Biosynthesis of silver nanoparticles using upland cress: purification, characterisation, and antimicrobial activity

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Silver nanoparticles have traditionally been synthesised using physical and chemical methods, often requiring expensive equipment and reagents that pose risks to the environment. This work provides a green method for the biosynthesis of silver nanoparticles using leaf extracts from upland cress: *Barbarea verna*. Natural reducing agents within the leaf extracts of upland cress reduce silver ions from silver nitrates, resulting in the formation of silver nanoparticles. The silver nanoparticles were purified using centrifugation and extraction using Triton X-114. The resulting nanoparticles were characterised using UV–Vis spectroscopy, dynamic light scattering, atomic force microscopy, and scanning electron microscopy. Silver nanoparticles were shown to have a diameter of 30–40 nm with a characteristic UV–Vis absorption peak at 420 nm. Antimicrobial properties of the synthesised silver nanoparticles were also confirmed using *S. epidermis* and *E. coli* bacteria.

1. Introduction: Nanoparticles, which are particles ranging from 1 to 100 nm in size, have attracted increasing interest in the last decade due to their variety of applications in fields such as electronics, cosmetics, energy, nanobiotechnology, and health care [1–5]. These particles exhibit different properties based on their size and composition [6]. The surface area to volume ratio of nanoparticles increases as the size of the particles decreases. Among the most commonly used nanoparticles are silver nanoparticles, which have been exploited for their antimicrobial properties to disinfect surfaces, such as medical equipment [1, 7]. Silver nanoparticles have also been used for water treatment, biosensors, and imaging [8–10].

Traditional techniques for synthesis of silver nanoparticles include physical methods such as evaporation condensation and laser ablation, chemical reduction by organic or inorganic reducing agents, UV-initiated photoreduction, and the electrochemical synthesis method [7, 11]. Many of these techniques are expensive and non-environment friendly due to the use of toxic chemicals during the synthesis [12]. Biosynthesis of silver nanoparticles has emerged as an alternative technique that is more environment friendly and cost-effective [13].

Biosynthesis of nanoparticles utilises the natural reducing agents in organisms such as bacteria, fungi, algae, and plants [7, 13]. Highly stable silver nanoparticles can be formed using biosynthesis, and the sizes of the nanoparticles synthesised vary depending on the organism used and the synthesis protocol [14]. The leaf extracts of many plants contain the reducing agents necessary for silver nanoparticle formation. These reducing agents have been identified as being phenolic acid-type biomolecules such as caffeine and theophylline and proteins with amine groups [15, 16]. Some of these plants that have been used for biosynthesis include *Camellia sinesis* (green tea) [15], *Ginko bilboa* (ginko tree) [14], *Magnolia kobus* (magnolia tree) [14], and *Capsicum annuum* (pepper) [17], among many others.

Barbarea verna, more commonly known as upland cress, belongs to the family of *Brassicacea* (order Brassicales) and is widely available across the globe [18, 19]. Upland cress, like watercress (*Nasturtium officinale*) and garden cress (*Lepidium nativum*), exhibits excellent antioxidant capacity due to its high levels of antioxidant phytochemicals such as ascorbic acid, carotenoids, tocopherols, and so on [20]. Upland cress was chosen for the biosynthesis of silver nanoparticles due to its availability, low cost, and potential reducing power.

Triton X-114 was used for nanoparticle purification to remove organic matter. Triton X-114 is a non-ionic detergent that allows for phase separation, in which the silver nanoparticles could be separated from organic materials in the leaf extracts [21–23].

2. Materials and methods

2.1. Materials: Silver nitrate and Triton X-114 were acquired from Millipore Sigma (St. Louis, MO). Upland cress (B&W Quality Growers, Fellsmere, FL) was obtained from a local Whole Foods market (Milwaukee, WI) and stored at 4°C. Seeds (Lot No. 47102-BSP17UC) were from Vilmorin North America (Salinas, CA).

2.2. Biosynthesis of the silver nanoparticles using upland cress: Ten grams of upland cress was cut and boiled in 100 ml deionised (DI) water (60°C) [24–26]. After 20 min, the upland cress extract was collected by vacuum filtration using Whatman filter paper. Upland cress extract (5 ml), DI water (35 ml), and 1 mM silver nitrate solution (0.2 ml) were mixed into a Erlenmeyer flask covered in aluminium and incubated in a shaker at 37°C for 6 h to allow for the nanoparticle synthesis [27, 28].

2.3. Purification of silver nanoparticles: After 6 h of incubation, the mixture was centrifuged for 20 min at 3000 rpm to collect the silver nanoparticles [29]. The supernatant was removed and the silver nanoparticles were then resuspended in a Triton X-114 solution (0.375 µl/ml DI water) [30]. The solution was vortexed for 5 min to fully resuspend the silver nanoparticles. The nanoparticles, collected through centrifugation, were dried in an oven at 60°C. The dried silver nanoparticles were then ground using a mortar and pestle to further prevent aggregation of the nanoparticles. Finally, the nanoparticles were rehydrated in DI water, vortexed,

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and sonicated to produce a silver nanoparticle colloid for further analysis.

2.4. Characterisation of silver nanoparticles: *UV–Visible spectroscopy*: UV-Visible spectroscopy (GENESYS™; Thermo Fisher Scientific, Waltham, MA) was performed to analyse the silver nanoparticles. A scanning from 350 to 650 nm was performed to find the maximum absorption wavelength of the nanoparticles. Both Triton-treated and non-treated (three washes in DI water during the purification process) were tested to elucidate if absorption intensity differences were present.

Atomic force microscopy (AFM): To prepare samples for AFM imaging, 25 µl of each silver nanoparticle colloid was pipetted onto a freshly cleaved mica disc. The discs with nanoparticles were then examined under a Bruker (Billerica, MA) MultiMode atomic force microscope with the Nanoscope IIIa controller (Veeco, Santa Barbara, CA) using contact mode.

Scanning electron microscopy (SEM): Nanoparticle colloid samples were dropped onto aluminium stubs and then air-dried. Samples were then examined under a Hitachi S-4800 Ultra High-Resolution Cold Cathode Field Emission Scanning Electron Microscope (FE-SEM; Hitachi S-4800; Krefeld, Germany). Energy dispersive X-ray (EDX, Noran Si:Li detector; Thermo Fisher Scientific, Waltham, MA) spectroscopy was also performed to verify the elemental composition of nanoparticles.

Size analysis: Dynamic light scattering (90 plus/BI-MAS; Brookhaven, Holtsville, NY) method was used to determine the size and the distribution of the nanoparticles. AFM and SEM images were also analysed by ImageJ software (US National Institutes of Health, Bethesda, Maryland, USA) for size measurement and size distribution analysis.

X-ray diffraction analysis (XRD): The powder X-ray diffraction analysis was carried out on a Bruker D8 Discover X-ray diffractometer (Billerica, MA) to confirm the crystal nature of the silver nanoparticles.

2.5. Antibacterial activity of silver nanoparticles: The antibacterial activity of the silver nanoparticles on both Gram-positive (*S. epidermidis;* Item #: 470176-542, Ward's Science, Rochester, NY) and Gram-negative (*E. coli;* Item #: 470176-528, Ward's Science, Rochester, NY) bacterial cultures was evaluated using the agar disc diffusion method [31]. Briefly, *S. epidermidis and E. coli* bacteria were cultured in nutrient broth media for 24 h at 37°C. Then, the discs (diameter of 6.35 mm) with varying dilutions of silver nanoparticles colloid (initial concentration: 0.2 mM) were placed on Mueller-Hinton agar plates inoculated with each of the previously mentioned microorganisms. A blank disc was used as a negative control, while an ampicillin disc (10 mcg, AMP10-1815; Carolina Biological Supply Company, Burlington, NC) was used as a positive control. After incubation for 24 h at 37°C, the zones of inhibition were observed and analysed [32–34].

3. Results and discussion

3.1. Effect of Triton X-114 on the nanoparticle purification process: As shown in Fig. 1, the upland cress extract/silver nitrate colour (golden/brown) deepens with time indicating the chemical formation of silver nanoparticles. Natural reducing agents in the upland cress extract reduced the silver ions to form silver nanoparticles. The effectiveness of this silver reduction could be attributed to the high concentration of carotenoids such as β-carotene and lutein, as well as other antioxidants and reducing agents in the upland cress [35, 36]. Aggregation of nanoparticles remains a challenge for nanoparticle synthesis. In this Letter, it was noticed that silver nanoparticles tended to aggregate and were covered by organic matter (Figs. 2*a* and 3*c*). Moreover, no characteristic silver nanoparticle absorption peak could be observed through UV–Vis spectroscopy (Fig. 4). As the optical properties of silver nanoparticles change when particles aggregate [31],

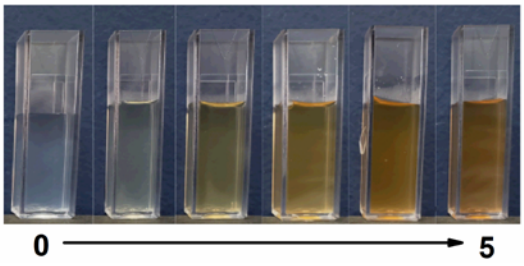

Fig. 1 *Images of the upland cress extract and silver nitrate mixture at different time points. Time interval: 1 h*

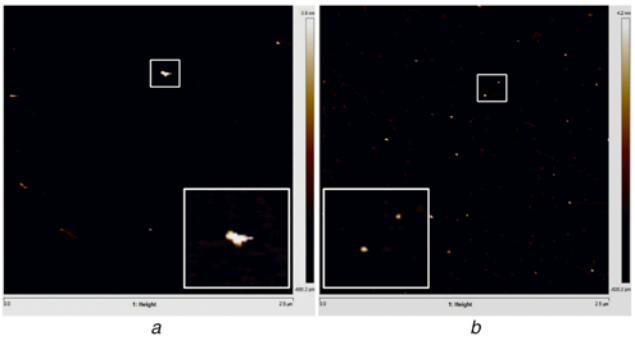

Fig. 2 *AFM images of silver nanoparticles. Inserts are the enlargements of the selected regions*
a Non-Triton X-114-treated silver nanoparticles
b Triton X-114-treated silver nanoparticles

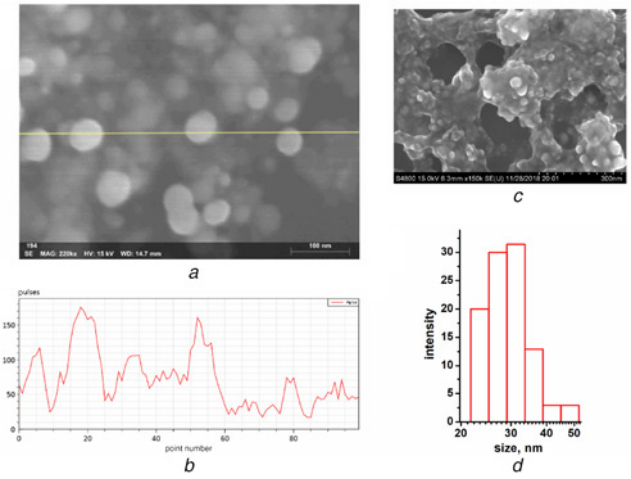

Fig. 3 *SEM-EDX analysis of silver nanoparticles*
a SEM image of Triton X-114-treated silver nanoparticles. Scale bar represents 100 nm
b EDX analysis result. The yellow line indicates where the EDX analysis was performed
c SEM image of non-Triton X-114-treated silver nanoparticles
d Size distribution of silver particles measured through DLS

Triton X-114, a non-ionic surfactant, was used in organic matter removal. Low concentration Triton X-114 solution was adopted to replace one of the three DI water wash steps. Triton X-114 has previously been shown to be effective in extracting organic materials [37, 38]. Silver nanoparticles reside in the aqueous phase while hydrophobic organic molecules from the leaf extracts are sequestered to the detergent-rich Triton X-114 phase after separation [38]. Triton X-114 treatment effectively improves the purification process. As shown in Fig. 2*b*, the nanoparticles were

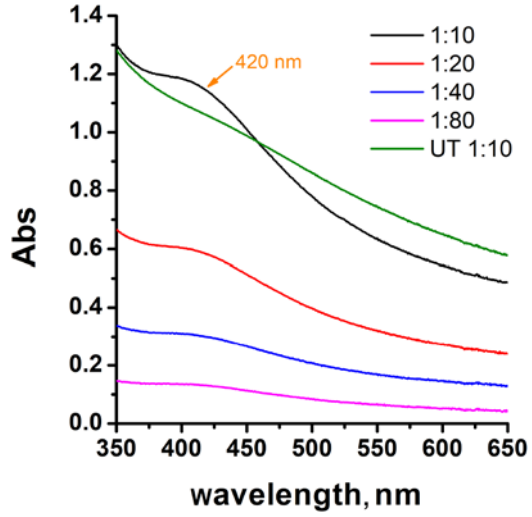

Fig. 4 *UV–Vis spectra of non-Triton X-144-treated (UT) and Triton X-114 silver nanoparticle colloids of different concentrations. The initial concentration: 0.2 mM*

well-dispersed showing individual particles. The UV–Vis spectra show a characteristic peak at around 420 nm, which is consistent with the literature [34]. Moreover, the intensity of the absorption peak shows concentration dependence.

3.2. Morphology, chemistry, size, and crystal structure of silver nanoparticles: SEM image of silver nanoparticles is shown in Fig. 3*a*. The particles exhibited a round shape with a uniform size distribution. EDX analysis (Fig. 3*b*) confirmed the chemical composition, specifically the Ag element, of the nanoparticles. The increased pulses of Ag element align perfectly with the appearance of the nanoparticles. Dynamic light scattering (DLS) indicated that the nanoparticles have a mean diameter of ∼30 nm with the size distribution shown in Fig. 3*c*, which is similar to the AFM and SEM image analysis results: 31.6 ± 5.6 and 40.1 ± 9.9 nm, respectively. The XRD pattern of synthesised silver nanoparticles (Fig. 5) showed Bragg reflection peaks at 38.14°, 44.28°, 64.44°, 77.4° which could be indexed to the (111), (200), (220) and (311) planes of the face-centred cubic crystals, respectively. This pattern matched well with the standard patterns of silver (JCPDS Card No. 04-0783). Based on the peak intensity, the (111) plane is the predominant orientation. The XRD pattern confirmed the crystalline nature of the Ag NPs formed in this study. The unassigned peak at 46.21° is thought to be related to crystalline and amorphous organic phase. It was found that the average size of

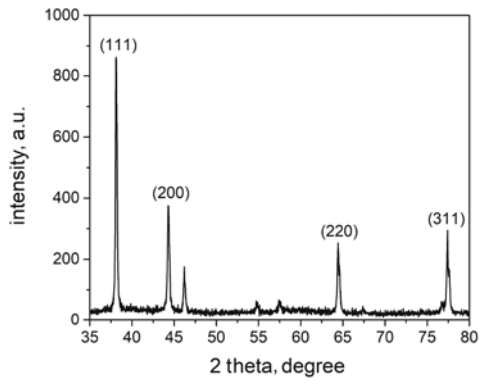

Fig. 5 *XRD patterns of silver nanoparticles*

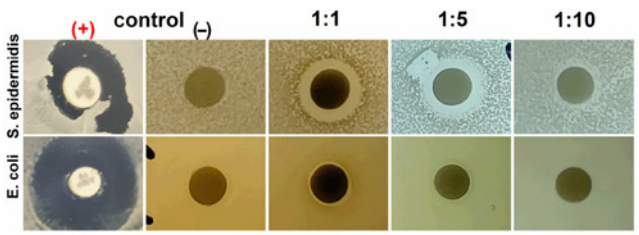

Fig. 6 *Antimicrobial results of synthesised silver nanoparticles: top: S. epidermidis and bottom: E. coli. Different nanoparticle concentrations were tested: 1:1, 1:5, and 1:10 dilutions. The ampicillin disc and blank discs were used as positive and negative control, respectively*

the nanoparticles from XRD data using the Debye-Scherrer equation was ∼34 nm [39].

3.3. Antibacterial activity of nanoparticles: Synthesised silver nanoparticles showed antibacterial activity in a dose-dependent and species-dependent manner on both Gram-positive *S. epidermis* and Gram-negative *E. coli* (Fig. 6). As expected, higher concentrations of silver nanoparticles exhibited larger zones of inhibition for bacterial growth. The diameter of the zones of inhibition for *S. epidermis* was 10.2, 9.8, and 7.8 mm for 1:1, 1:5, and 1:10 dilutions, respectively. The inhibition diameters for *E. coli* were 7.3, 7.0, and 6.7 mm for 1:1, 1:5, and 1:10 dilutions, respectively. The biosynthesised nanoparticles exhibited the desired antimicrobial activity typical of silver nanoparticles. The possible antimicrobial mechanisms are: causing physical changes in the bacterial envelope, producing high levels of reactive oxygen species, and releasing Ag^{+} which interact with cellular structures and biomolecules [40]. The species dependency may be explained by the envelope structural differences [41].

4. Conclusion: This Letter showed that upland cress *(Barbarea verna)* leaf extracts could be used for the green biosynthesis of silver nanoparticles. The leaf extracts acted as reducing agents for silver, forming nanoparticles ∼30–40 nm in diameter. Silver nanoparticles showed minimal aggregation upon purification and exhibited antimicrobial properties in bacterial cultures, showing promise for use as antimicrobial agents. Biosynthesis using upland cress, which is readily available and inexpensive, provides an alternative method of synthesising silver nanoparticles that is more environment friendly than traditional methods.

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