

Green synthesis of silver nanoparticles using fruit extract of bitter gourd and evaluation of their antibacterial activity against human and plant pathogenic bacteria

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ABSTRACT

The conventional methods such as physical and chemical are used for the biosynthesis of silver nanoparticles. Biological methods of nanoparticle synthesis are cost effective, easily scaled up and environmental friendly. A green synthesis of silver nanoparticles by the reduction of silver ions in the solution by fruit extract of bitter gourd (*Momordica charantia* L.) has been demonstrated. The biosynthesised silver nanoparticles were characterized by UV-Visible spectrophotometer, X-Ray Diffraction and Scanning Electron Microscopy analysis. Nanoparticles were crystalline in nature with diameter of 24.21 nm. Antibacterial activity of synthesized silver nanoparticles was screened. The silver nanoparticles have shown antibacterial activity against *Bacillus subtilis* NCIM 2063 and *Proteus morgani* NCIM 2719; plant pathogenic *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicae bacteria.

Keywords: Silver nanoparticles, antibacterial activity, *Momordica charantia* L.

INTRODUCTION

Nanotechnology is intensively developed during the last decade and represents one of the most important dimensions in the technological developments of the developed countries. Employment of nanoparticles opens new perspective in electronics, chemical industry, energetic, biology and medicine. Nanotechnology influences all aspects of our life. The field of nanotechnology is the most important area of research in modern material science. Nanoparticles exhibit completely new and improved properties based on specific

characteristics as size, distribution and morphology. New applications of nanoparticles and nanomaterials 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 biologist towards nanobiotechnology. Properties of these particles have diverse application including electronic devices [1], chemical and biological sensing [2] and surface enhanced Raman spectroscopy [3].

Nanoparticles can be synthesized by various conventional methods. Some of these are chemical [4] and physical [5], but biological methods of nanoparticle synthesis using microorganisms, enzymes and plant extracts are possible and eco-friendly alternatives to chemical and physical methods [6]. Living organisms have huge potential for the production of nanoparticles of wide applications. Use of plants for nanoparticles synthesis can be advantageous over other biological processes by eliminating elaborate process of maintaining cell cultures. 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 [7].

Bitter melon is a popular vegetable in some Asian countries, where the health benefits of the plant are well-known particularly, its ability to lower blood glucose in diabetics. It has been used to treat diabetes in traditional medicine and is now commercially available as tea (from fruits or leaves), juice, extracts, and pills. Although these products promise health benefits, most of the manufacturers do not offer scientifically proven data on the effectiveness of bitter melon or their products. However, in recent years researchers worldwide have started to focus on the antidiabetic effects of bitter melon. Bitter melon reduces the amount of glucose that is released into the blood by inhibiting the enzymes that break down disaccharides to two monosaccharides (e.g. glucose) [8,9]. It can influence the transport channels for glucose, which also reduces glucose transport into the blood [10]. This effect is important for the treatment of both Type I and Type II diabetic patients and helps to prevent high blood sugar levels after meals.

Insulin is necessary to lower high blood sugar levels. In Type I diabetes, also called insulin-dependent diabetes, the pancreas does not produce or secrete enough insulin to keep blood sugar levels low. Bitter melon has been shown to be effective in treating Type I

diabetes in rats or mice by increasing pancreatic insulin secretion [11,12]. Additionally, scientists found an insulin-like molecule in bitter gourd [13].

Silver has been recognized as having inhibitory effect on microbes [14]. 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 [15]. Silver nanoparticles have been found effective against *E. coli* and *Pseudomonas aeruginosa* [16]; *Bacillus cereus*, *Staphylococcus aureus* [17].

In the present study we report the biosynthesis of silver nanoparticles using fruit extract of bitter gourd (*Momordica charantia* L.) and its antibacterial activity against the human pathogenic bacteria *Bacillus subtilis* NCIM 2063 and *Proteus morgani* NCIM 2719 as well as plant pathogenic bacteria *Xanthomonas axonopodis* pv *citri* and *Xanthomonas axonopodis* pv *punicae*.

MATERIALS AND METHODS

Plant material and preparation of extract

Unripe fruits of bitter gourd (*Momordica charantia* L.) were procured from the campus of Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar 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 bitter gourd (*Momordica charantia* L.) fruit extract and 90 ml of 1mM aqueous solution of silver nitrate. The reaction was continued for 2 h at room temperature.

UV-Vis Spectra analysis

UV-Vis spectroscopy is commonly used to examine size and shape controlled nanoparticles in aqueous suspensions [18] (Wiley *et al.*, 2006). 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-Vis Spectrophotometer (ELICO-SL-191).

XRD measurement

The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10,000 rpm for 20 min. It was 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 studied by XRD (RIGAKU-D Machine). The data was collected in the 2θ range. The crystalline domain size was calculated from the width of XRD peaks using Scherrer's equation.

Dabye- Scherrer's equation

$$D = K \lambda / \beta \cos \Theta$$

Where, D = average crystalline domain size; β is the Full Width at Half Maximum (FWHM), $K = 0.94$, $\lambda = 1.540598 \text{ \AA}$ and Θ is the diffraction angle.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using PHILIPS-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.

Antibacterial assays :

The antibacterial activity of synthesized silver nanoparticles was studied against human pathogenic *Bacillus subtilis* NCIM, 2063 and *Proteus morgani* NCIM 2719; plant pathogenic *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicae bacteria. The bacterial cultures of *Bacillus subtilis* NCIM, 2063 and *Proteus morgani* NCIM 2719 were procured from National Chemical Laboratory, Pune and plant pathogenic *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicae bacteria were isolated from diseased plant materials. Nutrient agar medium was used to cultivate 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. *Bacillus subtilis* NCIM 2063, *Proteus morgani* NCIM 2719, *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicae were grown in nutrient broth for 24 h. A 100

ml nutrient broth culture of bacterial organism (1×10^5 cfu/ml) was used to prepare bacterial lawn. Sterile paper discs of 6 mm diameter were prepared. One disc was loaded with 30 μ l of silver nanoparticles suspended 'hydrosol' and others with 60 μ l. 30 μ l each of ampicilline and tetracycline were used as positive control. These plates were incubated at 37 °C. The plates were examined for evidence of zones of inhibition, which appear as a clear area around the disc [19]. The diameter of each zone of inhibition was measured after 24h of incubation.

RESULTS

It was observed that aqueous Ag ions when exposed to fruit extract of bitter gourd (*Momordica charantia* L.) were reduced in solution, thereby leading to formation of hydrosol. Silver nanoparticles exhibited dark yellowish brown colour in aqueous solution within 2 h. It indicated the formation of silver nanoparticles. (Fig. 2). Silver nanoparticles formed in the reaction media has absorbance peak at 440 nm, broadening of peak indicated that the particles are polydispersed (Fig. 3).



Fig 1. Bitter gourd (*Momordica charantia* L.)

Fig.2 Photograph of bitter gourd (*Momordica charantia* L.) fruit extract , (B) 1.0 mM AgNO₃ solution without leaf extract, (C) Colloidal solution of silver nanoparticles

The dry powder of the silver nanoparticles was used for XRD analysis. The diffraction intensities were recorded from 10° to 80° at 2 θ angles. Figure 4 reveals four intense peaks in the whole spectrum of 2 θ value ranging from 10° to 80°, corresponding to four diffraction facets of silver. Four peaks at 2 θ values of 32.54, 46.62, 67.54 and 77.05

corresponding to (111), (200), (220) and (311) Bragg reflections respectively, which may be index based on the face-centered cubic structure of silver. This reveals that particles are crystalline in nature. The unassigned peaks at $2\theta = 28.11$, 55.09 and 57.83 may be due to crystalline and amorphous organic phases. The particle size ranges between 19 to 25 nm with an average of 24.21 nm and these were spherical in shape. A comparison of our XRD spectrum with the standard confirmed that the silver nanoparticles formed in the experiment were in the form of nanocrystals.

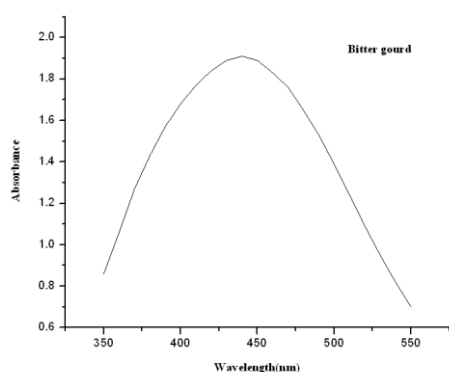


Fig. 3 UV-Visible spectra of silver nanoparticles

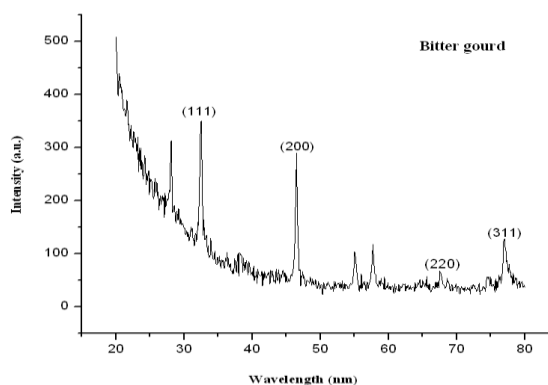


Fig. 4 XRD pattern of silver nanoparticles

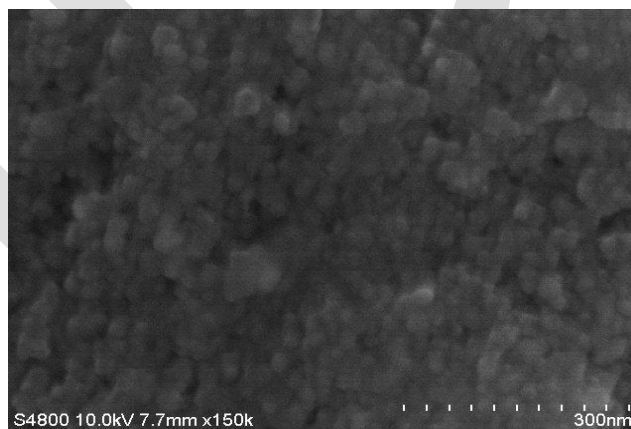


Fig. 5 SEM image of silver nanoparticles synthesized using bitter gourd (*Momordica charantia* L.) fruit extract

The biosynthesized silver nanoparticles by employing bitter gourd (*Momordica charantia* L.) was further demonstrated and confirmed under scanning electron microscope.

The SEM image (Fig. 5) shows the high density and relatively spherical nanoparticles synthesized by the bitter gourd (*Momordica charantia* L.) fruit extract .

Silver nanoparticles synthesized by green route using fruit extract of bitter gourd (*Momordica charantia* L.) were found to be highly toxic to bacteria *Bacillus subtilis* NCIM 2063, *Proteus morgani* NCIM 2719, *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicae. The highest inhibitory activity was recorded for *Xanthomonas axonopodis* pv punicae (15 mm) and lowest for *Bacillus subtilis* NCIM 2063 (8 mm).

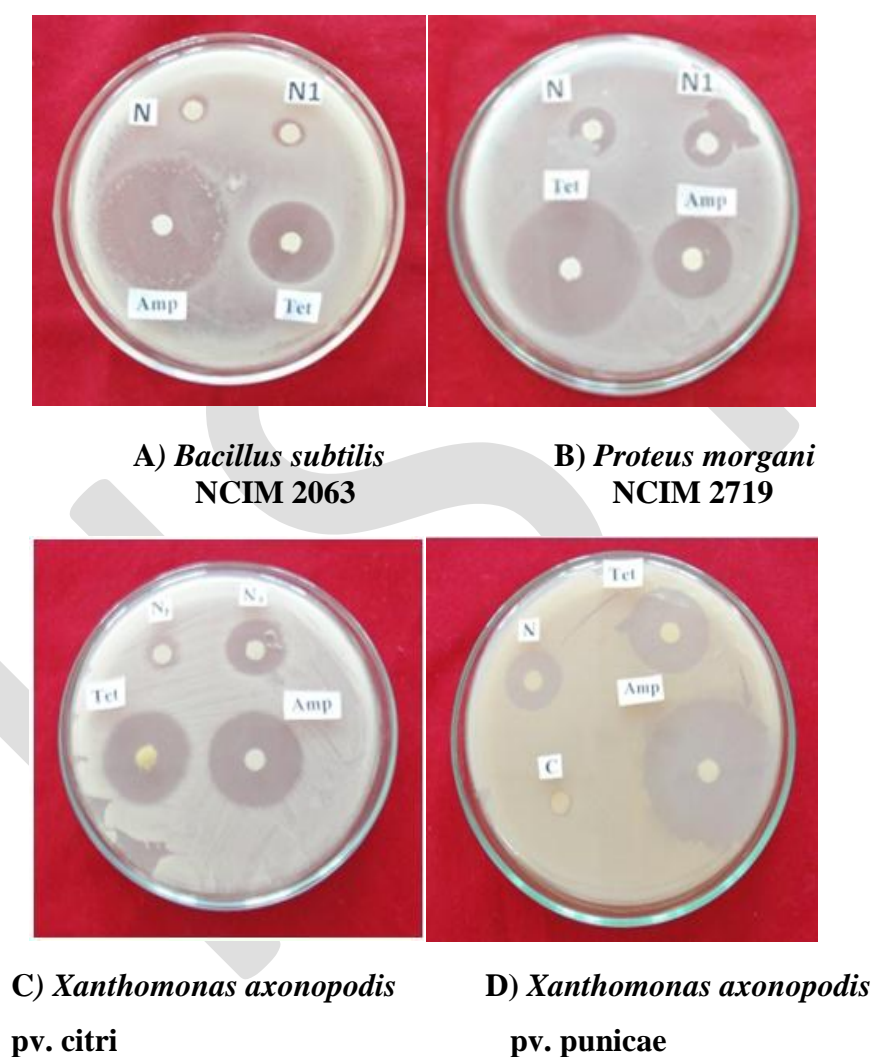


Fig. 6 Antibacterial activity

DISCUSSION

When bitter gourd (*Momordica charantia* 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 (Fig. 2); which indicated formation of silver nanoparticles. Reduction of silver ions exhibited brown colour in aqueous solution due to surface plasmon vibrations in silver nanoparticles [20]. Silver nanoparticles have been synthesized using leaf extracts of several plants such as *Parthenium* [7]; *Ocimum sanctum* [21]; *Trienthera decandra* [22]; *Euphorbia hirta* [17]; *Murraya koenigii* [23]; *Citrullus colocynthis* [19]; *Moringa oleifera* [24]; *Rhinacanthus nasutus* [25]; *Ipomoea carnea* [26]; *Sasbania grandiflora* [27]. The fundamental mechanism of biosynthesis of silver nanoparticles is not fully understood [6]. It was 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 [28]. According to another report, proteins are found to be responsible for the reduction of metal ions when plant extracts are used for the synthesis of silver nanoparticles [29]. Secondary metabolites present in plant systems may be responsible for the reduction of Ag^+ and synthesis of silver nanoparticles [30].

UV-Vis spectroscopy could be used to examine size- and shape – controlled nanoparticles in aqueous suspensions [18]. Absorption spectra of silver nanoparticles formed in the reaction media after 2 h has absorption peak at 440 nm, broadening of peak indicated that the particles are polydispersed (Fig. 3).

The diffraction intensities were recorded from 10° to 80° at 2θ angles. Figure 4 revealing four intense peaks in the whole spectrum of 2θ value ranging from 10° to 80° , corresponding to four diffraction facets of silver. A number of Bragg reflections corresponding to the (111), (200), (220), (311) 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 24.21 nm. The silver nanoparticles were spherical in shape. Hence from the XRD pattern it is clear that silver nanoparticles formed using fruits of bitter gourd (*Momordica charantia* L.) were essentially crystalline in nature.

The SEM showing the high density silver nanoparticles synthesized by bitter gourd (*Momordica charantia* L.) fruit extract further confirmed the development of silver nanostructures. The SEM image showed relatively spherical shape nanoparticles. (Fig 5). Similar phenomenon has been reported [31].

Silver nanoparticles synthesized by using plant extracts exhibited antibacterial activity against *E. coli* and *Pseudomonas aeruginosa* [16]; *E. coli*, *Proteus* and *Pseudomonas* species [32]; *E. coli*, *Salmonella*, *Shigella* and *Vibrio cholera* [33]; *Salmonella enterica* and *Staphylococcus aureus* [27]; *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *E. coli* [25]; *Staphylococcus aureus* and *E. coli* [34]; *Staphylococcus aureus* NCIM 2079 and *Pseudomonas aeruginosa* NCIM 2200 [26]; *E. coli* and *Staphylococcus aureus* [35]. In the present investigation silver nanoparticles synthesized by using bitter melon fruit extract showed antibacterial activity against *Bacillus subtilis* NCIM 2063, *Proteus morgani* NCIM 2719, *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicea. The highest inhibitory activity was recorded for *Xanthomonas axonopodis* pv punicea (15 mm) and lowest for *Bacillus subtilis* NCIM 2063 (8 mm).

Fruit extracts of bitter melon (*Momordica charantia* L.) is capable of synthesizing silver nanoparticles in aqueous solution. These silver nanoparticles revealed to possess an antibacterial activity against *Bacillus subtilis* NCIM 2063, *Proteus morgani* NCIM 2719, *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicea. The mechanism of synthesis of silver nanoparticles by plants can be used in the drug production for human and plant diseases which are caused by multidrug resistant microorganisms.

CONCLUSION

In conclusion, silver nanoparticles can be synthesized by using fruit extract of bitter melon (*Momordica charantia* L.). 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 bitter melon (*Momordica charantia* L.) can be used by nanotechnology processing industries. Silver nanoparticles have toxic effect on human pathogenic bacteria such as *Bacillus subtilis* NCIM 2063 and *Proteus morgani* NCIM 2719; plant pathogenic bacteria *Xanthomonas axonopodis* pv citri and *Xanthomonas axonopodis* pv punicea. They can be used in medical applications and in the management of plant diseases.

ACKNOWLEDGEMENT

Authors are thankful to the Principal, Annasaheb Awate Arts, Commerce and Hutatma Babu Genu Science College, Manchar (Affiliated to University of Pune) and Rayat Shikshan Sanstha, Satara for providing laboratory facilities.

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