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Rapid biosynthesis of silver nanoparticles using bottle gourd fruit extract and potential application as bactericide

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Synthesis of silver nanoparticles by biological route represents as economic alternative for physical and chemical methods of nanoparticles formation. Biosynthesis of silver nanoparticles using bottle gourd (*Cucurbita lagenaria* L.) fruit extract was investigated. Silver nanoparticles were characterized using UV-Vis absorption spectroscopy, absorption peak was obtained at 435 nm. X-Ray diffraction and Scanning Electron Microscopy analysis showed that silver nanoparticles were spherical with average particles size of 27.4 nm. These were found to be toxic against *Klebsiella pneumoniae* NCIM 2719 and *Clostridium acetobutilicum* bacteria.

Keywords : Silver nanoparticles, biosynthesis, antimicrobial activity.

Introduction

Nanoparticles exhibit completely new or improved properties based on specific characteristics as size, distribution and morphology. New applications of nanoparticles and nano materials are employed. Development of nano-devices using biological materials and their use in wide array of applications on living organisms has recently attracted the attention of biologists towards nanobiotechnology. Living organisms have huge potential for the production of nanoparticles of wide applications. A wide variety of physical, chemical and biological processes results in the synthesis of nanoparticles, some of these are novel and others are quite common.

Green synthesis of nanoparticles is an emerging branch of nanotechnology. The use of environmentally benign materials like plant extracts, bacteria and fungi for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical, biomedical and agricultural applications as they do not use toxic chemicals in the synthesis protocols (Parshar *et al*. 2009). Biosynthesis of nanoparticles provides advancement over chemical and physical methods as it is cost effective and environmental friendly method.

A bottle gourd (*Cucurbita lagenaria* L.) fruit a vegetable is cooling, diuretic, sedative and antibilious. Bottle gourd is very valuable in urinary disorders. The juice of bottle gourd is a valuable medicine for excessive thirst due to severe diarrhea, diabetes and excessive use of fatty or fried foods. The gourd fruit juice is used in the treatment of insanity, epilepsy and other nervous diseases.

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Silver has been recognized as having inhibitory effect on microbes (Lok *et al*. 2007). The most important application of silver and silver nanoparticles is in medical industry such as topical ointment to prevent infection against burns and open wounds (Ip *et al*., 2006). Silver nanoparticles have been found effective against *E. coli* and *Pseudomonas aeruginosa* (Jain *et al*. 2009); *Bacillus cereus*, *Staphylococcus aureus* (Elumalai *et al*. 2010).

In the present study we report the rapid synthesis of silver nanoparticles using fruit extract of bottle gourd (*Cucurbita lagenaria* L.) and its antibacterial activity against *Klebsiella pneumoniae* NCIM 2719 and *Clostridium acetobutilicum*.

Material and Methods

Plant material and preparation of extract

Unripe fruits of bottle gourd (*Cucurbita lagenaria* L.) were procured from the local market and used to prepare an extract. The fruit rind was removed; 25 g pulp was cut into pieces and boiled in 80 ml sterile distilled water for 30 min. Extract was filtered through Whatman No 1 filter paper. The volume of the filtrate was adjusted to 100 ml by sterile distilled water.

Synthesis of silver nanoparticles

1 mM aqueous solution of silver nitrate was prepared and used for the synthesis of silver nanoparticles. The reaction medium contained 10 ml of bottle gourd (*Cucurbita lagenaria* L.) fruit extract and 90 ml of 1mM aqueous solution of silver nitrate. The reaction was continued for 2 h at room temperature. The colour of reaction mixture changed from colourless to brown.

UV-Vis Spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 2 h, after diluting a small aliquot of sample into distilled water. UV-Vis spectral analysis was done by using UV-2450 (Simatzu).

XRD measurement

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min followed by re-dispersion of the pellet of silver nanoparticles into 10 ml of sterile distilled water. After freeze drying of purified silver nanoparticles, the structure and composition were analyzed by XRD (RIGAKU-D Machine). The crystalline domain size was calculated from the width of XRD peaksusing Scherrer'sequation.

Scherre'requation

$D = K\lambda / \beta \, Cos\Theta$

Where, $\beta = \pi / 180 * FWHM$ (FWHM = Full Width Half Maximum), $\lambda = 1.540598\ A^o$,

$K\lambda = 0.94*1.540598\ A^o = 1.4482$

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done usingPHILIPS-XL-30SEM machine. Thin films of sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the films on the SEM grid were allowed to dry by putting under a mercury lamp for 5 min.

Antibacterialassays

The antibacterial assays were done on *Klebsiella pneumoniae* NCIM 2719 and *Clostridium acetobutilicum*. The bacterial culture of *Klebsiella pneumoniae* NCIM 2719 was procured from National Chemical Laboratory, Pune and that of *Clostridium acetobutilicum* was obtained from Modern College, Pune. Nutrient agar medium was used to culture bacteria. 20 ml molten and cooled media (Nutrient agar) was poured in sterilized petridishes. The plates were left overnight at room temperature to check for any contamination to appear. *Klebsiell apneumoniae* NCIM 2719 and *Clostridium acetobutilicum* were grown in nutrient broth for 24 h. A 100 ml nutrient broth culture of bacterial organism (1 x 10^5cfu/ml) was used to prepare bacterial lawn. Sterile filter paper discs of 6 mm diameter were prepared. One disc was loaded with 30 µl of silver nanoparticles suspended 'hydrosol' and others with 30 µl of positive control drugs were used as positive control. These plates were incubated at 37 ^{0}C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc (Satyavani *et al*. 2011). The diameter of each zone of inhibition was measured after 48 h of incubation.

Results

It was observed that aqueous Ag ions when exposed to fruit extract of bottle gourd (*Cucurbita lagenaria* L.) were reduced in solution, thereby leading to formation of hydrosol. Silver nanoparticles exhibited dark yellowish brown colour in aqueous solution within 2 h (Figure 1). It indicated the formation of silver nanoparticles. Silver nanoparticles formed in the reaction media has absorbance peak at 435 nm on UV Vis-spectrophotometer. The peak was broadened (Figure 2).

The dry powder of the silver nanoparticles was used for XRD analysis. The diffraction intensities were recorded from 10^o to 70^o at 2Θ angles. Figure3 reveals three intense peaks in the whole spectrum of 2Θ value ranging from 10^o to 70^o, corresponding to three diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220) sets of lattice planes were observed. The particles have size ranging between 11.2 to 50.12 nm. An average size of silver nanoparticles synthesized was 27.4 nm and these were spherical in shape.

Figure 1. Photograph of (A) *Cucurbita lagenaria* L. fruit extract, (B) 1.0 mM $AgNO_3$ solution without fruit extract, (C) Colloidal solution of silver nanoparticles

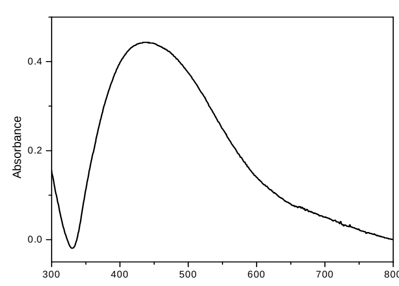

Figure 2.UV-Visible spectra of Ag nanoparticles

The biosynthesized silver nanoparticles by employing bottle gourd (*Cucurbita lagenaria* L.) was further demonstrated and confirmed under scanning electron microscope. The SEM image (Figure 4) showing the high density nanoparticles synthesized by the bottle gourd (*Cucurbita lagenaria* L.) fruit extract were relatively spherical in shape. Silver nanoparticles synthesized by green route using nanoparticles fruit extract of bottle gourd (*Cucurbita lagenaria* L.) were found to be highly toxic to bacteria *Klebsiella pneumoniae* NCIM 2719 (Zone of inhibition 18 mm in diameter) and *Clostridium acetobutilicum* (Zone of inhibition 15 mm).

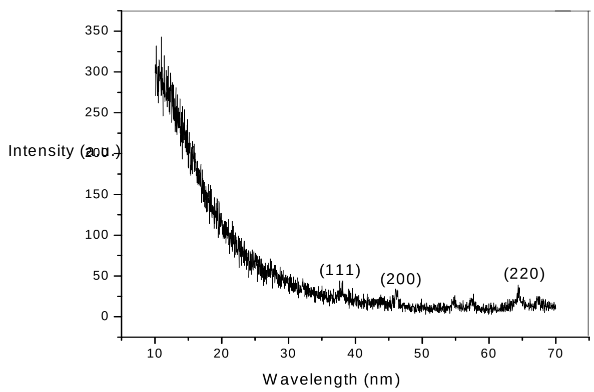

Figure3. XRD pattern recorded for the silver nanaoparticles

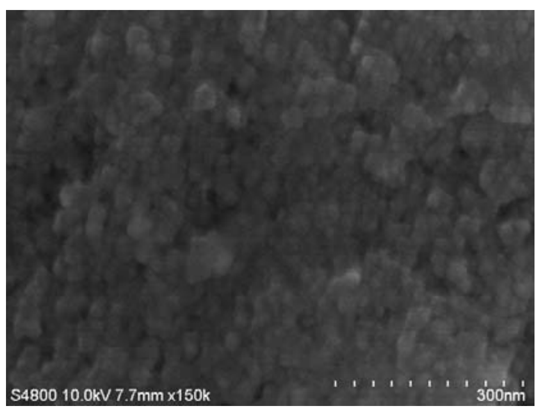

Figure 4. SEM image of silver nanoparticles synthesized using *Cucurbita lagenaria* L. fruit extract

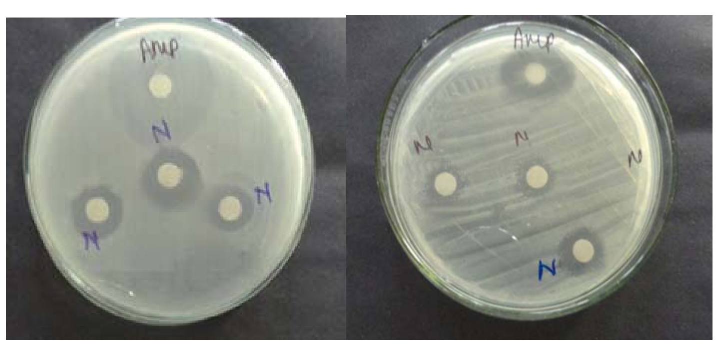

Figure 5. A) *Klebsiella pneumoniae* NCIM 2719 B) *Clostridium acetobutilicum*

Discussion

Reduction of silver ions exhibited brown colour in aqueous solution due to surface plasmon vibrations in silver nanoparticles (Shankar *et al*., 2004). When bottle gourd (*Cucurbita lagenaria* L.) fruit extract was added in the aqueous solution of the silver ion complex, it started to change the colour from watery to yellowish brown due to the reduction silver ion (Figure 1).The fundamental mechanism of biosynthesis of silver nanoparticles is not fully understood (Song and Kim, 2009). Huang *et al*. (2007) reported that polyol compounds and the water soluble heterocyclic compounds are mainly responsible for the reduction of silver ions and the stabilization of the nanoparticles respectively. According to Bajaj *et al*. (2008), proteins are found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles. According to LingaRao and Savitramma (2012), secondary metabolites present in plant systems may be responsible for the reduction of Ag^+ and synthesis of silver nanoparticles. Electrons released during glycolysis for the conversion of NAD to NADH led to transformation of silver nitrate to form silver nanoparticles (LingaRao and Savithramma, 2012).

UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time. Absorption spectra of silver nanoparticles formed in the reaction media after 2 h has absorption peak at 435 nm, broadening of peak indicated that the particles are polydispersed (Figure2). UV-Vis spectroscopy could be used to examine size- and shape – controlled nanoparticles in aqueous suspensions (Wiley *et al*. 2006).

The diffraction intensities were recorded from 10^o to 70^o at 2Θ angles. Figure 3 revealing three intense peaks in the whole spectrum of 2Θ value ranging from 10^o to 70^o, corresponding to three diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220) sets of lattice planes were obtained, which may be index based on the face centered cubic structures of silver nanoparticles. The average size of silver nanoparticles calculated using Scherrer's formula was 27.4 nm. The silver nanoparticles were spherical in shape. Hence from the XRD pattern it is clear that silver nanoparticles formed using fruits of *Cucurbita lagenaria* L. were essentially crystalline in nature.

The SEM image showing the high density silver nanoparticles synthesized by bottle gourd (*Cucurbita lagenaria* L.) fruit extract further confirmed the development of silver nanostructures. The SEM image showed relatively spherical shape nanoparticles formed with range 11.2 to 50.12nm (Figure4). Similar phenomenon was reported by Chandran *et al* (2006).

Silver nanoparticles synthesized using papaya fruit extract showed antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* (Jain *et al*. 2009). Antimicrobial activity of silver nanoparticles has also reported against *Vibrio chorerae*, *Proteus vulgaris, Staphyllococcus aureus* and *Pseudomonas aeruginosa* (Prabhu *et al*. 2010). Silver nanoparticles demonstrated greater bactericidal efficiency compared to penicillin against *E. coli* (ATCC 10536) and *Staphylococcus aureus* (ML 422) (Sarkar *et al*. 2007). In the present investigation, silver nanoparticles have exhibited antibacterial activity against *Klebsiella pneumoniae* NCIM 2719 and *Clostridium acetobutilicum*. Our results are consistent with the earlier reports. With the detailed study of DNA / Protein migration profiles, Gogoi *et al.* (2006) demonstrated that silver nanoparticles have no direct effect on either cellular DNA or protein, although the silver nanoparticles were more efficient bactericidal agent compared to the silver ions (Lok *et al*. 2006). They reported that cell wall rupture due to silver ions and silver nanoparticles. The attachment of both silver ions and nanoparticles to the cell wall caused accumulation of envelops protein precursors which resulted in dissipation of the proton motive force. Silver nanoparticles also exhibited destabilization of the outer membrane and rupture of the plasma membrane there by causing depletion of intracellular ATP. Morones (2005) proposed that the bactericidal mechanism of silver nanoparticles and silver ions are distinctly different. For treatment with silver nitrate, a low molecular weight central region was formed within the cell as a defense mechanism, whereas for treatment with nanoparticles, no such phenomenon was observed, although the nanoparticles were formed to penetrate to the cell wall.

Conclusions

In conclusion, the biosynthesis of silver nanoparticles using fruit extract of bottle gourd (*Cucurbita lagenaria* L.) has been demonstrated. The reduction of metal ions through fruit extracts leading to the formation of silver nanoparticles of fairly well defined dimensions. This green route of silver nanoparticle synthesis has many advantages such as, ease with which process can be scaled up, economic viability and eco-friendliness. The use of easily available and cheap fruits of bottle gourd (*Cucurbita lagenaria* L.) can be used by nanotechnology processing industries. Silver nanoparticles have toxic effect on *Klebsiella pneumoniae* NCIM 2719 and *Clostridium acetobutilicum*. They can be used in medical applications.

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