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Green silver nanoparticles from novel Brassicaceae cultivars with enhanced antimicrobial potential than earlier reported Brassicaceae members

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ABSTRACT

In the present study, we report perhaps for the first time the use of novel varieties of *Brassica oleracea* var. *botrytis* and *Raphanus sativus* as potential bioreductant, to synthesize highly stable silver nanoparticles (AgNPs, no aggregation observed for six months), which is a significant finding as plant extract-directed AgNPs are intrinsically unstable and tend to aggregate. The reduction of Ag^{+} to Ag^{0} nanostructures was confirmed using UVVis spectroscopy showing SPR spectra at 400–435 nm. Nanosight and transmission electron microscope (TEM) analysis showed monodisperse spherical AgNPs (4–18 nm). Fourier transform infrared spectroscopy (FTIR) analysis revealed that the polyphenolics and other secondary metabolites including glucosinolates in the aqueous extracts may act as reducing/capping agent for the nanoparticle synthesis. X-ray diffraction (XRD) confirmed the face centered cubic crystalline (fcc) structure of AgNPs. Controlled synthesis of AgNPs was achieved by varying experimental parameters ($AgNO_3$ concentration, extract volume, pH and temperature). These AgNPs exhibited strong antibacterial activity at significantly lower concentration (5 ppm) against both Gram negative (*Escherichia coli, Myroides, Psuedomonas aeruginosa*) and Gram positive (*Kocuria and Promicromonospora*) bacteria. In the present study, the green AgNPs showed (10–30%) better antimicrobial efficacy than chemical AgNPs and AgNPs from other Brassicaceae members. These green AgNPs may have promising application in nano-drug formulation to combat bacterial infections, in future.

1. Introduction

Nanotechnology is a cutting-edge field of science which involves synthesis of nanoparticles of variable sizes, shapes, chemical compositions with controlled dispersity and multiple potential applications [1]. Amongst all noble metal nanoparticles, silver nanoparticles (AgNPs) are of prime significance due to their unique structural, plasmonic and physical properties like surface functionalization and controlled drug release [2].

AgNPs have major applications in the area of electronics, food industry [3], fabrics, pharmaceuticals, cosmetics, packaging and biomedicine [4]. Strong antibacterial, antiviral, antifungal as well as anti-inflammatory activities, makes these a good choice for wound dressings, topical creams and antiseptic sprays [5].

Emergence of multi-drug resistant bacteria (due to antibiotics overuse) is a severe global threat of the present times. Therefore, novel antimicrobial agents with enhanced broad-spectrum potency are required. AgNPs could provide an efficient alternative. In the recent past, AgNPs were also utilized for disinfecting filters, wastewater treatment, toxic dyes degradation and coating for various materials/surfaces as an antifouling biofilm [6,7].

In the last few decades, several conventional physical and chemical methods involving the use of sodium borohydride, hydroxylamine hydrochloride, trisodium citrates, dimethyl-formamide as reducing agents have been employed for the production of smaller (< 10 nm) monodispersed metal nanoparticles. However, these methods are expensive, involves higher energy and contributes to environmental and biological toxicity which limits their biomedical applications [8]. Till date, chemically synthesized AgNPs are preferred as potential antimicrobial agents due to the ease of controlled synthesis.

Nature is an invaluable source of unexplored medicinal plants including vegetables which could be utilized to convert inorganic metallic ions into metal nanoparticles with numerous benefits like eco-friendliness, low cost, energy efficient and with novel biomedical properties. Plant secondary metabolites acts as reducing as well as capping agents and may impart antioxidant and antimicrobial properties to NPs. [9]. Green synthesis of AgNPs has earlier been attempted with plants from Apiaceae, Asteraceae, Solanaceae, Zingiberaceae and Brassicaceae.

Amongst Brassicaceae, cruciferous vegetables have earned the status of “Functional Foods” being nutrient rich and could provide

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several health benefits [10]. Owing to their redox properties these bioactive compounds also have the capability to neutralize the free radicals and prevent oxidative stress induced cardio vascular and other degenerative diseases like cancer [11].

The synthesis of AgNPs as well as other metallic nanoparticles using different parts of the Brassicaceae members (Broccoli, Cabbage, Cauliflower and Radish) is already reported and their potential as antioxidant, antimicrobial, anticancer and antifungal agents is known. However, all these studies are reported either using the Brassicaceae members from local market or the South Indian (SI) varieties [12–17].

Moreover, a comparative analysis of different Brassicaceae members for their reducing capacity, for controlled synthesis of AgNPs and to explore better performing varieties with higher antimicrobial potential is lacking. Therefore, antimicrobial screening of the AgNPs synthesized using the North Indian (NI) varieties and their comparison with AgNPs from other SI Brassicaceae members was done, to provide a green alternative to the commonly used chemical AgNPs.

In the present investigation, nine NI varieties belonging to Brassicaceae family including six different cultivars of *Brassica oleracea var. botrytis* (Ashwini, Deepali, Kartik Sankar, Meghna, Paushja and Sharad) and three different varieties of *Raphanus sativus* (Himani, a white variety and colored varieties Gulabi and Jamuni) were explored for green synthesis of AgNPs. AgNPs with high yield, uniform spherical shape and narrow size distribution, were successfully synthesized and characterized using UV–Vis spectroscopy, TEM, XRD and FTIR. Our goal was also to find if there is any difference in reducing capacities of the colored cultivars of the Radish due to high carotenoids and anthocyanins. Moreover, controlled synthesis of AgNPs by tuning size, shape and surface modification was also attempted for enhanced antimicrobial activity. To the best of our knowledge, the nanobiotechnological potential of these nine cultivars including the colored ones has not been reported earlier.

2. Experimental section

2.1. Materials

For this study, silver nitrate ($AgNO_3$, purity 99%) was obtained from Merck Pvt. Ltd., India. Nutrient agar (NA containing Peptone, beef extract/yeast extract, agar, NaCl at pH 7.4), Nutrient broth and Muellor Hinton Agar (MHA containing Beef, Casein acid hydrolysate, Starch, Agar at pH 7) were procured from HiMedia laboratories Pvt. Ltd., India. All chemicals were of analytical grade. Bacterial pathogens: *Escherichia coli, Bacillus* sp. SV1*, Psuedomonas aeruginosa* sp. AHM*, Kocuria* sp. UK S5*, Myroides indicus* and *Promicromonospora* sp. VP111 were kindly obtained from Prof. Ved Pal Singh, Department of Botany, University of Delhi.

2.2. Plant material

Recently, Indian Agricultural Research Institute (IARI), New Delhi, India has released new NI varieties and cultivars of *Brassica oleracea* var. *botrytis* and *Raphanus sativus*. Cauliflower head of six cultivars of *Brassica oleracea* var. *botrytis* cv. Pusa Ashwini, Pusa Deepali, Pusa Kartik Sankar, Pusa Meghna, Pusa Paushja and Pusa Sharad and three varieties of tuber *Raphanus sativus* Pusa Gulabi, Pusa Himani and Pusa Jamuni were obtained from IARI (Fig. S1). These varieties differ significantly in their characteristic, size, yield and maturing period as mentioned in the Supplementary Information Table S1.

The material was thoroughly washed using tap water to remove the adhered dust and rinsed twice with deionised water. The tissue was completely dried at room temperature on a blotting paper. Further, the material was frozen and stored at − 80 °C for further use.

2.3. Preparation of aqueous plant extract

Tissue was ground to fine powder and aqueous extract was prepared (20% w/v) using deionized water. The mixture was agitated for an hour and centrifuged at 10,000 rpm for 15 mins at 4 °C. Clear supernatants were collected, stored at 4 °C and used for AgNP synthesis.

2.4. Green synthesis of silver nanoparticles

For AgNPs controlled synthesis (shape and sizes) effect of different salt (0.3–0.9 mM) concentrations ($AgNO_3$), extract volume (50–400 µL), temperature (RT–80 °C), and pH (8, 10 and12) was observed.

Briefly, to optimize the conditions for enhanced production of AgNPs, different volumes (50, 100 and 200 µL) of the extracts (20% w/v) were mixed with 3 mL of 0.3, 0.6 and 0.9 mM $AgNO_3$ solution maintained at different pH (8, 10 and 12). pH was adjusted with 1 M NaOH. To analyse the effect of temperature on AgNPs synthesis a separate set of reactions at optimized conditions was kept at different temperatures RT (overnight), 40 °C, 60 °C and 80 °C (for 20 mins).

2.5. Chemical synthesis of nanoparticles

Chemical synthesis of AgNPs using sodium citrate as reductant was performed following the method of Turkevich et al. [18]. Briefly, 1 mM $AgNO_3$ solution (60 mL) was boiled and 10 mM trisodium citrate (6 mL) was added dropwise. Change in color of solution to light golden confirmed the synthesis of AgNPs.

2.6. Characterization of nanoparticles

The excitation spectrum of the green synthesized silver nanoparticle (AgNPs) was recorded in the range 200–800 nm at a resolution of 2 nm using UV–vis spectrophotometer (DU 800, Beckman & Coulter). Morphology and size of the nanoparticles was analysed using TEM. AgNPs were drop coated on the carbon-coated copper grids and observed under FEI Tecnai TEM operating at 200 kV. A NanoSight (NanoSight Limited, Amesbury, UK) was used to carry out nanoparticle tracking analysis (NTA) for size distribution of AgNPs system (NANOSIGHT, NTA version 2.3). Nanoparticle Tracking Analysis (NTA) is a particle characterization technique where the individual particles can be visualised to provide single-particle resolution. It is based on the principle that nanoparticles move in Brownian motion where smaller particles move faster than the larger ones. Diffusion Coefficient is calculated by analysing the movement of each particle simultaneously. Further the particle size is calculated by applying the Stokes-Einstein equation. AgNPs (100 µL) were syringed into the viewing unit of the NanoSight and a red (638 nm) laser was used to illuminate the particles to be tracked. Measurements were recorded at 20 °C. NanoSight's NTA software was used to analyse the AgNPs and determine the particle size distribution.

XRD analysis was performed to examine the crystallographic structure of the AgNPs using a Bruker D8 Advance diffractometer operating in the reflection mode with Cu-Kα radiation (40 kV, 40 mA) in the 2 theta range of $20-80^{\circ}$ and diffracted beam monochromator. The samples for XRD measurements were prepared by casting the AgNPs solution on a glass substrate and subsequently air-drying under ambient conditions. FTIR spectra were recorded using Shimadzu FTIR 8400, over the range of 650–4000 cm^{-1} to investigate the biomolecules involved in synthesis and capping of AgNPs.

2.7. Phytochemical screening of aqueous extracts

For estimation of total phenolic content, cauliflower or radish extracts (40 µg, 200 µL of 20% w/v) and standard gallic acid (5–40 µg/ml) were mixed with Folin-Ciocalteu (FC) reagent, followed by addition of (20%) saturated sodium carbonate after 5 mins [19]. Absorbance was