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Green Synthesis, Characterization and Antimicrobial Evaluation of Nano Silver on Mastitis Causing Microorganisms

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A B S T R A C T

Nanotechnology is now creating a growing sense of excitement in the field of life science. Nano particles can be synthesized by physical and chemical method but green synthesis method is the most emerging approach of preparation due to eco friendly and less time consuming procedure. This study aimed at green synthesis, characterization and evaluation of antibacterial effect of nano silver on mastitis causing bacteria. Aqueous coriander leaf extract with certain concentration of reducing agent were used for the production of silver nano particles. Characterization of synthesized silver nano particles was done by Zeta sizer analysis. The result of zeta sizer analysis reveals that the diameters of nano particles are within the range of nano scale. Transmission electron microscopy (TEM) analysis of silver nano particles was done to confirm the shape, size and morphology. Antibacterial activity of nano silver was investigated on mastitis causing bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus* by well diffusion method. The antibiosis test result indicated that silver nano particles are able to inhibit *E. coli* and *S. aureus* but unable to inhibit *P. aeruginosa*. Therefore green synthesized nano silver can be used as anti microbial agent for mastitis causing bacteria.

Keywords

Bacteria, Mastitis, Nanotechnology, Silver

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Introduction

A nano material is defined as elements having at least one dimension in length, ranges between 1 nm and 100 nm. Nano particles (NPs) have wide range of applications in areas such as health care, cosmetics, food and feed, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics and space industries (Nel *et al.*, 2006). NPs can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time for synthesis of large quantity of NPs, the chemicals used are toxic and lead to non- eco friendly by products. Thus, there is an increasing demand for green synthesis of NPs (Dietz and Herth, 2011).

Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, tollens, biological, and irradiation method which have advantages over conventional

methods involving chemical agents associated with environmental toxicity. Among plants, *Coriandrum sativum* are easily available and has been used in synthesis of various nano particles. Silver (Ag) nano particles have been widely used as an effective antimicrobial agent against bacteria, fungi, and viruses (Aziz *et al.,* 2015). Their effect was recognized already in ancient times. Ag and its compounds have long been used for the disinfection of medical devices and water purification. In medicine, Ag compounds are commonly applied to treat burns, wounds, and a variety of infectious diseases ((Aziz *et al.,* 2015). Considering the advantages of green synthesis, the present investigation was aimed at production of silver nano particles. Its characterization by TEM and its effect on mastitis causing microorganisms like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Materials and Methods

Green synthesis of AgNps by using *Coriandrum sativum*

Leaf extract of *Coriandrum sativum* was prepared by boiling chopped coriander leaves with distilled water. 50ml of 1mili molar silver nitrate ($AgNO_3$) solution was prepared by taking 50 ml distilled water with 0.016gm silver nitrate. Prepared plant extract were added at different concentration to the $AgNO_3$ solution. This setup was incubated in a dark chamber to minimize photo-activation of silver nitrate at room temperature. Reduction of $AgNO_3$ was observed on the basis of colour change from green to reddish brown (Ahmed *et al.,* 2015) (Fig. 1).

Characterization of AgNps

TEM analysis was performed to determine the shape and size of synthesized nanoparticles. NPs samples were diluted in ependroff tubes using double distilled water and placed in an ultrasonic bath for 15 minutes. After that one drop of NPs samples were placed over a copper grid and leave for overnight drying and finally observed under TEM (JEOL-2010)**.**

Collection of bacterial pathogens

Mastitis causing microorganisms likes *staphylococcus aureus* (*S. aureus) (MTCC-1144), Escherichia coli (E. coli) (MTCC-443), Pseudomonas aeruginosa (MTCC-1019)* were obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India

Evaluation of antibacterial activity of AgNPs

Antibacterial effect was observed on mastitis causing microorganisms like S. *aureus, P. aeruginosa, E. coli* by well diffusion method. Mueller Hinton agar medium (Hi-media) was prepared for lawn culture of the supplied microorganisms. Then by using a puncher well diffusion method was done by adding 50µl plant extract solution as control. Synthesized nano particle solution in same amount also added to each culture plate. Incubation was done at 37^{o}C for 24 hour. Antibacterial activity was measured on the basis of the inhibition zone around the well.

Results and Discussion

The first indicator of synthesis of AgNPs using *C. sativum* leaf extract in the form of greenish and then dark brown coloured pigment. The change in colour from greenish to brown confirmed a quick reduction of silver ions (Ag) to zero valent silver ($AgNO_3$). This difference in colour could be considered as a primary evidence of reducing potential of *C. sativum* leaf. Plants leaves contain a substantial number of organic constituents, like phenolic compounds and various types of glycosides that help in synthesis of metal nano particles (Lin *et al.,* 2016) The TEM analysis result of AgNPs demonstrated that the silver

nano particles are predominantly spherical in shape. These are well dispersed and homogenous. The average diameter of AgNPs was around 40-60nm (Fig. 2). Result indicate *C. sativum* synthesized NPs having average size range bellow 100 nm with spherical in shape. That denote the quality of the NPs is as good as synthesized by chemical or biological process (Zhang *et al.*, 2011; Shirsat *et al.*, 2015).

The antimicrobial activity of newly synthesized AgNPs was assessed against mastitis causing bacterial pathogens (Fig. 3). Well diffusion method was performed against mastitis causing bacteria such as *E.coli*, *S. aureus*, *P. aeruginosa*. Inhibition zone well observed in case of *E. coli* and *S. aureus* but no inhibition zone was observed against *P. aeruginosa.* Results from the present study demonstrate that the antibacterial activity of NPs may be due to the generation of reactive oxygen species (ROS), malondialdehyde (MDA), and leakage of proteins and sugars in bacterial cells ((Aziz *et al.*, 2015). Furthermore, low concentration of silver nano particles were found to be highly effective in inducing loss of cell viability in both gram positive and gram negative bacteria. Ag NPs at a concentration of 1mM prevent the growth of mastitis causing bacteria.

Fig.1 Synthesis of silver nano particles by using *Coriandrum sativum*

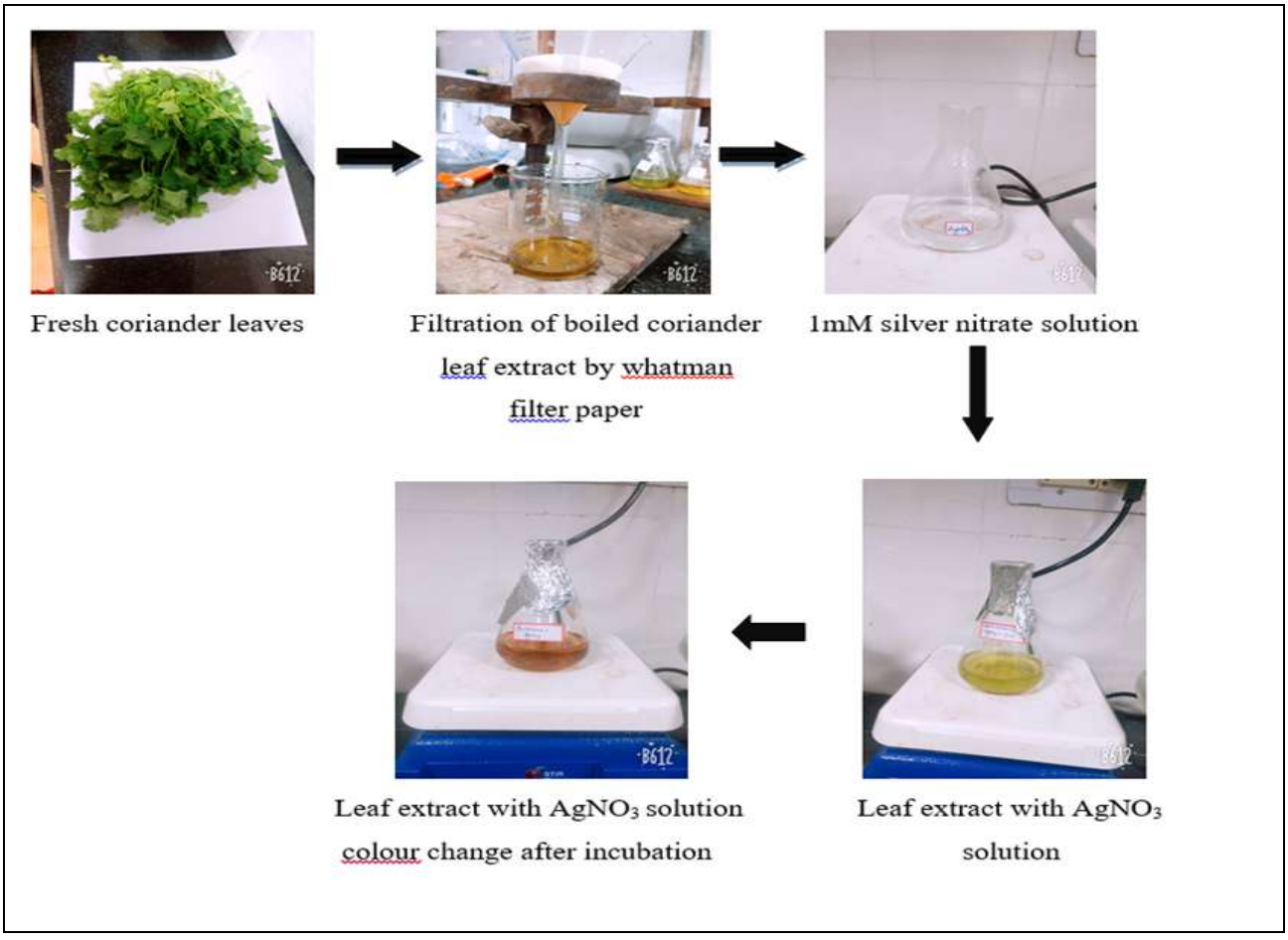


Fig.2 Size of the silver nano particles by using TEM

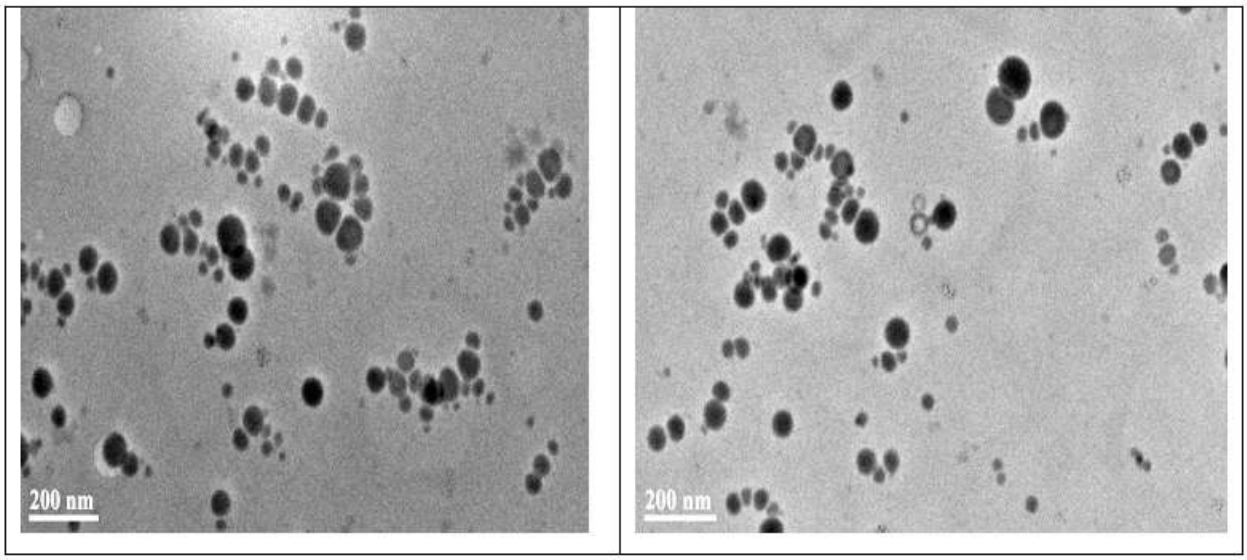
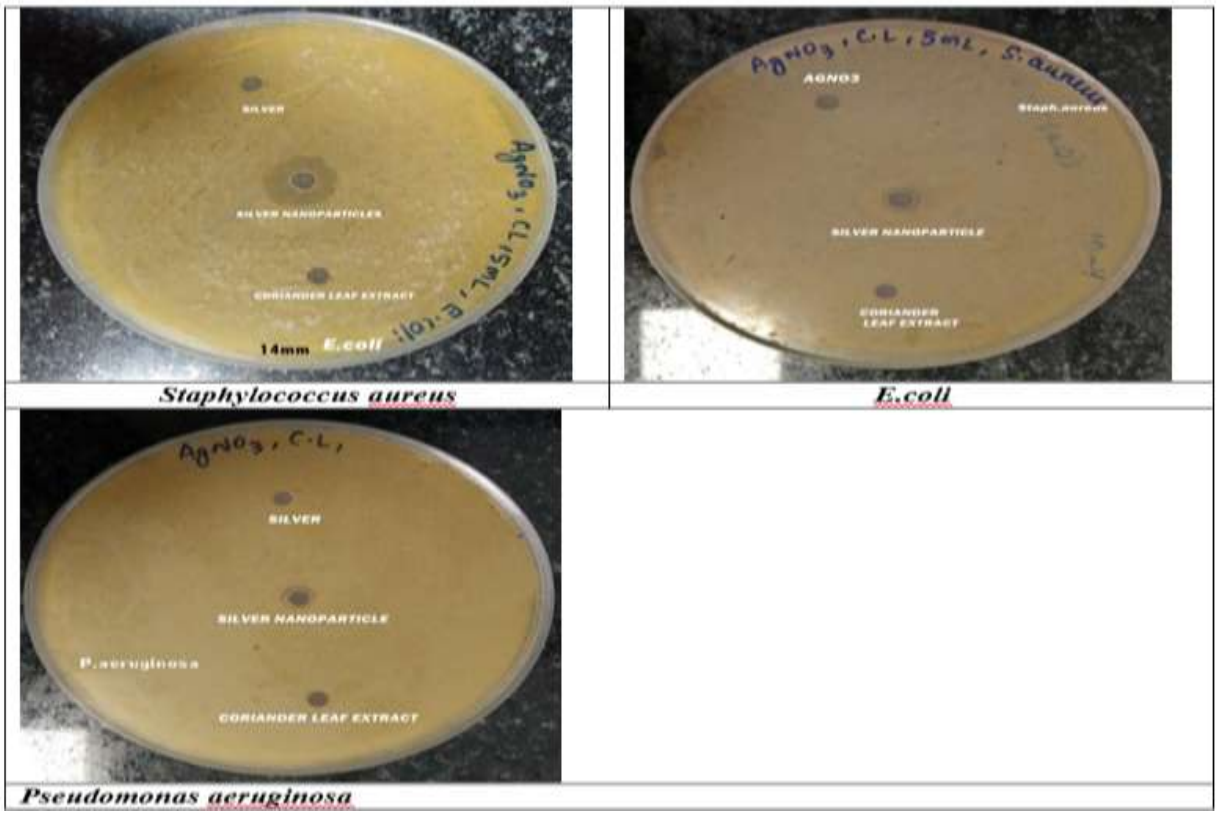


Fig.3 Effect of nano silver infusion on mastitis causing microorganisms



Similarly Ahmed *et al.*, (2015) and Mirzajani *et al.*, (2011) observed that Ag NPs showed antibacterial activities against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*).

In conclusion, AgNPs can be synthesized by using *Coriandrum sativum leaves* and may be highly beneficial for prevention of growth of gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*) bacteria.

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