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Green synthesis of silver nanoparticles using *Allium cepa* extract and their antimicrobial activity evaluation

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Nanoparticles synthesis route is one important aspect for practical application and easy, eco-friendly, non-toxic and low-cost methods are preferred in this regard. In this study, we described the fabrication of silver nanoparticles utilizing *Allium cepa* (Onion) extract, which were characterized by SEM, EDX, and XRD technique. The biosynthesized Ag NPs was face-centered, spherical in shape, and particle size was in nanometer range of 30-100 nm (with average size of 84.8 nm), which were in aggregated form. The EDX analysis revealed the formation of highly pure form of the silver nanoparticles. The antibacterial activity was assessed against *E. coli* and *S. aureus* microbes and results revealed that the silver nanoparticles are highly active against the selected microbes and silver nanoparticles have potential for application in biomedical field.

Capsule Summary: Silver nanoparticles were prepared by green route using *Allium cepa* extract. The Ag NPs are in pure form and highly active against *E. coli* and *S. aureus* microbes.

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INTRODUCTION

Nanoscience is the study of materials with diameters ranging from 1 to 100 nm. This is an area of materials science research that is quickly expanding. Metal nanoparticles are widely used in pharmaceutical and nanotechnology research. To cover textiles and store food, natural ingredients are employed (Arya et al., 2019; Das et al., 2019; de Jesus Oliveira et al., 2019; Girón-Vázquez et al., 2019; Raj et al., 2018; Rolim et al., 2019).

The use of silver nanoparticles (Ag NPs) is becoming increasingly common. Among the applications are solar energy absorption, storage batteries, optical receptors, catalysis, bio-labelling, and food preparation. The Ag NPs are biocompatible and have a wide range of possible applications, including cancer therapy and diagnosis, as well as healthcare, pharmaceutical delivery, cosmetics, and food and feed. The Ag NPs contain Ag ions, which interfere with DNA replication, increase membrane permeability, and cause cell content leakage (de Souza et al., 2019; Hemmati et al., 2019; Michna et al., 2019; Otari et al., 2019; Shukla and Iravani, 2018; Soto et al., 2019).

There are several methods for producing nanoparticles, the majority of which are excessively expensive, require a lot of energy, or are toxic to humans and the environment. Natural antioxidants are used in place of conventional reducing agents in green synthesis. Green

chemistry concepts underpin these "green synthesis" techniques. Microorganisms or biological products such as fruit, leaf, and seed extracts are used in green synthesis. This method was eco-friendly, one-step, cost-effective, and non-toxic. It substitutes more efficient and eco-friendly alternatives for all commodities that are harmful to the environment or human health (Alsammarraie et al., 2018; Rivera-Rangel et al., 2018; Vedelago et al., 2018).

Using contemporary procedures, Ag NPs and particles were synthesized from *G. pectinata, M. officinalis, A. reticulate, F. benjamina,* and *D. viscosa* etc leaf extracts. Chlorophyll is the primary component of a leaf; it creates food through photosynthesis and also gives plants their green color to absorb light energy. Additionally, leaf includes organic acids, nitrogen, carbon, sugars, and minerals, in addition to organic molecules such as carbon, carbon monoxide, nitrous oxide, potassium, magnesium, calcium, and zinc. The produced Ag NPs exhibit a variety of morphologies, including worm-like, irregular flower, spherical, and dendritic, with a range of diameters, when dissolved in methanol, acetone, acetonitrile, and water. These conclusions are drawn utilizing a variety of characterization techniques, including UV-Vis, XRD, SEM, TEM, and FT-IR (Anandan et al., 2019; de Jesús Ruíz-Baltazar et al., 2017; Femi-Adepoju et al., 2019; Parthiban et al., 2019; Puente et al., 2019).

The Ag NPs were crystalline and spherical in shape, with diameters ranging from 3-14 nm for grape extract nanoparticles and 5-50 nm for orange extract nanoparticles. To produce a green, simple, and low-cost technique, we employed wolfberry fruit extract in an aqueous medium. Another study used R. glaucus fruit to create spherical agar nanoparticles (Dong et al., 2017; Kumar et al., 2017; Soto et al., 2019). A new method has been established that employs seed extract of *C. sativum* and *I. verum* seeds at 25 °C to synthesis Ag NP. The typical particle size of Ag NP was 15-30 nm, while shape was spherical with size of 50 nm respectively. The development of Ag NPs was recognized by instrumental examination comprising of UV-Vis, FTIR, SEM, TEM and particle size analysis (Luna et al., 2015; Nazeruddin et al., 2014). The Ag NPs were produced from Coriandrum sativum seeds using an ecologically safe and degradable approach. They were found to be efficient against E. coli and L. monocytogenes. Correspondingly, Parkia speciosa Hassk was used to create the Ag NPs. These nanoparticles have more antibacterial activity than other extracts and have no harmful side effects (Fatimah, 2016; Nazeruddin et al., 2014).

Based on aforementioned facts, present study was aimed to prepare silver nanoparticles (Ag NPs) by a green approach using *Allium cepa* as a stabilizing agent, capping, and reducing agent. XRD, SEM, and EDX techniques were for characterization of Ag NPs and antibacterial activity was evaluated against *E. coli* and *S. aureus* microbial strains.

MATERIAL AND METHODS

Chemical and reagents

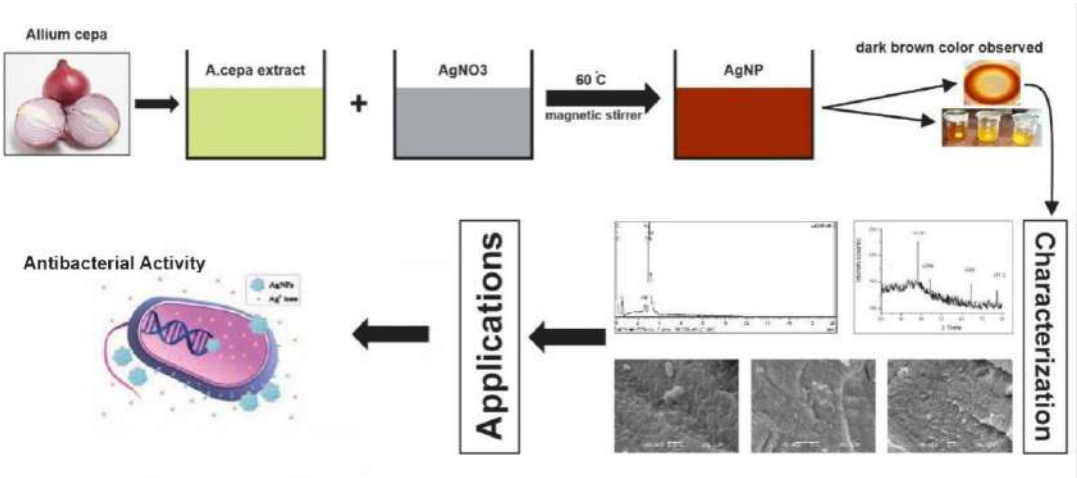
The chemicals used during the synthesis of silver nanoparticles (Ag NP) are analytical graded. Fresh *Allium Cepa* was purchased from local market of Sialkot, Silver nitrate of 0.1M (AgNO3). The sodium hydroxide (NaOH), distilled water and ethanol all were purchased from Sigma Aldrich.

Preparation of extract

A 100 g of *Allium Cepa* was taken and chopped into thin pieces and dipped into the distilled water in a flask of 200 ml at ambient temperature along with continues stirring, which was then filtered after 24 h. The obtained extract of *Allium Cepa* in the form of filtrate was used for Ag NPs preparation.

Preparation of nanoparticles

For the preparation of silver nanoparticles, we use extract of *Allium Cepa* that act as a reducing as well as stabilizing agent. The extract was mixed by heating at 60 °Cwith 50 ml of 0.1 M



Scheme. 1: Schematic presentation for Ag NPs preparation and application

concentration of silver nitrate into a flask of 100 ml, this process takes place in the presence of sunlight which act as a catalyst. The conformation of silver nanoparticles is done by the appearance of dark brown color of the solution after sometime. The solution is then centrifuge at 4000 rpm for 15 minutes. The obtained silver nanoparticles are washed with distilled water and further with ethanol to remove any impurities. After washing, drying of nanoparticles was done at 60°Cand stored for further analysis (Scheme 1).

Characterization of nanoparticles

The final dried form of prepared Ag NP from the extract of Allium cepa are used for advanced characterization techniques including Scanning Electron Microscope (SEM) for morphological analysis by employing Nova Nano SEM 450 at 10,000x, and Xray-Diffraction (XRD) was carried out on X-ray diffraction device with sort D8, Bruker, Germany at 298K with range 2θ = 20 to 80 for crystal structural analysis also Energy Dispersive Xray (EDX) for identification, conformation and distribution of nanoparticles.

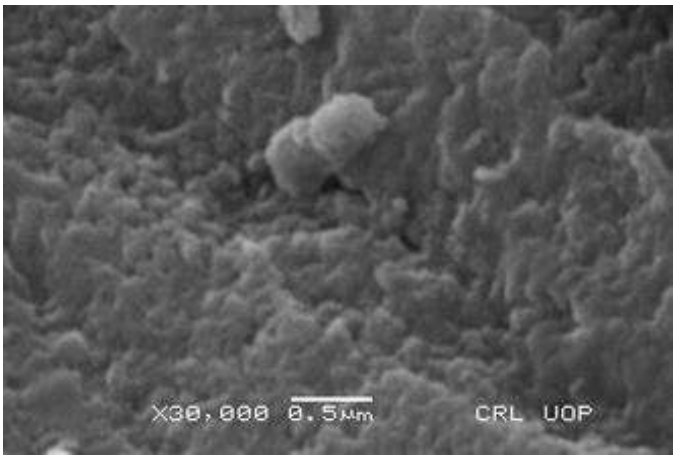


Fig. 1: SEM analysis of the Ag NPs prepared using *Allium cepa* extract

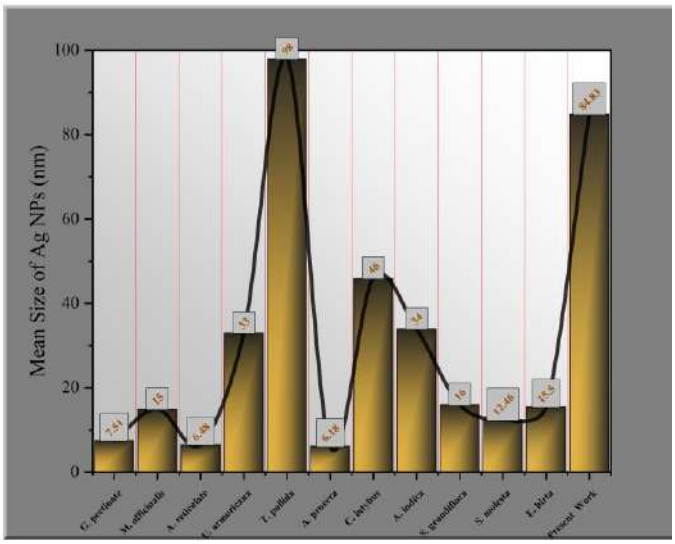


Fig. 2: Comparison of Ag NPs size (nm): Present study versus previous reports

Antibacterial activity analysis

For assessing the anti-bacterial activity of Ag NPs from A. cepa extract, we employed bacterial strains *E. coli* and *S. aureus*. To establish fresh cultures of bacterial and fungal growth, we used standard growth media. To avoid contamination, bacterial (nutrient broth, Oxoid, UK) and fungal growth (potato dextrose broth, Oxoid, UK) were grown in a well sterilized environment. All tested cultures were stored at 4 °C prior to use, and the following number (1 x 10^8 CFU/mL) of cell cultures were used in disc diffusion experiments. To avoid contamination, fresh growth media was prepared and autoclaved for 15 minutes at a standard temperature of 121°C. Wicks paper discs with a diameter of 9 mm was prepared and sterilized. 20 mL of autoclave medium (nutrient agar) was poured into each petri plate and mixed well to homogenize before waiting for the media to harden. Each sterilized disc contained 100 µL of the antibiotic Rifampicin and Ag NPs (different concentrations) were put flat on the growth medium with the use of forceps discs. After that, the Petri plates were kept at 37 °C for 24 h and zone of inhibition was measured using zone reader in mm.

RESULTS AND DISCUSSION

Surface morphology and elemental composition

The surface morphology and elemental analysis determined by the Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX), which confirmed the formation and purity of the prepared samples of Ag NPs. SEM gave the evidence of surface characteristics of NPs. SEM results are shown in Figure 1, the Ag NPs are spherical in shape with size range 30-100 nm with an average size 84.83 nm. Also, the NPs are in agglomerated form due to interaction with biomolecules and having uniform distribution. Different mean sizes are shown in Figure 2 for present study and previous studies reporting green route for Ag NPs synthesis (Ajitha et al., 2016; Anandan et al., 2019; Gomathi et al., 2017; Kumar et al., 2014; Kumar et al., 2016).

Energy dispersive X-ray spectroscopy was used to investigate the elemental composition and stoichiometry of the produced silver nanostructures in Figure 3. The Ag NPs produced a distinctive silver signal about 3 keV, which is characteristic for metallic silver absorption due to surface plasmon resonance (SPR). In addition, O peaks were observed as a result of biomolecules sticking to the surface of Ag NPs. The carbon peak in the spectrum was caused mostly by the carbon tape used in the spectrum analysis. It has been discovered that nanoparticles made from plant extracts are encased in a thin coating of some capping organic component derived from the plant extract, which

results in the formation of morphologically distinct nanoparticles. It can be seen that major XRD peak produced for silver is observed as the (111) plane, the development process is more practicable in the (111) direction than in others.

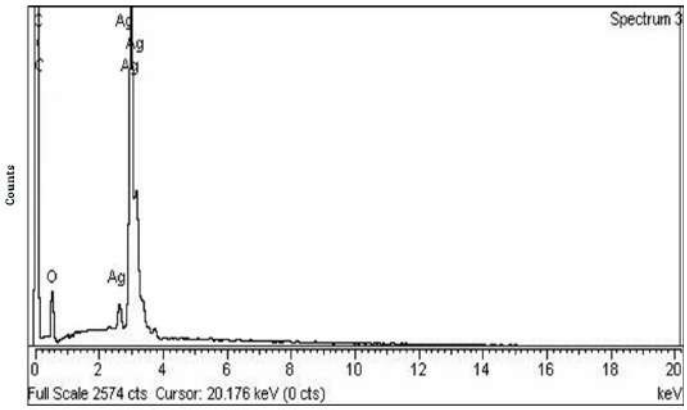


Fig. 3: EDX analysis of Ag NPs prepared using *Allium cepa* extract

Structural analysis

The XRD analysis was employed to evaluate the size and crystal structure of Ag NPs (Fig. 4). This shows that the Ag NPs have four distinct diffraction peaks at 2θ = 38.21°, 43.23°, 64.66°, and 77.32°, indicating that the metallic silver crystal lattice is a face-centered cube with hkl values (111), (200), (220), and (311), which match the standard values provided by the Joint Committee on Powdered Diffraction Standards (JCPDS) file no. 893722 In addition to these prominent peaks, the XRD pattern revealed a number of smaller peaks. These tiny peaks are caused by organic compounds found in herbal extracts, which are responsible for the reduction of silver ions and the stability of the resulting nanoparticles (Verma et al., 2016). The size of particles can easily be found using Eq. 1 (where D = crystalline size, β = broadening of peak, λ = wavelength of X-ray in 0.15406 nm, k = constant (0.89) and θ is the diffraction angle.

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

Antibacterial activity

For assessing the antibacterial activity of Ag NPs prepared using *A. cepa* extract, we employed bacterial strains *E. coli*, and *S. aureus* and responses thus overseed are shown in Fig. 4. The Ag NPs prepared using *A. cepa* extract were tested at different concentration against *E. coli*, and *S. aureus* strains. The Ag NPs showed promising antibacterial activity and the zones of inhibition were in 12-20 mm range for *S. aureus* and in 14-22 mm range for *E. coli.* The zone of inhibition against Rifampicin (positive control) were recorded to be 32 and 30 (mm) for in case of and *S. aureus* and *E. coli*, respectively.

These findings revealed that the Ag NPs prepared via green route using *A. cepa* extract are highly active antibacterial agents and could be used for biomedical application as an antibacterial agent. All of the results are also consistent with previous research indicating that Ag NPs synthesized via a green method is a potential antibacterial agent, i.e., the prepared Ag NPs with *Sesbania grandiflora* plant extract showing highest antibacterial activity against Pseudomonas spp. and better antifungal activity against *Penicillium spp.,* (Ajitha et al., 2016). In addition, leaf extracts of *Dodonaea viscosa* were treated with methanol, acetone, acetonitrile, and water to produce Ag NPs with worm-like, irregular floral, spherical shaped nanoparticles. When compared to other extracts, Ag NPs generated using methanol extract displayed greater antibacterial activity in terms of zone of inhibition against the test bacterium Streptococcus pyogenes (Anandan et al., 2019). In another study, it was found that Ag NPs isolated from green and black tea leaves had antibacterial activity with a zone of inhibition of 19–21 mm against *Staphylococcus aureus* (Asghar et al., 2018). Furthermore, using a disk diffusion test, simple and green fabrication of Ag NPs with spherical shapes and sizes values of up to 70 nm using *Berberis vulgaris* leaf and root extract was tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* bacteria, yielding successful results with MICs of 1.3 and 5 mM (Behravan et al., 2019). Besides, using methanolic fruit extract of *Aegle marmelos* to make ecofriendly Ag NPs had the strongest inhibitory effect against *B. cereus*, *P. aeruginosa* and *E. coli* (Devi et al., 2020). Withal, the synthesized Ag NPs had a well-defined spherical morphology with an average crystallite size of 18 nm generated from *Datura stramonium* leaf extract and

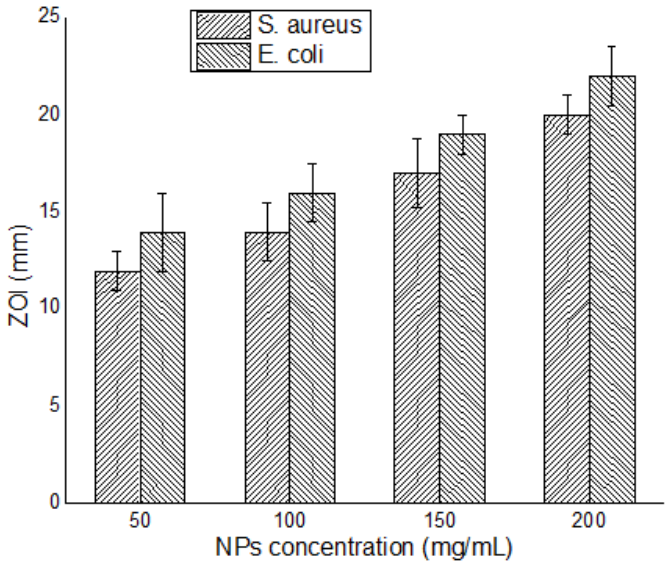


Fig. 4: Antibacterial activity (zone of inhibition, mm) of Ag NPs against bacterial strains

demonstrated antibacterial activity against *E. coli* and *S. aureus* bacteria. However, there was a lot of activity against *E. coli* (Gomathi et al., 2017). The Ag NPs were tested against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Flavobacterium branchiophilum* for antibacterial activity, with *F. branchiophilum* demonstrating the highest sensitivity when compared to the other two bacterial pathogens (Vijay Kumar et al., 2014).

CONCLUSIONS

The Ag NPs were synthesized successfully using *Allium cepa* extract as a reducing, capping, and stabilizing agent. The Ag NPs were studied using XRD, SEM, and EDX techniques The biosynthesized Ag NPs was face-centered, spherical in shape, with average size of 84.8 nm and in aggregated form. The antimicrobial activity was evaluated against *S. aureus* and *E. coli* microbial strains and their response was highly promising, which revealed that Ag NPs prepared via green route using *Allium cepa* have potential for biomedical applications. Future studies will be extended on cell lines to check their biocompatibility.

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