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Research Article

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Green Synthesis and Characterization of *Urtica dioica* Mediated Silver Nanoparticles for Antibacterial Application

Al-Shehri HS*

Abstract

Green synthesis of Ag-NPs (silver nanoparticles) has revolutionized the area of nanotechnology consistently and bio-based silver nanoparticles have emerged as efficient therapeutic tool in the field of biomedical science. The nanoparticles were synthesized via green route approach by *Urtica dioica* leaf extract. The biosynthesized Ag Nanoparticles (NPs) were analyzed by Powder XRD, FTIR, SEM with EDX, UV-vis and HRTEM analysis. The antimicrobial studies of the synthesized Ag-NPs were tested against *Bacillus subtilis*, and *Staphylococcus aureus* (gram-positive) and *Psuedomonas aeruginosa*, and *Shigella dysenteriae* (gram-negative) bacterial pathogens using the disc diffusion method. The prepared nanoparticles demonstrated strong antimicrobial activity against all microbial strains examined with varying concentrations.

Keywords

Green synthesis; Ag-NPs; TEM; Bactericidal activity

Introduction

The field of nanobiotechnology and nanomedicine has opened the path for use of green-synthesized nanoparticles. In biomedical applications, palladium nanoparticles (Pd-NPs), silver nanoparticles (Ag-NPs) and gold nanoparticles (Au-NPs) are mostly used among other nanoparticles [1].

Nanoparticles can be engineered to desired dimensions and shape depending on their applications in optical, electronics, biological fields [2]. Nanoparticles have gained a wide area of interest because of their surface properties which differ from the bulk material [3]. Diverse types of physical and chemical methods have been employed for the preparation of the nano-materials [4]. All these methods have some disadvantages like the use of toxic chemicals and environmental issues. So an eco-friendly approach called green synthesis is currently being used in order to nullify the toxic effect of the synthesis process [5]. The green synthesis method has advantages like non-toxicity, low cost, eco-friendly and biocompatibility. This approach employs extra/intra cellular matrix of biological entities (bacteria, fungi, algae, other microorganisms and plants) as reducing and capping agents [6].

Metal NPs have attracted research community because of their exceptional and tuneable physical and chemical properties suitable for different biological applications such as drug carrier, bio-imaging probe and antimicrobial agents [7]. Silver nanoparticles are also known for their excellent antimicrobial activity over a wide spectrum of microbes and are being used in cosmetics [8]. Silver exerts its antimicrobial activity by adhering to the bacterial cell membrane and disturbing the cellular respiration [9]. It is recognized as safe and nontoxic but precautions have to be taken in terms of the dosages of silver nanoparticles and cost-effectiveness [10].

Urtica dioica (nettle plant) has been a staple in herbal medicine since ancient times. The nettle plants edible portion is enriched with variety of phytochemicals with tremendous antioxidant potential. These plants consist of numerous phytochemicals (glycosides, glucosinates, polyphenols, sterol, terpenoids, phenolic acids, alkaloids, flavonoids and carotenoids). The strong antioxidant effect of these plants occur via reduction and chelation of metal ions, free radical scavenging action, reduction of lipid and positive interaction with the antioxidant enzymes [11].

Here we repoprt the fabrication of Ag-NPs through environment friendly method using *U. dioica* leaf extract. Prepared nanomaterials were characterized using PXRD, FTIR, SEM with EDX, UV-Visible and HR-TEM techniques. The inherent bactericidal activity of Ag-NPs (Ag^+ ions) have the synergistic effect against *B. subtilis,* and *S. aureus* (gram-positive) and *P. aeruginosa*, and *S. dysenteriae* (gram-negative) bacterial pathogens.

Experimental Section

Materials used

All chemicals used were of analytical grade. Silver nitrate [$AgNO_3$] was purchased from Sigma Aldrich.

Collection, processing and synthesis of *Urtica dioica* leaf extract

Fresh green leaves of *Urtica dioica* (thirty grams) were collected from a Tumkuru (devarayanadurga) forest, INDIA. The leaves were thoroughly rinsed with double-distilled water to get rid of dust particles and subsequently air-dried. For preparation of extract, the leaves were boiled for 10 min at 50°C -60°C in 250 mL of ultrapure water, sieved and stored (Figure 1) [12].

Preparation of silver nanoparticles (Green synthesis)

10 ml of *U. dioica* leaves extract were made to mix in 90 mL of 0.01 M $AgNO_3$ at ambient temperature. The reduction of silver NPs were clearly seen within thirty minutes. The *U. dioica* leaves extract reduced $AgNO_3$ into AgNPs, this resulted in brownish yellow solution, thus confirming the formation of Ag-NPs [13]. Gotten silver nanoparticles was purified by Remi Cooling Centrifuge (Figure 2) [14].

Nanoparticles characterization

Green synthesized silver nanoparticles characterized by X-ray diffractometer (Rigaku Smart Lab model). Nanoparticles FTIR spectra were scanned from 400 cm^{-1}-4000 cm^{-1} by BRUKER-ALPHA spectrometer. The Technologies-Cary 60 instrument used for UV-Vis

***Corresponding author:** Al-Shehri H.S., Chemistry Division, King Khalid, Military Academy, Riyad, Kingdom of Saudi Arabia, E-mail: alshehrih@kkma.edu.sai

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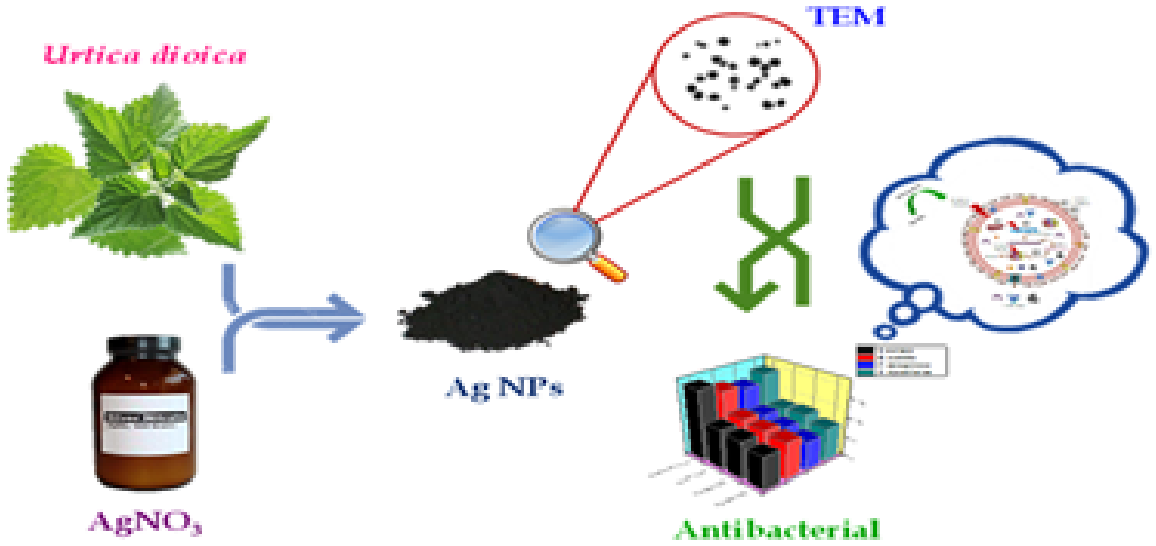

Figure 1: Graphical abstract of the process.

Figure 2: Schematic representation for the preparation of time of leaves extract for the synthesis of AgNPs.

measurements. The presence of silver and other elements in particles was confirmed by EDX (OXFORD XMX N) analysis. The structure and morphology were examined by scanning electron microscope (JEOL model JSM6390LV) and HR-TEM (JEOL/JEM 2100).

Antibacterial action

The disk diffusion method was allowed to study the antibacterial activity against gram positive and gram negative bacteria [15]. The

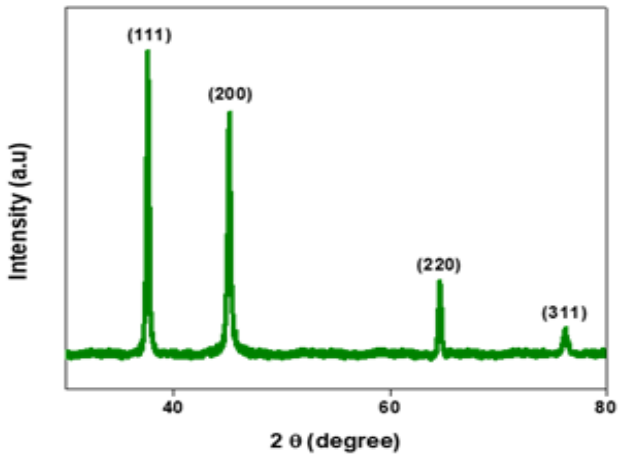

Figure 3: PXRD pattern of AgNPs.

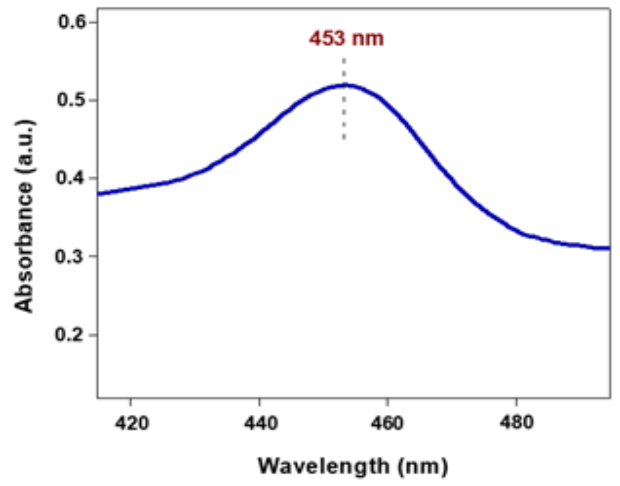

Figure 4: UV visible spectrum of AgNPs.

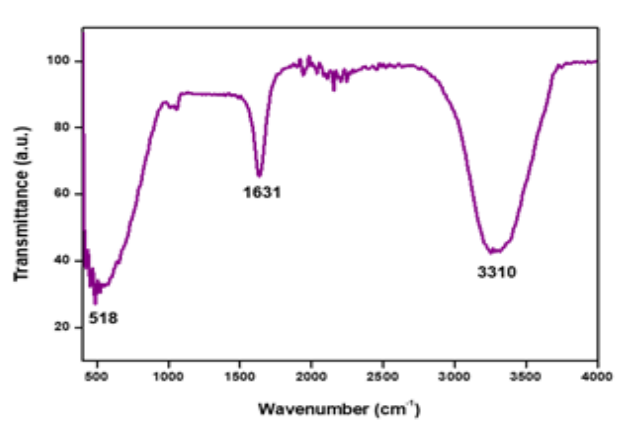

Figure 5: FTIR spectrum AgNPs.

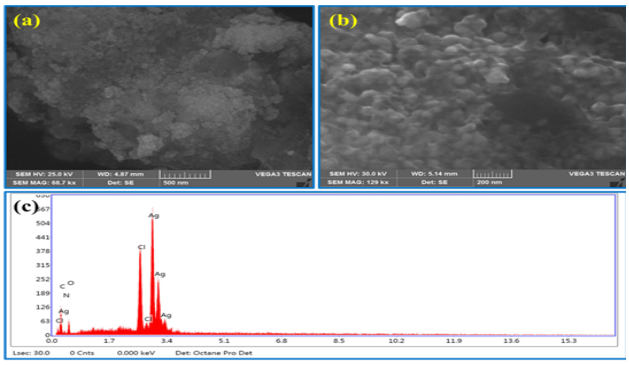

Figure 6: SEM and EDAX studies; (a,b): SEM images; (c): EDAX spectrum of synthesis AgNPs.

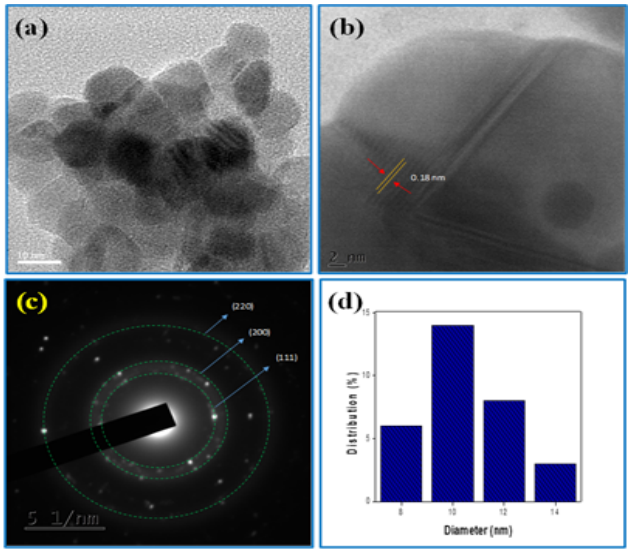

Figure 7: TEM studies; (a): TEM image; (b): HRTEM image; (c): SAED image; (d): Histogram show the range of particle size distrubution.

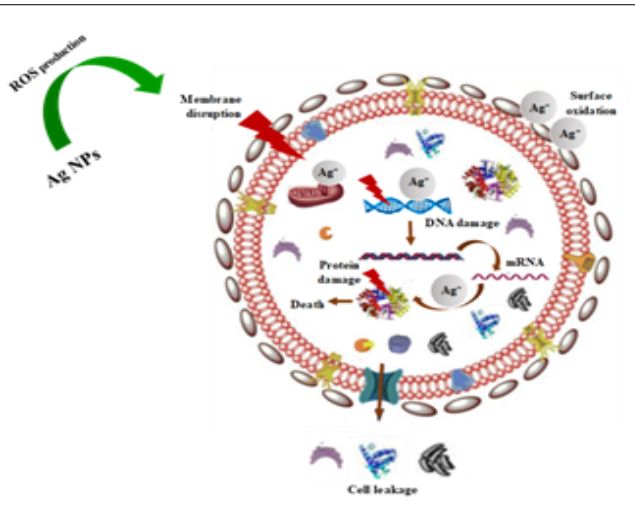

Figure 8: Antibacterial mechanism of Ag-NPs with bacterial cells.

pathogenic strains of *B. subtilis*, and *S. aureus* (gram positive) and *P. aeruginosa,* and *S. dysenteriae* (gram negative) were chosen to access the antibacterial activity of the prepared samples by Kirby-Bauer disk diffusion method. Known weights of the prepared samples were

Table 1: Zone of inhibition observed in various bacteria treated with green synthesized Ag-NPs.

Sl.No.	Tested microbes	Ciprofloxacin* 10 µg/mL (mm)	Ag-NPs		
			40 µg/mL (mm)	50 µg/mL (mm)	60 µg/mL (mm)
1	*S. aureus*	18	8.5	9.8	10.2
2	*B. subtilis*	16	9.6	10.1	10.9
3	*P. aeruginosa*	15	7.5	8.8	10.1
4	*S. dysenteriae*	17	8	9.1	9.8

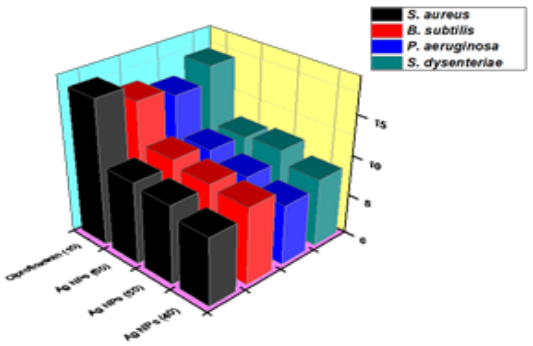

Figure 9: Bar diagram of inhibition zone of antibacterial activity of Ag-NPs.

Table 2: Zone of inhibition observed in various bacteria treated with green synthesized Ag-NPs.

Sl. No.	Extracts	Nanoparticle	Bacteria	Zone of Inhibition (mm)	References
1	Banana (leaf)	Ag-NPs	*Bacillus*	16 ± 0.016	32
			E. coli	14 ± 0.02	
2	Neem (leaf)	Ag-NPs	*Bacillus*	14 ± 0.008	
			E. coli	12 ± 0.007	
3	Tulsi (leaf)	Ag-NPs	*Bacillus*	14 ± 0.021	
			E. coli	14 ± 0.017	
4	*Eucalyptus tereticornis*	Ag-NPs	*E. coli*	6.0 ± 0.32	28
			S.Aureus	7.22 ± 0.44	
5	*Skimmia laureola*	Ag-NPs	*E. Coli*	12	33
			K. Pneumoia	13	
			S.Aureus	14	
			P. Aerugenosa	13	
			P. Vulgaris	13	
6	Lantana camara leaf extract	Ag-NPs	*E. Coli*	3	34
			Staphylococcus	3	
			Pseudomonas	6	
7	*Parkia speciosa*	Ag-NPs	*S.aureus*	6.9	35
			E.coli	7.6	
			P.aeruginosa	8.6	
8	*Tragopogon Collinus* leaf	Ag-NPs	*S.aureus*	2	36
			E.coli	4	
9	*Ocimum canum*	Ag-NPs	*E.coli*	24.5	37
10	Givotia moluccana leaf extract	Ag-NPs	*E.coli*	14 ± 0.98	38
			P. vulgaris	151 ± 06	
			K. pneumoniae	16 ± 0.45	
			S. aureus	16 ± 0.32	
			S. aureus	10.2	
11	*Urtica Dioica*	Ag-NPs	*B. subtilis*	10.9	Present Work
			P. aeruginosa	10.1	
			S. dysenteriae	9.8	

diluted in 1 ml of double distilled water followed by sonication for 3-5 min for uniform distribution of NPs. Nutrient agar was added into the sterilized petri plates and was allowed to settle followed by inoculation with the bacterial cultures of gram-positive and gram-negative. Circular Whatman filter paper disks of size 5 mm were placed on the solid agar. These disks were filled with 40 µg/mL, 50 µg/mL and 60 µg/mL of nanoparticles from the sample solution followed by incubation for 24 h at 37°C. Disks loaded with 10 µg/mL of ciprofloxacin were used as the positive control. Formation of a clear zone around the disks is evidence of antibacterial activity of the test sample. The diameter of the zones of inhibition was analysed using antibiotic zone scale [16-21].

Results and Discussion

Powder X-ray diffraction

Figure 3 giving detailed PXRD patterns of silver NPs. From the obtained PXRD data, the 2 theta peaks around 38.92°, 45.12°, 64.81° and 77.23° which are corresponding to (111), (200), (220) and (311) planes respectively [22]. In this obtained plane revealed that the synthesized Ag-NPs having Face Centered Cubic (FCC) crystalline structure of Ag. The average crystallite sizes of prepared nanoparticles were calculated using the Scherrer's equation, $D=k\lambda/\beta\cos\theta$; Where average crystallite size is denoted by D, wavelength is referred as λ, 'θ' is the Bragg's angle and 'β' is the full width at half maxima. The average crystallite size of the prepared sample is 18 nm. The strong peak observed at (111) suggested that the polycrystalline structure of synthesized AgNPs.

UV-Visible spectroscopy

The synthesis of Ag-NPs was detected with the help of UV-visible spectroscopy. The prepared nanomaterial exhibited higher UV-spectral absorbance peak at 453 nm and the obtained spectral data confirmed the formation of Ag NPs (Figure 4). The UV-absorbance at 453 is the characteristic feature of AgNPs and that was attributed to the SPR (Surface Plasmon Absorption) of Ag [23].

FTIR spectroscopic studies

Functional groups in Ag-NPs were conferment by FT-IR analysis. FTIR spectrum for *U. dioica* leaves extract synthesized Ag-NPs showed the O-H stretching band at 3310 cm^{-1}, N-H symmetric stretching at 1631 cm^{-1}, C-I bending vibration observed at 518 $^{cm-1}$ for silver nanoparticles (Figure 5). From the FTIR studies, it was noticed that the presence of O-H, N-H and C-I functional groups [24].

SEM and EDAX studies

The morphology and structure of synthesized Ag-NPs was analyzed by SEM. The SEM micrographs of the Ag-NPs were depicted in Figure 6a and Figure 6b. The particles are looking like spherical pellets, and are agglomerated. Figure 6 (c) represents the EDAX spectrum of Ag-NPs, elemental analysis of Ag-NPs confirmed the formation and purity of synthesized material silver (Ag) peak around 2 keV [25].

TEM studies

The TEM and histogram of material was examined and depicted in Figure 7. The particles were dispersed neatly, and obtained the diameter about 10 nm and every particle looks like spherical shape. And high resolution TEM images shows d spacing value and it was found to be 0.18nm of plane (111) from XRD analysis. SAED pattern

specifies the occurrence of (111), (200) and (220) planes in silver nanoparticles [26].

Antibacterial activity

Antibacterial activity and its mechanism were greatly studied by various scientists in order to detect particular action of AgNPs on cell. Due to the Ag^+ ion discharge and production of oxygen reactive group (O_2, O_2^-, OH, H_2O_2) cell and its membrane may get ruptured. For effective interaction with bacteria, size of the particle should be small and it could give excellent antibacterial activity [27]. At the time of light irradiation on bacterial cell wall, oxidative stress leads to the generation of oxygen species and which causes cell death. Negative charge of cell membrane and positive charge of Ag attract mutually. The demolition and death of cell membrane is the most important and activated step which occur when Ag^+ penetrate cell membrane and reacts with thiol group on it (Figure 8) [28].

The antimicrobial action of silver NP towards gram-positive, gram-negative bacterial pathogens were evaluated by BauerKirby disc diffusion method and presented in figure 8. The presence or absence of the Zone of Inhibtion (ZOI) around the sample impregnated disc indicated the bactericidal potential of the test compounds. From the obtained results, it is evident that the ZOI increases with increase in Ag-NPs concentration in the samples. The values of ZOI of Ag-NPs against *B. subtilis*, and *S. aureus* (gram-positive) and *P. aeruginosa*, and *S. dysenteriae* (gram-negative) bacterial pathogens are tabulated in Table 1 and Table 2, Figure 8 and Figure 9 respectively. Ag nanoparticles exhibit antibacterial activity by various means such as by the release of reactive oxygen species; destruction of cell structure by direct contact with the bacterial cell wall; inhibiting the protein synthesis which ultimately leads to cell death [29-31]. Also, the synthesized nanoparticles from leaf extract of *Urtica dioica* have exhibited higher bactericidal efficiency against gram-negative and gram-positive bacterial pathogens.

Conclusion

In the present work, Ag-NPs were prepared by green synthesis method by *Urtica dioica* leaves extract which proves to be an eco-friendly, cost-effective and non-toxic method for the nanoparticle synthesis. The Ag NP was confirmed by XRD study and SEM, TEM images shows particles are virtually agglomerated, crystalline and spherical shapes with 10 nm average particle size. The antibacterial activity of prepared NPs was analyzed by the disc diffusion method and found that the zone of inhibition of Ag-NPs (three different concentrations) against various bacterial pathogens. Increased concentrations of silver nanoparticles showed increased zone of inhibition. Therefore, based on the observed results we can conclude that, this is a fast, effective and environment friendly method which include additional asset of less reaction time and controlling shape, size of nanoparticles.

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Author Affiliations Top

Department of Mechanical Engineering, The University of Tokyo, Japan