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Green Synthesis, Characterization and Antimicrobial Evaluation of Selenium Nano Particles on Mastitis Causing Bacteria

Adyasha Debata, Kamdev Sethy*, R.K. Swain, S. Maity, S. Pati, D. Jena and S. Tripathy

Department of Animal Nutrition, College of Veterinary Science and Animal Husbandry,
Odisha University of Agriculture and Technology, Bhubaneshwar-751 003, India

ABSTRACT

Nanotechnology is now creating a growing sense of excitement in the field of life science. Nano particles can be synthesized by chemical, biological and green methods, 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 selenium on mastitis causing bacteria. Aqueous extract of *Coriandrum sativum* leaf extract were used for the production of selenium nano particles. Characterization of synthesized nano particles was done by zeta sizer analysis and transmission electron microscope. The result of zeta sizer analysis reveals that the diameters of nano particles are within the range of nano scale *i.e.* less then 100 nm. Transmission electron microscopy analysis of selenium nano particles was done to confirm the shape, size and morphology. Antibacterial activity of nano minerals was investigated on mastitis causing bacteria such as *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* by well diffusion method. The antibiosis test result indicated that selenium nano particles were able to inhibit *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa.* So, it may be concluded that the advances in nanotechnology has enabled us to utilize particles of nano size of selenium as a therapeutic application against mastitis causing bacteria in the coming years.

Key words: Antibiosis, Green synthesis, Nanoparticles, Selenium

INTRODUCTION

A nano material is defined as elements having at least one dimension in length, ranges between 1 nm and 100 nm (Khan, 2017). Nanoparticles (NPs) have wide range of applications in areas such as health care, cosmetics, food, environmental health, mechanics, optics, biomedical sciences, chemical industries, electronics and space industries (Zhang *et al.,* 2012). It creates and uses structures that have novel properties because of their small size. It has been referred to as the next industrial revolution. NPs can be synthesized using various approaches including chemical, physical, and biological. Although chemical method of synthesis requires short period of time, but chemicals used for NPs synthesis and stabilization are toxic and lead to non-eco friendly by products (Elumalai and Velmurugan, 2015). Thus, there is an increasing demand for green nanotechnology. Green synthesis of nano particles makes use of environment friendly and non-toxic. In other words, Green nanotechnology is the utilization of various plant resources for the biosynthesis of metallic nano particles. Green synthesis approaches include mixed-valence polyoxometalates, polysaccharides, tollens and biological method, which have advantages over conventional methods involving chemical agents associated with environmental toxicity (Solanki *et al.,* 2008). Among plants, *Coriandrum sativum* are easily available and has been used in synthesis of various nano particles including selenium (Zhang *et al.,* 2012). Selenium nano particles (SeNPs) have been widely used as an effective antimicrobial agent against bacteria, fungi, and viruses (Zhang *et al.,*2004). Their effect was recognized already in ancient times. Although the SeNPs mechanism of action is still not clear, small diameter SeNPs have a superior antimicrobial effect to those of a larger diameter. Considering the advantages of green synthesis, the present investigation was aimed at production of SeNPs by green synthesis method, its characterization and evaluation of its anti microbial effect on mastitis causing microorganisms such as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.

*Corresponding Author: E mail: babuivri@gmail.com

MATERIALS AND METHODS

Coriandrum sativum leaves were washed thoroughly with the tap water followed by double distilled water to remove debris and other contaminants. The leaves were heated with distilled water at 60 ºC for 15min. Filtration was done by using Whatman filter paper (0.2µm). 5ml prepared leaf extract were added to 100ml of 1mM sodium selenite in an Erlenmeyer flask. The flask was placed on the magnetic stirrer for 24 hours for constant stirring. Reduction of selenite into elemental selenium was confirmed by appearance of red colour of the solution. In order to determine the particle size and potential, Zeta sizer (Malvern Zetasizer Nano ZS90) was used under room temperature and the peak was observed. Transmission electron microscopy (TEM) analysis was performed to determine the shape and size of synthesized nanoparticles. NPs samples were diluted in eppendorf 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, Japan).

Mastitis causing microorganisms likes *staphylococcus aureus* (*S. aureus)* (MTCC-1144), *Escherichia coli (E. coli) (MTCC-443), Pseudomonas aeruginosa (P. aeruginosa)* (MTCC-1019) were obtained from Institute of Microbial Technology (IMTECH) Chandigarh, India. Antibacterial effect was observed on mastitis causing microorganisms like S. *aureus, P. aeruginosa, E. coli* by well diffusion method. Muller Hinton agar medium (Hi-media) was prepared for lawn culture of the supplied microorganisms. Then by using a puncher well diffusion method 50µl of synthesized NPs was added. Incubation was done at 37ºC for 24 hours. Antibacterial activity was measured on the basis of the inhibition zone around the well.

RESULTS AND DISCUSSION

The first indicator of synthesis of SeNPs using *C. sativum* leaf extracts is the formation of dark red coloured pigment. The change in colour from pale yellow to red confirmed a quick reduction of Se ions to nano selenium. This difference in colour could be considered as a primary evidence of reducing potential of *C. sativum* leaf extract (Fig. 1). The particle size of the synthesized SeNPS were analysed by using Zeta potential, particle size analyzer which measure the average particle size. For this study the SeNPs solution was placed in a particle size analyser (Malvern Zetasizer Nano ZS90) at 25 ºC shows the average particle size of nanoparticle below 100 nm. Whereas the high intensity peak observed at 79.8 nm denotes that SeNPs synthesized by coriander leaf extract have high poly-disparity index and standard in size (Fig. 3). The TEM analysis result of SeNPs demonstrated that the particles are predominantly spherical in shape. These are well dispersed and homogenous. The average diameter of SeNPs was around 40-60nm (Fig. 2). The red coloured formation confirmed a quick reduction of Se ions to nano selenium. The transmission electron

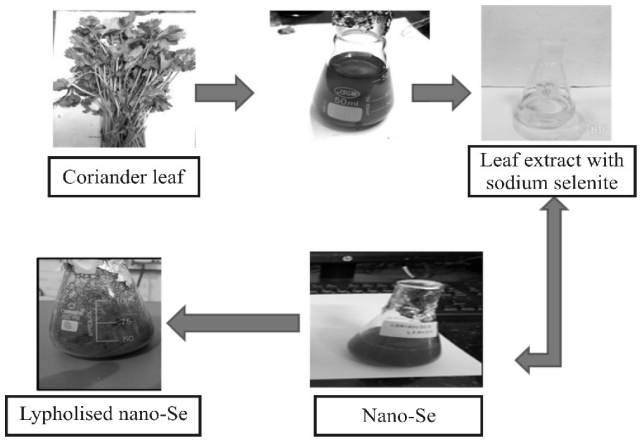


Fig 1. Green synthesis of nano se by using Coriandrum sativum

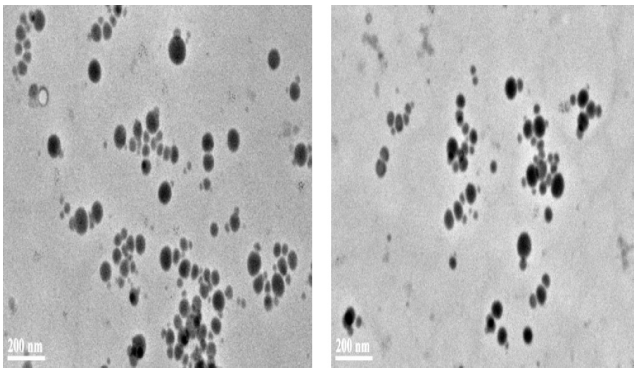


Fig 2. Transmission electron microscopy of synthesized nano-Se

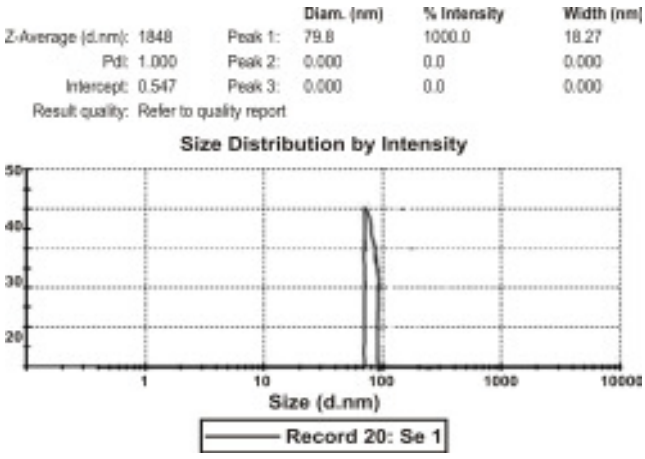
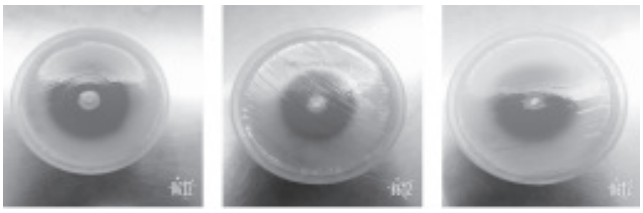


Fig 3. Zeta sizer analysis of synthesized nano-Se

microscope (TEM) analysis demonstrated that the particles are predominantly spherical in shape. The average diameter of SeNPs was around 40-60nm. Ingole *et al.* (2010) also reported that by using glucose as a reducing agent, the size of SeNPs synthesized, as determined by TEM, was in the range of 20-80 nm. Dwivedi *et al.* (2011) also reported that by using an organic acid as a reducing agent, the size of the Se nanoparticles synthesized as determined by TEM was in the range of 40-100 nm.

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. Therefore, research work of Balamurugan and Sami Nathan (2014), Ahmed *et al.* (2015) and Santhoshkumar *et al.* (2014) taken as a reference to synthesize Se nano particles using various leaves extract. However, for the first time *Coriandrum sativum* was used for the synthesis of Se nano particles.

Zeta potential analysis of synthesized nano particles was done which indicate the average size of the particles and determine the surface charge of nano particles in solution. This study revealed that *Coriandrum sativum* leaf extract is a good candidate for synthesizing metal nanoparticles high stabilization properties. TEM analysis of NPs determines the size and shape of individual particles. Result indicate *Coriandrum sativum* synthesized NPs having average size range bellow 100 nm with spherical in shape. That denote the quality of the NPs synthesized by green method is as good as synthesized by chemical or biological process (Zhang *et al.,* 2011).



Staphylococcus aureus | Pseudomonas aeruginosa | Escherichia coli

Fig 4. Antibiosis of Se- nano particles on mastitis causing bacteria

The antimicrobial activity of newly synthesized SeNPs was assessed against mastitis causing bacterial pathogens showed well observed inhibition zone in case of *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Fig. 4). Nano- Se at a concentration of 1mM prevent the growth of mastitis causing bacteria namely *Pseudomonas aeruginosa, Staphylococcus aureus* and *E. coli* which were commonly isolated from milk samples produced by mastitis infected cows. The minimum inhibitory concentrations (MICs) of NPs against *P. aeruginosa* and *S. aureus* were found to be 1 and 2ìg/mL, respectively. Our findings suggest that NPs exert antibacterial effects in a dose and time dependent manner. 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 (Zhang *et al.,*2004). Se NPs at a concentration of 1mM prevent the growth of mastitis causing bacteria. When selenium nanoparticles were mixed with bacterial solution, the growth of bacteria was inhibited after 3 hours (compared with the control). The inhibitory effects continued after 4 and 5 hours. Similarly, Erskine *et al.* (1989) reviewed Selenium is a cofactor for enzyme glutathione peroxidase. Inside the cell, cytoplasm contains (70 %) and mitochondrial matrix contains (30 %) glutathione peroxidase activity. Feeding cows 2mg of supplemental selenium/day starting 3 months before calving and throughout lactation reduced the severity and duration of mastitis

caused by experimentally challenging cows with *Escherichia coli.* Malbe (2006) suggested dietary Se supplementation to diets deficient in Se reduced the rate of new intra-mammary gland infections in dairy cows.

CONCLUSION

Coriandrum sativum can be used as a suitable medium for green synthesis of nano Se and the synthesized nano Se have antibacterial affects against mastitis causing bacteria.

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