracts Silver Nanoparticles and Investigation of in Vitro Antibacterial Activity

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Abstract: This study presents a novel and green approach for the synthesis and stabilisation of silver nanoparticles (M_AgNPs) using a water-based stem extract of Salvadora persica. As evidenced by the development of a brownish-yellow colour, the biosynthesis of green synthesised M_AgNPs was further investigated using UV-Visible spectroscopy and ATR analysis (attenuated total reflectance). M_AgNPs were discovered to be spherical and 52 ±2 nm in size after SEM and DLS investigation. Zeta potential of -31mV supported M_AgNPs' stability. Additionally, they showed inhibitory zones against S. aureus and E. coli of 12.5 mm and 13.5 mm, respectively. Additionally, it was found that the MIC of M_AgNPs for S. aureus (MTCC 25923) and E. coli (MTCC 25922) were 6.0 g/ml and 6.7 g/ml, respectively. This indicates that the plant mediated M_AgNPs depict positive response for antibacterial activity. Therefore, these may be developed as novel medicines for the treatment of bacterial infections, particularly those that are multi-drug resistant.

Keywords: Antibacterial activity, Green synthesis, M_AgNPs, novel therapeutics, silver nanoparticles, Salvadora persica.

Introduction

Nanotechnology has revolutionized diverse areas of the healthcare sector through the deployment of effective drug delivery systems with minimal side-effects. Nanostructures ranging from 1 to 100 nm in diameter are generated, manipulated, and imaged using nanotechnology[1–3]. Numerous sectors, including food packaging, livestock farming, electronics, agriculture, medicine, and health care, have benefited

from the development of nanotechnology[4-12]. Due to atomic interactions that take place on their surfaces, nanoparticles (NPs) have unique features compared to bulk materials, leading to improved characteristics and less coordination. Metals or non-metals can be used to make nanoparticles, depending on how they are produced. The primary components of metallic nanoparticles include semiconducting and magnetic (cobalt, nickel) materials as opposed to non-metallic nanoparticles that are generally prepared of materials with a carbon basis. Metallic nanoparticles have garnered attention owing to their unique electrical, optical, and catalytic capabilities[13–15]. They have shown promising applications in the areas of cancer therapeutics, radiation therapy, antibacterial & antifungal agents, and gene and drug delivery[16]. However, the chemical synthesis of nanoparticles generate toxic by-products. To address this issue, "biosynthesis" has emerged as an eco-friendly approach. It includes the use of plants & micro-organisms to produce low-cost, stable and durable metal nanoparticles.

Small evergreen *Salvadora persica* trees, also known as toothbrush trees, are indigenous to India, Africa and the Middle East. Traditionally, its sticks are called "miswak" and it's commonly used for dental cleaning. Almost all parts of *Salvadora persica* have useful medicinal properties including its stem, roots and leaves. Stem displays anti-caries, antiplaque, anticonvulsant and antispasmodial effects. It is also used for gastric troubles and is pharmaceutically important[17-23].

In this paper, we have used the stem extract from *Salvadora persica* for the bio fabrication of M_AgNPs. Silver particles were chosen as they have remarkable antimicrobial properties. Using UV-Vis, ATR, and zeta potential, the physicochemical characteristics of the green fabricated nanoparticles were characterised. In order to ascertain the antibacterial capabilities of M_AgNPs, both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria were utilised.

Material and Methods

Plant material collection

Fresh stem of *Salvadora persica,* were gathered from Kupwara District, Jammu and Kashmir, India. Fresh water was used to wash the stems three times, which were then dried for a week at room temperature in the shade. The dried stem of *Salvadora persica* was crushed using an electric grinder into a fine powder. The powder obtained was subsequently dried once more and stored in an airtight container.

Preparation of plant extracts

10 g of *S. persica* stem powder was boiled in 100 ml of distilled water at 100°C for 1h in a water bath. Centrifugation was performed at 10,000 rpm for 15 min once the solution cooled. Supernatant was collected and passed through a 0.2µM cellulose nitrate membrane filter to acquire a clear solution. The solution was stored in refrigerated conditions (4°C).

Synthesis of M_AgNPs

Preparation of M_AgNPs was done using 9 ml of 0.01M silver nitrate concentration and 1 ml plant extract. The incubation time for the creation of NPs was 20 min. The temperature for the formation was 30°C to achieve maximum yield of NPs. The transformation of the silver nitrate and stem extract solution from yellow to brown was observed as an initial hint of synthesis.

Characterization of M_AgNPs

Using a 1900i Shimadzu spectrophotometer, UV-vis spectrum with a range of 300 to 800 nm was collected to verify the synthesis of M_AgNPs. Additionally, functional group determination made use of a JASCO FT-IR 4100 spectrometer in diffuse reflectance mode with a resolution of 4 cm^{-1}. A German Carl Zeiss Supra 55 microscope was utilized for SEM analysis in order to decipher the morphology and size of the M_AgNPs. For assessing the stability of the M_AgNPs, the zeta potential values were calculated using Malvern Panalytical apparatus.

Antimicrobial activity

The antimicrobial potential of the synthesized AgNPs was checked against *Escherichia coli* (MTCC 25922), and *Staphylococcus aureus* (MTCC 25923) [procured from IMTECH, Chandigarh] using agar well plate diffusion assay. In brief, the above mentioned bacterial cultures were activated in Mueller Hinton broth tubes and 10^8 CFU/ml of *S. aureus* culture and 10^6 CFU/ml of *E. coli* were spread on Mueller Hinton agar plates. Wells with roughly 8 mm diameter were created. Each well (50 µl) was filled with varying doses of AgNPs (15-75 g/ml), and control wells with just the *S. persica* aqueous extract without M_AgNPs were also included. The cultures were kept at 37°C for the following day. In order to validate the results, three replicates of the experiments were run, and the inhibitory zones were measured.

Determination of MIC

To establish a new bacterial culture, a freshly grown bacterial cells was kept in 5 ml of sterilized broth for 6 hours at 37 °C. Two test tubes were kept as controls and 0.9 ml of normal saline (0.85% NaCl) was poured in nine tubes. 0.1 ml was taken from the freshly grown culture and poured in the first tube containing saline solution ($1x10^9$ CFU/ml). The final seven test tubes were serially diluted. Each tube received 10 ml of M_AgNPs, and each tube underwent an overnight incubation at 37°C. A control tube containing *S. persica* aqueous extract without M_AgNPs was also kept to see if aqueous extract alone could kill bacteria. On the plates, a drop was added from each tube. Colony growth on the plates was monitored for 18 to 24 hours while they were incubated at 37 °C.

Results and Discussion

Synthesis of silver nanoparticle was achieved with 0.01M silver nitrate and 10% of plant extracts of *S. persica* at pH 8, 30ºC (as depicted in Fig. 1a-c). In this study, UV–Vis spectroscopy (400-800 nm) was used for the analysis of the M_AgNPs. The absorption maxima of M_AgNPs was obtained at 425 nm (Fig. 1d). Previous studies describing synthesis of M_AgNPs using various plant extracts highlighted that M_AgNPs shows maximum reading around 400 nm[24, 25]. Hence, the current synthesis of M_AgNPs is consistent with the existing reports. M_AgNPs synthesized using *Piper chaba* stem extracts have been found to exhibit absorption maxima around 445 nm[26]. Similarly, AgNPs synthesized using *Piper chaudocanum* stem extracts exhibited absorption maxima at 400 nm[27]. Likewise, M_AgNPs synthesized using *Clinacanthus nutans* aqueous extracts from leaf and stem have been observed to exhibit absorption maxima around 450 nm[28]. The ATR data of the M_AgNPs was recorded to detect functional groups present on the nanoparticles. The existence of proteins as a capping agent for M_AgNPs stabilizes the synthesised nanoparticles as depicted by the transmission peak at 1637.50 cm-1. The occurrence of this peak could be attributed to the C H O stretching mode in the amine group, which is frequently seen in proteins (Fig. 2a). Furthermore, the peak at 3330.99 cm-1 was due to OH

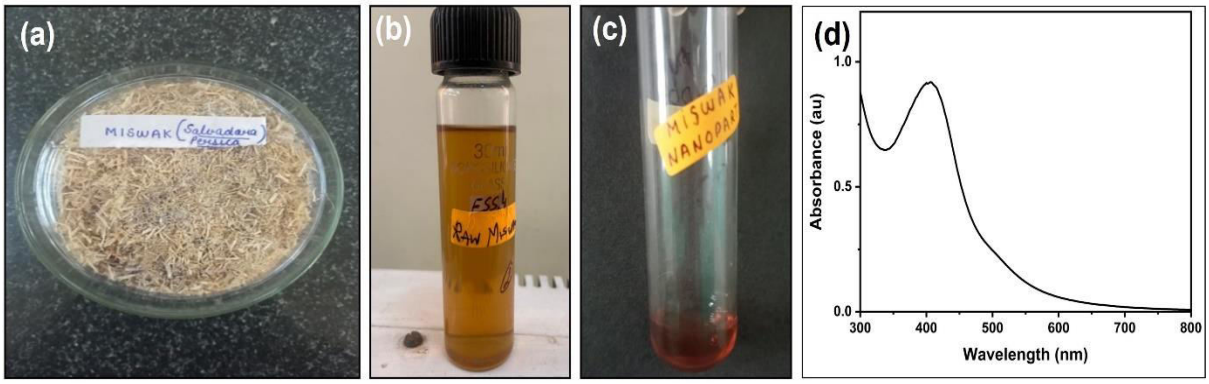

Fig. 1: (a) *Salvadora persica* grinded and dried stem, (b) *Salvadora persica* aqueous extract, (c) synthesized M_AgNPs, and (d) UV-vis spectrum of M_AgNPs.

group present in the plant metabolites and aqueous solution. The results of FTIR analysis are well supported by existing reports[26, 28]. SEM analysis of M_AgNPs established the spherical morphology of nanoparticles and the calculated average size of nanoparticles was 52 ± 2 nm (Fig. 2b). Biosynthesized M_AgNPs in the diameter range of 5-50 nm were reported to possess good antibacterial properties[29]. M_AgNPs synthesize using *Berberis vulgaris* leaf extract were also found to be spherical in morphology with size around 50 nm[24]. Zeta potential is a

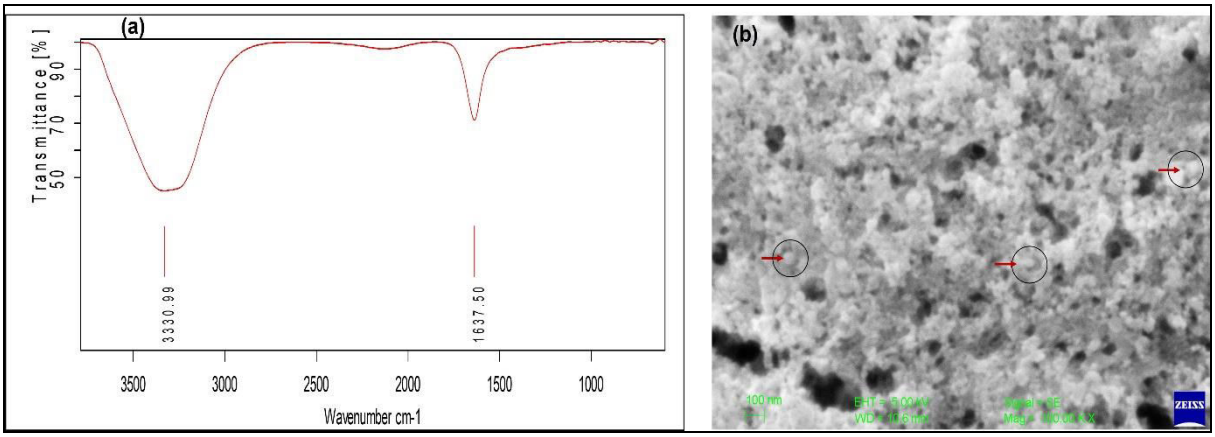

Fig. 2: (a) ATR spectrum of M_AgNPs and (b) SEM of M_AgNPs

fundamental parameter for characterizing the charge on the surface of nanoparticles, which determines their electrical potential. The magnitude and sign of the zeta potential is primarily affected by the composition of the particles and the medium in which they are suspended. Generally, zeta potential values > (+33) mV or < (-30) mV are an indication of the high stability of synthesized AgNPs. In the present study, the zeta potential of M_AgNPs was measured and found to be -31mV, which indicates that the particles are stable under the given conditions (Fig 3). This result suggests that M_AgNPs have a negative surface charge and are thus likely to repel each other, preventing agglomeration and improving their stability in the suspension medium. The results attained in this study are in accordance with previous documented data that has established an association between the zeta potential of M_AgNPs and their stability[24-32]. These studies have shown that nanoparticles with higher zeta potentials are more stable and less prone to agglomeration in the suspension medium. Therefore, the observed zeta potential of M_AgNPs (-31mV) in our study supports

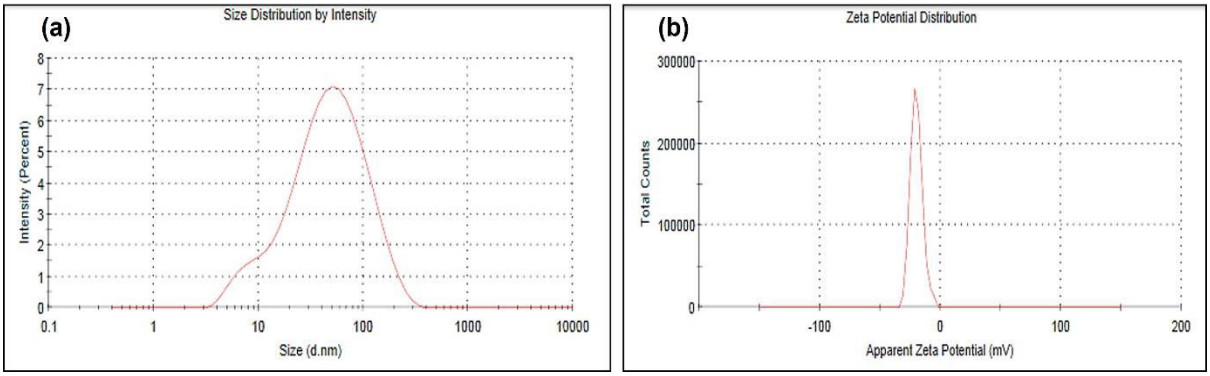

Fig. 3: (a) Particle size distribution of M_AgNPs, and (b) Zeta potential analysis of M_AgNPs

the notion that these particles are stable under given conditions. Antimicrobial effect of M_AgNPs was established using disc diffusion method at working concentration of $AgNO_3$ and *S. persica* stem extract and was compared with synthesized M_AgNPs. The results for the same have been mentioned in Fig.4. Kanamycin disc (30µg/disc) was used as a standard for antimicrobial analysis. The results highlighted that M_AgNPs exhibited synergistic antimicrobial effect on *E. coli* and *S. aureus*. M_AgNPs exhibited inhibition zones measuring 13.5 mm and 12.5 mm on plates growing *E. coli* and *S. aureus*, respectively (Fig.4a). The minimum inhibitory concentration of M_AgNPs against *E. coli* and *S. aureus* cultures were 6.0 µg/ml and 6.7 µg/ml, respectively (Fig.4b). Another study evaluated the susceptibility of M_AgNPs produced from the stem extract of *Zanthoxylum armatum* against four pathogenic microorganisms, namely *S. aureus*, *E. coli*, *P. aeruginosa* and *Salmonella* enteric, where it exhibited the strongest antimicrobial activity against *S. aureus*[33]. A comparison of the antibacterial activity of M_AgNPs synthesized using saffron extract versus commercially purchased nanoparticles against six pathogenic bacteria revealed no significant antibacterial effects of the saffron extract and purchased nanoparticles, whereas the biosynthesized nanoparticles were able to prevent the growth of the six bacteria studied[34]. Similarly, AgNPs synthesized using a green method with *Pedalium murex* leaf extract were capable of curbing the growth of bacteria such as *E. coli, Klebsiella pneumoniae, Mariniluteicoccus flavus, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus pumilus* and *S. aureus* [35].

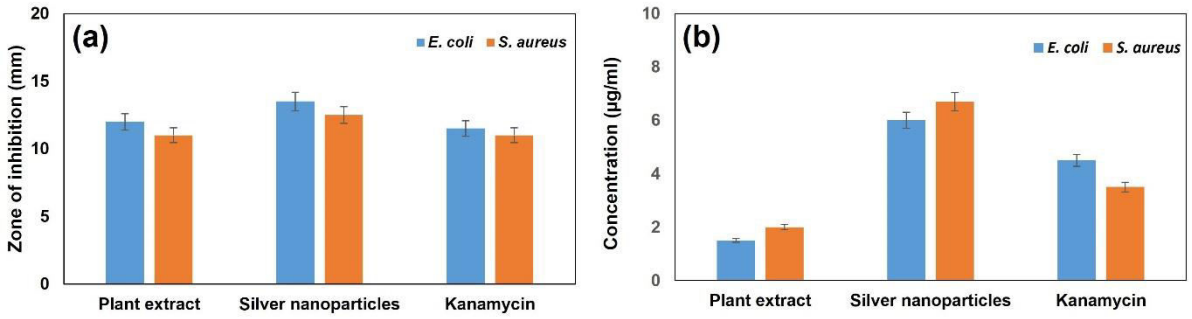

Fig. 4: (a) Zone of inhibition exhibited by M_AgNPs, plant extract and kanamycin against *E. coli* and *S. aureus* (b) MIC of M_AgNPs, plant extract and kanamycin against *E. coli* and *S. aureus*.

Conclusion

This research study utilized an aqueous extract of *Salvadora persica* (*S. persica)* stem to provide a unique and environmentally friendly method for the manufacture stabilization of silver nanoparticles (M_AgNPs), biofabrication and

evaluation of its antimicrobial activity. Overall, the results suggest that the production of M_AgNPs through biosynthesis is a simple, low-cost, and risk-free process that uses no harmful materials and has no negative side effects.

The current work used stem extract as a capping and reducing agent to create silver nanoparticles (M_AgNPs'). Because the stem extract contains a variety of phytochemicals and bioactive substances that are advantageous for biological and catalytic applications, the generation of non-toxic M_AgNPs' has been greatly enhanced. M_AgNPs' as synthesised were examined using scanning electron microscopy (SEM), and dynamic light scattering (DLS) to determine their structure, shape, elemental makeup, and size. SEM and DLS analysis revealed that M_AgNPs were spherical and 52 2 nm in size. M_AgNPs' stability was maintained by a zeta potential of -31mV. Additionally, they demonstrated 12.5 mm and 13.5 mm, respectively, inhibitory zones against *S. aureus* and *E. coli.*

Significant antibacterial activities were portrayed by the synthesised nanocrystals, and they showed promise for application as efficient antimicrobial agents to replace conventional antibiotics. To corroborate the present findings, more cytotoxicity analysis and *in vivo* research must be carried out. This suggests that the M_AgNPs produced by plants exhibit favourable antibacterial action. The development of these as new therapeutics for the treatment of bacterial infections, especially those that are multi-drug resistant, is therefore possible.

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