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Research Article

VEGETABLE ASSISTED SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL ACTIVITY AGAINST TWO HUMAN PATHOGENS

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ABSTRACT

Biological methods are considered safe and ecologically sound for the nanomaterial fabrication as an alternative to conventional, physical and chemical methods. Thecauliflowerbroth was found to be a suitable plant source for the green synthesis of silver nanoparticles. In the process of synthesizing silver (Ag) nanoparticles we observed a reduction of silver ions leading to the formation of stable crystalline silver nanoparticles in the solution. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to nano-sized Ag particles.Energy dispersive X-ray spectrometers (EDAX), UV-Vis spectroscopy analysis of these particles, XRD and Particle size distributions results confirmed the presence of nano-crystalline Ag particles. The antibacterial effect of silver nanoparticles produced in this study was studied against two human pathogens *Escherichia coli (E. coli)* and *Staphylococcus aureus (S.aureus).*

Key words: Cauliflower,silver nanoparticles, biological method, antibacterial activity.

INTRODUCTION

Now a days, nanotechnology has grown to be an important research field in all areas. For several years, scientists have constantly explored different synthetic methods to synthesize nanoparticles. The green method of synthesis of nanoparticles is easy, efficient, and eco-friendly in comparison to chemical-mediated or microbe mediated synthesis. Chemical and physical methods may successfully produce pure, well defined nanoparticles, these method involves toxic solvents, high pressure, energy and high temperature conversion which are potentially dangerous to the environment [1]. Use of biological organisms such as microorganisms, plant extract or plant biomass could be an alternative to chemical and physical methods for the production of nanoparticles. The silver and certain other noble metal nanoparticles have several important applications in the field of biolabelling sensors; drug delivery system, filters and also possesses antimicrobial activity. These metal nanoparticles exhibit new physico-chemical properties, which are not observed in the bulk [2]. The synthesis and characterization of nanoparticles is being an important area of research as selection of size and shape of nanoparticles provide an efficient control over many of the physical and chemical properties and their potential application in optoelectronics, recording media, sensing devices, catalysis and medicine.

Biological routes to the synthesis of metal particles have been proposed by exploiting bacteria [3-6], yeast [7-9], fungi [10-15], actinomycetes [16-17] and virus [18-20] microbe involved synthesis is not feasible industrially because of their pathogenicity and due to its lab maintenance in an eco-friendly manner. Recently plant-mediated biological synthesis of noble nanoparticles is gaining importance due to its simplicity, eco-friendliness and it eliminates the elaborate process of maintaining cell cultures. Although biosynthesis of silver nanoparticles by plants viz. *Pinus desiflora*, *Diopyros kaki*, *Ginko biloba*, *Magnolia kobus* and *Platanus orientalis*[21], *Cycas* leaf [22], *Moringa oleifera* leaf[23], *Shorea tumbuggaia* stem bark [24], *Cinnamon zeylanicum* stem bark [25], *Carica papaya* fruit[26], *Trianthema decandr* roots[27], *Jatropha curcas* latex [28], *Artocarpus heterophyllus* leaf[29], *Lantana camara* fruit[30], *Coriandrum sativum* leaf[31]*and Baliospermum montanum* leaf[32]have been reported, the potential of the plants as biological materials for the synthesis of nanoparticles is yet to be fully explored.

In this work we report the synthesis of silver nanoparticles, reducing the silver ions presents in the solution of silver nitrate by the aqueous extract of cauliflower this method yields faster and stable \ silver nanoparticles compared to other methods. Cauliflower is one of several vegetables in the species *Brassica oleracea*, in the family Brassicaceae. Cauliflower is low in fat, low in carbohydrates but high in dietary fiber, folate, water, and vitamin C, possessing a high nutritional density. Cauliflower contains several phytochemicals , common in the *Brassica oleracea* family, that may be beneficial to human health like sulforaphane, glucosinolates, carotenoids, indole-3-carbinol, estrogen. It is a common plant available in all tropical regions.

Its quantitative formation was monitored by UV-Visible spectroscopy. Also the silver nanoparticles formations were confirmed by reddish brown color formation. The antibacterial effect of Ag/cauliflower was evaluated against two human pathogenic bacteria, including *E.coli* (Gram negative) and *S. aureus* (Gram positive) using the agar disc diffusion method.

MATERIALS AND METHODS

Materials

Silver nanoparticles were synthesized by using cauliflower extract as reducing and capping agents which are procured from the market and silver nitrate (99.8%) was supplied by S.D.Fine Chem. Ltd., India. The strains employed in this work were procured from Microbial Type Culture Collection Center (MTCC) located at the Institute of Microbial Technology (IMTECH) Chandigarh, India. The strains employed were *E. coli* (MTCC 433), *S. aureus* (MTCC 3160). Luria Bertani broth (LB) and Nutrient broth were procured from Hi-Media Laboratories, Mumbai.

Synthesis of nano-scale silver particles

For preparing plant broth solution, 20 g of finely cut cauliflower was thoroughly washed with double distilled water. This was taken in a 250 ml Erlenmeyer flask with 100 ml of double distilled water and then boiled the mixture for 5 min. The extract was decanted slowly and then was stored at 4°C and used within a week. In a typical synthesis of silver (Ag) nanoparticles, 5ml of cauliflower extract was added to 45 ml of 1mM $AgNO_3$ aqueous solution and kept at room temperature. The experiment was done in triplicate for reproducibility. After 30 min, the color of the solution changed from colorless to reddish brown indicating the formation of Ag nanoparticles (Fig. 1).

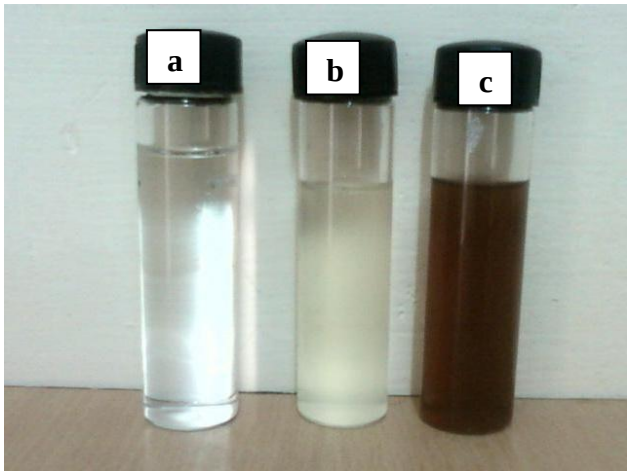


Figure 1: Optical photograph of (a) 1 mM AgNO3 solution (b)Cauliflow erextract (c) Silver nanoparticles produced.

Characterization

Reduction of Ag+ ions during exposure to the extract of cauliflower was easily followed by UV-Vis spectroscopy (Varian, Cary 5000; with wavelength accuracy of ±0.1nm and 0.025nm as wavelength reproducibility). Absorption measurements in the UV and Visible regions provided information about electronic transitions in the samples. The UV–Visible spectra of the resulting solutions were monitored as a function of reaction time. Energy dispersive X-ray spectrometers (EDAX) take advantage of the photon nature of light. In the X-ray range the energy of a single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra low noise preamplifier connected to the low noise are a statistical measure of the corresponding quantum energy. The presence of elemental silver signal was confirmed in the sample by using EDAX (JEOL-JED-2300). Particle sizing experiments were carried out by means of Zetasizer 3000 HSA, (Malvern, Worcestershire, UK) using a detection angle of 90° and a 60 mW He-Ne laser operating at a wavelength of 633 nm. XRD data were obtained using Siemens X-ray diffractometer (Japan), operated at 30 kV and 20 mA current with Cu Kα (l=1.54 Å) radiation.

Antibacterial activity

The antibacterial activity of silver nanoparticles was carried out on human pathogenic *E. coli* and*S. aureus* by standard disc diffusion method. LB broth/agar medium was used to cultivate bacteria. Fresh overnight inoculum (100µl) of each culture was spread on to LB agar plates. Sterile paper discs of 5mm diameter (containing 50mg/lit. silver nanoparticles) along with four standard antibiotic containing discs were placed in each plate. The plates containing bacterial and silver nanoparticles were incubated at 37°C; the plates were examined for zones of inhibition. The clear area was appeared around the well, the diameters of such zones was measured using meter ruler and expressed in millimeter.

RESULTS AND DISCUSSION

UV-Vis Spectra analysis

Cauliflower extract when added to the aqueous solution of 1mM $AgNO_3$ solution, started to change its color from colorless to reddish brown. It indicated the formation silver nanoparticles with the reduction of silver ion. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time with water as reference from 300 to 700nm. The absorption spectra of silver nanoparticles formed in the reaction media at different duration of 1, 15, 30, 45, 60 and 75 minutes (Fig. 2), it was noted that the evolution of absorption spectra of the particles has increasingly sharp absorbance at 424 nm with increase in time, which steadily increased in intensity as a function of time of reaction without showing any shift of the wavelength maximum. According to Mie's theory [33], only a single surface Plasmon resonance (SPR) band is expected in the absorption spectra of spherical nanoparticles, whereas anisotropic particles could give rise to two or more SPR bands depending on the shape of the particles. The number of SPR peaks increases as the symmetry of the nanoparticle decreases. In the present Ag/cauliflower investigation, the reaction mixtures showed a single SPR band revealing spherical shape of silver nanoparticles. The inset of Figure 2 shows that the reduction of the silver ions taking place at a faster rate and that saturation of data occurs at 60 minutes thus indicated the completion of reaction.

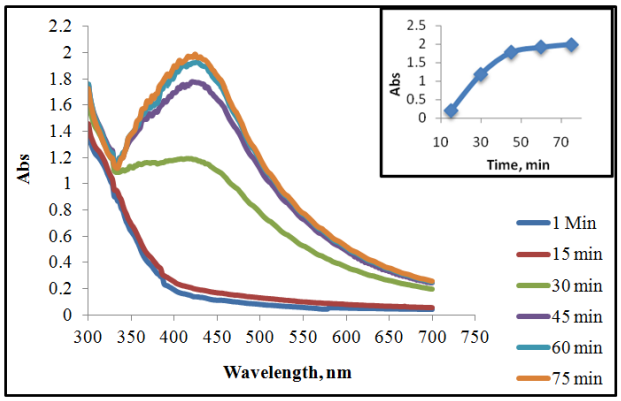


Figure 2: UV-Vis spectra of reduction of Ag ions to Ag nanoparticles.

It could be referred that these results have been supported in previous reports, where the addition of aqueous leaf extract of various plants to 1mM aqueous $AgNO_3$ solution started its color change due to the excitation of SPR in the production of silver nanoparticles [34, 35]. SPR is caused due to collective oscillations of the conduction electrons of nanoparticles upon irradiation with visible light [36]. The SPR is highly influenced by shape and size of the nanoparticles. This is accordance with the results obtained from bioreduction of silver nanoparticles using different plant extracts, which showed that SPR silver band occurred at 400–480 nm [35]. The reduction of silver ions and the formation of stable nanoparticles occurred rapidly within an hour of reaction, making it one of the fastest bio-reducing methods to produce Ag nanostructures reported earlier [37, 38]. In the analysis of the silver nanoparticles by EDAX, the presence of elemental silver signal was confirmed in the sample (Fig. 3). The vertical axis displays the number of X-ray counts whilst the horizontal axis displays energy in keV. The Ag nanocrystallites display an optical absorption band peak at 2.984 – 4.0 keV which is typical of the absorption of metallic silver nanocrystallites [39].

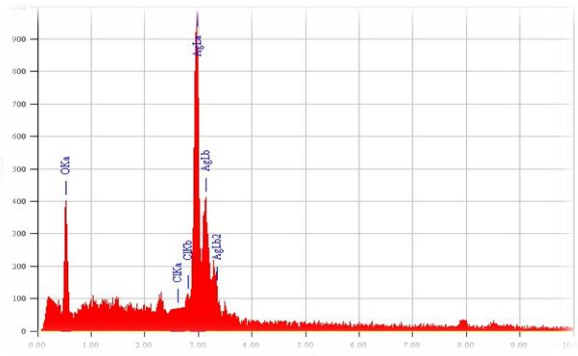


Figure 3: EDAX spectra of silver nanoparticles prepared by usingcauliflower. Strong signals from the atoms in the nanoparticles are observed in spectrum and confirm the reduction of silver ions to silver nanoparticles.

***Energy Dispersive* X-Ray *Spectroscopy* (EDAX) measurements**

The EDAX pattern thus clearly shows that the silver nanoparticles are crystalline in nature by the reduction of silver ions made in this study using cauliflower broth. The EDAX analysis obtained in the present study also confirmed the presence of silver nanoparticles

synthesized from cauliflower extract (Fig. 3). Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface Plasmon resonance. In an earlier study, individual spherical-shaped silver nanoparticles were obtained in the range 2.5–4 keV by using *Memecylon edule* leaf extract [35]. The EDAX analysis confirmed the presence of 91.05wt% of silver nanoparticles against earlier reports demonstrating only 33.52 wt% of silver nanoparticles by using *Shorea tumbuggaia*[24].

XRD analysis of silver nanoparticles

The presence of water soluble antioxidant, vitamin and a reducing agent like ascorbate at high levels in plants may also be the reason for the reduction, the ascorbate neutralizing reactive oxygen species leading to formation of ascorbate radical and an electron that reduces Ag^{+} ion to Ag^{o}[40]. The synthesized Ag nanoparticles using cauliflower extracts was further confirmed by the characteristic peaks observed in XRD image (Fig. 4). The XRD pattern showed different intensity peaks in the whole spectrum of 2θ values ranging from 0 to 80 for the cauliflower.

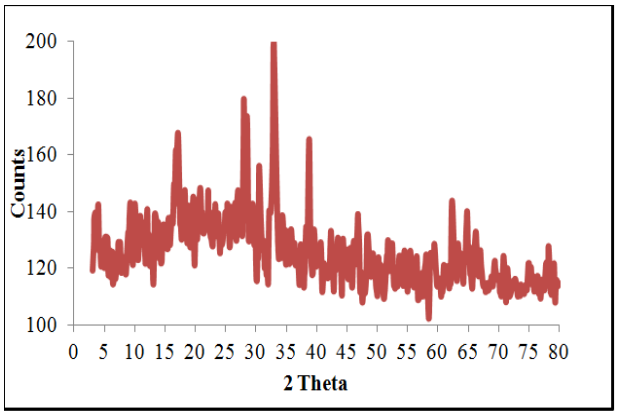


Figure 4: XRD pattern of silver nanoparticles formed after reaction of plant extracts of cauliflower.

The silver nanoparticles produced in our experiments were in the form of nanocrystals as evidenced by the peaks at 2θ values of 28.03 32.9, 38.7, 44.15 and 64.49. XRD pattern thus clearly illustrate that the silver nanoparticles formed in this present synthesis are crystalline in nature. Other peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles [31].

Particle size Distribution

Particle sizing experiment was carried out by means of laser diffractometry, using Zetasizer nano series (Malvern).Particle size determination of the formulated nanoparticles was shown under categories like size distribution by intensity. The size of Ag nanoparticles dispersed was ranged widely from 42 nm to 83 nm (Fig. 5), the average particle size (d_{50}) is expected in the range of 53.8 nm and the peaks width was found 12.8.

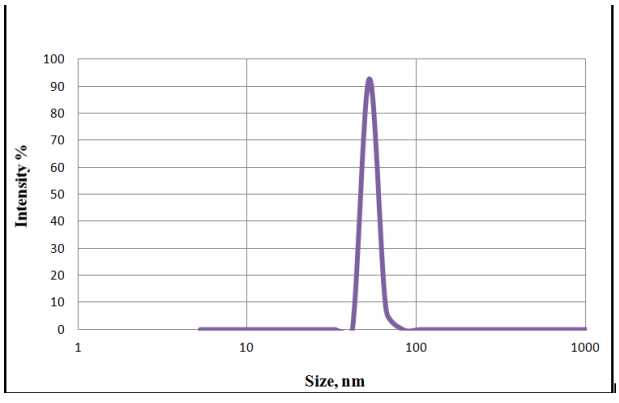
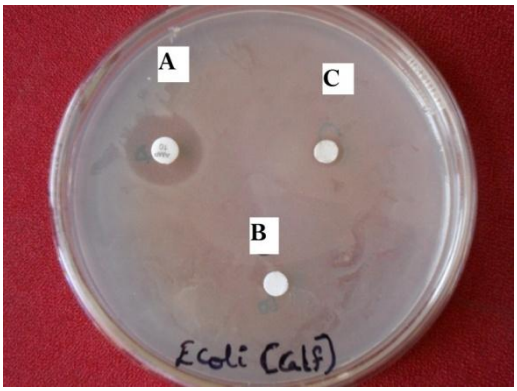


Figure 5: Particle size distributions of silver nanoparticles.

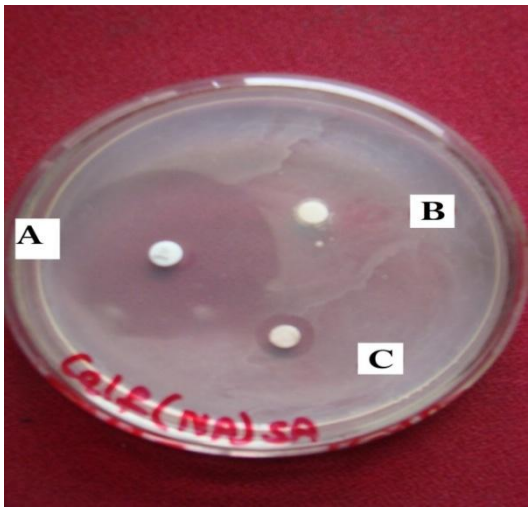
The size distribution by intensity gives a bell shaped pattern which indicates the wide range size distribution of nanoparticles in the sample formulation. In an earlier study, silver nanoparticles size distributions were obtained in the range of 18.03 to 148.7nm by using *Saururus chinensis* leaf extract [41].

Antibacterial activity studies

The antibacterial activity of silver nanoparticles was carried out on human pathogenic *E.coli* and *S.aureus,* by standard disc diffusion method. Luria Bertani (LB) broth/agar medium was used to cultivate bacteria. The bacterial activity of synthesized Ag nanoparticles against different bacteria such as *E.coli and S- aureus* showed a clear inhibition zone (Fig. 6 and Table 1); the synthesized Ag nanoparticles were highly effective in their activity against these bacteria. Standard antibiotic disc (100µg/ml) Ampicilin was used as reference drug and plant extract used as a control. Surfaces of silver nanoparticles affect / interact directly with the bacterial outer membrane, causing the membrane to rupture and killing bacteria.



(a)



(b)

Figure 6: Bacterial activities of synthesized Ag nanoparticles against (a) *E.coli* (b) *S.aureus*: A- Ampicilin, B-Leaf extract, C- Silver nanoparticles.

Table 1: The antibacterial activity of silver nanoparticles synthesized using cauliflower.

Name of Species	Zone of Inhibition, mm	
	Ag nanoparticles	Ampicilin
Escherichia coli	8.8	23.5
Staphylococcus aureus	13.4	40

Bacterial membrane proteins and DNA make preferential sites for silver nanoparticles interaction as they possess sulphur and phosphorus compounds. Further, silver has higher affinity to react with these compounds. Although the exact mechanism of inhibition

by silver ions on microorganisms is partially known, many possible mechanisms have been proposed. In general, it is believed that DNA loses its replication ability and cellular proteins become inactivated on silver ion treatment. The attachment of either Ag ions or nanoparticles to the cell wall causes accumulation of envelope protein precursors, which results in dissipation of the proton motive force. Ag nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane, thereby causing depletion of intracellular ATP. Another proposed mechanism involves the association of silver with oxygen and its reaction with sulfhydryl (–S–H) groups on the cell wall to form R–S–S–R bonds, thereby blocking respiration and causing cell death [25].

CONCLUSION

The bio-reduction of aqueous Ag+ ions by the cauliflower extract as a reducing and capping agent is described in this study was confirmed by color changes and was characterized by UV-visible spectrophotometer; the UV-Visible spectra showed a broad peak located at 424nm for silver nanoparticles. These nanoparticles at concentration of 50mg/liter were shown complete antibacterial activity against *E.coli* and *S.aureaus,*the zones of inhibition were found to be 8.8 and 13.4mm respectively. Hence, the synthesized nanoparticles are more efficient in the drug delivery process. In the present study we found that cauliflower can be also a good source for synthesis of silver nanoparticles. This green chemistry approach toward the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability, etc., and the present study exploited the same.

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