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Research Article

Antibacterial Effect of Green Synthesized Silver Nanoparticles Against *Vibrio* sp. Isolated from Broiler Chicken

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ABSTRACT

In the present study, *Ocimum sanctum* plant leaf extract was used for extracellular synthesis of metallic silver nanoparticles (AgNps) to investigate antibacterial activity against *Vibrio* sp. isolated from broiler chicken. Stable AgNps were formed after adding aqueous solution of 0.75 mM $AgNO_3$ with the *O. sanctum* plant leaf extract. Quantitative analysis of green synthesized AgNps was monitored by UV-visible spectroscopy. The size and shape was characterised by atomic force microscopy (AFM). In UV-visible spectroscopy, the peak length was reduced within three hours and the average size of the nanoparticles ranged from 10 to 15 nm was observed in the AFM. Bacteriological test were performed in luria-bertani (LB) medium on solid agar plates supplemented with different concentration of 100 to 400 µl green synthesized AgNps. Among the various concentration, 300 µl and 400 µl of nanoparticles showed higher inhibitory activity against the *Vibrio* sp. The present study clearly indicated that the green synthesized AgNps has potent antibacterial activity against *Vibrio* sp. *O. sanctum* based AgNps may be suitable for the formulation of new types of bactericidal activity and which can be effectively used in poultry farms for treating the *Vibrio* disease.

Keywords: Green synthesis, *Ocimum sanctum, Vibrio* sp, poultry, broiler chicken.

1. INTRODUCTION

Nanobiotechnology is the budding field in biological sciences and biosynthesis of nanoparticles has received increasing attention due to the growing need to develop environment friendly and sustainable methods. The cost effective bioreduction of various metals to nano-sizes with various shapes, size and control disparity is an important aspect of nanotechnology. Green synthesis of nanoparticles is capable to meet the requirements of diverse industrial application [1-3]. It has been known that silver (Ag) and its compound processes broad spectrum of antibacterial activities since ancient times [4, 5]. Silver has been used as a disinfectant to prevent the human infection in medical filed because of its natural antimicrobial activity towards many pathogens such as bacteria, viruses and fungi [6]. It is well known that silver ions and silver based compounds are highly toxic to microorganism [7, 8] due to the toxic effect of their ions; it shows strong biocidal effects on 16 species of bacteria [9]. However, silver exhibits fairly low toxicity against human [10]. Both elemental silver and silver compounds have been used as an antimicrobial coating materials for catheters [11], wound dressings [12], bone cements [13], and dental materials [14]. Silver particles can provide a large reservoir of silver ions that has been released gradually and support for long-term antimicrobial activity [15].

Eco-friendly materials like plant leaf extract [16], bacteria [17], fungi [18], actinomycetes and yeasts have been used for the green synthesis of silver nanoparticles as it offers numerous benefits. Thus the biologically synthesized nanoparticles are highly useful for biomedical applications and also it act as an alternative to physical and chemical control. In India, poultry industry has grown tremendously and shown steep rise in production in the last three decades because of the improved technologies in intensive culture practices and health management. Poultry production has faced many challenges when over intensification, poor management practices, farm environment degradations and variety of other factors lead to various diseases. Disease is the one of major concern and it has a greatest challenge for the poultry producers. Antibiotics and vaccine have been used by poultry farmers for treating the diseases. However antibiotics and bactericidal resistance are the major issues of recent days in poultry industry. To our knowledge there is not much more report on the antibacterial activity of biosynthesised silver nanoparticles using *O. sanctum* against *Vibrio* sp. isolated from broiler chicken. Keeping the above facts in mind, the present study was designed to find out the antibacterial efficacy of green synthesised silver nanoparticles as an alternative control measure against the bacterial diseases in poultry production.

2. MATERIAL AND METHODS

2.1. Green synthesis of silver nanoparticles from *O. sanctum* leaves

Ocimum sanctum plants leaves were collected from Karaikudi, Sivagangai district, Tamil nadu, India. The plants were examined and identified by a student indentifying sheet and species specific characters were confirmed in consultation with a botanist *O. sanctum* leaves extract (Fig. 1) was used to synthesis of silver nanoparticles based on the green synthesis procedure [16]. 7 gm of *O. sanctum* leaves was washed thoroughly with sterile distilled water and air dried. The air dried leaves were cut into fine pieces and mixed with 350 ml of sterile distilled water and then the mixture was boiled for 5 min. After boiling, the extract was filtered through Whatman filter paper no.1 to obtain a clear leaf broth. An aqueous solution of 0.75 mM $AgNO_3$ was added to the leaf broth for reducing Ag^+ ions into Ag^o and incubated in dark room. The reaction was observed by monitoring the colour change in the solution, which indicates the formation of green synthesized silver nanoparticles (AgNps).

2.2. Characterization of green synthesized silver nanoparticles

The green synthesis of AgNps was monitored periodically (0 to 24 hrs) in a UV-visible Spectrophotometer (Shimadzu UV-1800) between the wavelength of 200-700 nm. The synthesized silver nanoparticles were collected from the second day onwards and centrifuged at 10,000 rpm for 15 mins. The purified pellets were dried at 45°C and the powder was dissolved in distilled water to obtain desired concentration for further studies. The size and the morphology of the AgNps were examined in the atomic force microscopy (AFM diCP II Veecs, USA).

2.3. Isolation of *Vibrio* sp.

Fresh intestine samples of broiler chicken were collected from the slaughter houses of various regions in Sivagangai district (Arenthangi, Karaikudi, Kundrakudi, Siruvatthi and Devakottai), Tamilnadu. Immediately after collecting the intestine, samples were immersed in normal saline in a sterile container. One gm of intestine sample was mixed with 1 ml of sterile distilled water for homogenization. The homogenized samples were serially diluted, plated on thiosulphate citrate bile salts sucrose agar medium and incubated for 24 hrs at 37°C followed by series of biochemical analysis to isolate the *Vibrio* colonies [19]. Selected strains were sub cultured on nutrient agar to get the pure strains.

2.4. Antibacterial activity of silver nanoparticles

Agar bioassay method was used to examine the antibacterial effect of green synthesized silver nanoparticles on *Vibrio* sp. isolated from broiler chicken. The test *Vibrio* strain was grown overnight at 37°C in a medium containing alkaline peptone water at pH 7.8 ± 0.2 and 100 µg of these *Vibrio* strains at the cell density of 10^4 CFU/ml seeded in luria bertani broth along with the four different concentrations of silver nanoparticles 100 to 400 µl. The petri plates were incubated at 37°C for 24 hrs and observed for the antibacterial effect. Positive and negative control plates were also maintained for the comparison and colony forming units per ml was measured.

3. RESULTS

3.1. Green synthesized silver nanoparticles from *O. sanctum* leaves

Reduction of silver ions to silver nanoparticles during the exposure of *O. sanctum* leaf extracts was observed visually by monitoring the colour changes (Fig. 2). After adding the *O. sanctum* leaf extract, within 15 minutes the reaction was started and colour was changed into reddish brown, which indicates the formation of green synthesized silver nanoparticles.

Fig. 1: Leaves of Ocimum sanctum plant

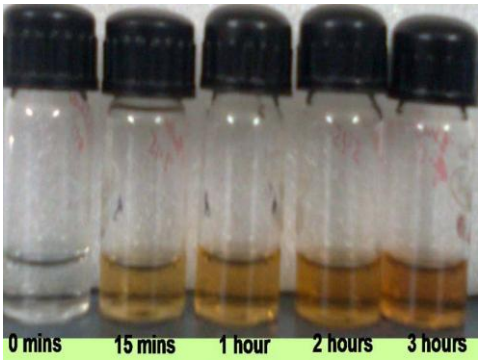

Fig. 2: The synthesis of AgNPs by Ocimum sanctum leaf extracts

3.2. Characterization of green silver nanoparticles

The spectrum of the AgNps samples were characterised by UV-visible spectroscopy. In UV-visible spectra of green synthesised silver nanoparticles showed peak values which was continued to increase for three hours. After three hours, the peak length was reduced and saturation phase was attained and this phase was observed up to 24 hrs (Fig. 3). Absorption spectra of silver nanoparticles formed in the reaction media

showed the highest peak at 450 nm (Fig. 3). The AFM technique used to visualize the size and shape of extracellular biosynthesized AgNps is shown in Fig. 4. The morphology of nanoparticles is variable with spherical shape and particle diameter ranged from 10 to 15 nm size.

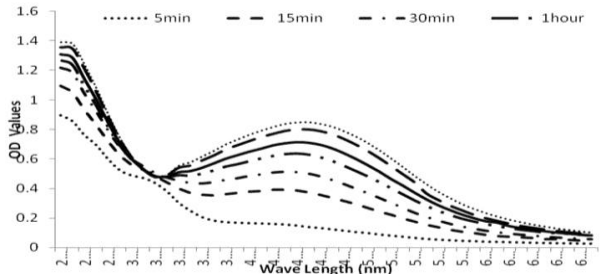

Fig. 3: UV-Spectra analysis of Ocimum sanctum leaf mediated silver nanoparticles synthesis with different time intervals

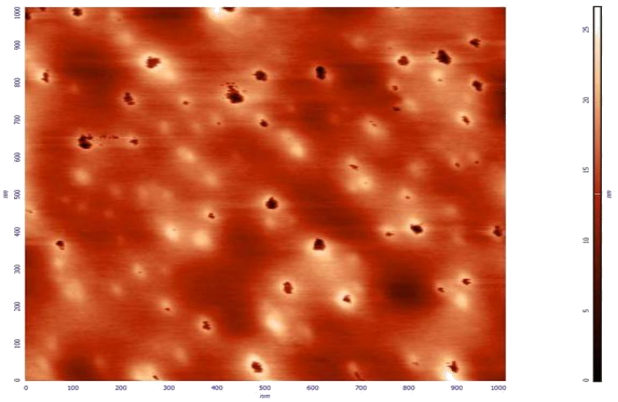

Fig. 4: Atomic force microscope photograph showing the AgNps with 10 to 15 nm size

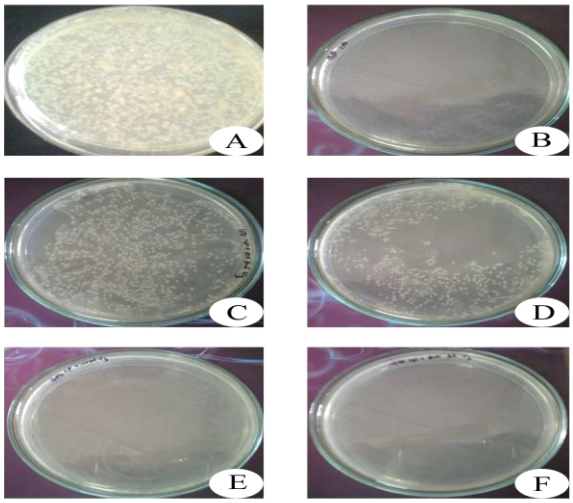

Fig. 5: Inhibition of Vibrio sp. at different concentration of AgNps using agar bioassay method

A-Vibrio culture, B-Control AgNps, C-100 µl AgNps with 100 µl of Vibrio culture, D-200 µl AgNps with 100 µl of Vibrio culture, E-300 µl AgNps with 100 µl of Vibrio culture, F-400 µl AgNps with 100 µl of Vibrio culture

3.3. Antibacterial activity of green synthesized AgNps against *Vibrio* sp.

Green synthesized AgNps *O. sanctum* showed a strong inhibitory activity against *Vibrio* sp. isolated from broiler chicken. LB agar medium with the different concentration of 100 to 400 µl silver nanoparticles showed different levels of inhibitory activity when compared with the positive control. The maximum inhibitory activity was observed against *Vibrio* sp. in plates containing 300 µl and 400 µl concentrations of green synthesized silver nanoparticles (Fig. 5).

4. DISCUSSION

The study of green synthesis of nanomaterials offers a valuable contribution to biomedicine at nanobiotechnology. Hence, the present study focused on the green synthesis of AgNps from *O. sanctum* leaf extract and its antibacterial effects on *Vibrio* sp. isolated from broiler chicken. The application of nanoparticles to control poultry bacterial diseases is largely unexplored. Number of methods has been employed to synthesize AgNps, including green chemistry. Although a number of physical and chemical methods [21] available for the synthesis of AgNps, there is a need to develop an eco-friendly process [22]. In this study, silver mediated aqueous leaf extracts of *O. sanctum* was used to synthesize AgNps which inhibited the growth of *Vibrio* sp. The plant extracts are widely being applied to synthesize AgNps by reducing Ag^{+} ions into Ag^{o} which increases the optical density of the solution [20]. This was due to the excitation of surface plasmon resonance (SPR) and the reduction of silver nitrate during the incubation period [23].

The silver nanoparticles exhibit reddish brown colour in aqueous solution due to excitation of surface plasmon vibrations [16]. In this study, we also observed reddish brown colour formation within 15 minutes after adding the clear leaf extract of *O. sanctum* with the aqueous solution of silver ion complex which indicated the synthesis of silver nanoparticles (Fig. 2). While clear leaf extract of *O. sanctum* was mixed in the aqueous solution of silver ion complex, it reduced the silver ion and produce reddish brown.

The absorption spectra of silver nanoparticles formed in the reaction media showed absorbance peak at 450 nm and within 3 hrs the peak length was reduced and saturation phase was obtained. The small size and morphology of surface planar accessibility of the AgNps dictated its efficient antibacterial potential [7]. The green synthesis of AgNps has been reported from bacteria [8] and angiosperms [24]. Several studies have confirmed the antimicrobial properties of AgNps [25, 26], physiochemical properties and partial oxidation surface (Ag^{1+}) of the spherical AgNps showed higher antibacterial activity than that of the totally oxidized surfaces (Ag) [27]. In the present

study, the size and morphology of nanoparticles was characterized by AFM. The results of the AFM clearly shown the formation of nanoparticles with spherical shape and the size of the nanoparticles varied from 10 to 15 nm (Fig. 4).

The application of antibiotic and bactericides are the common method for managing poultry bacterial diseases. Respected applications of antibiotics may develop the resistance to the bacteria and that may produce virulent bacteria to the industry. Therefore, it is very much essential to develop new type of alternative methods to control bacterial disease in poultry. The concentration of the nanoparticles and increase in CFU influences the complete inhibition [28]. Nanosilver particles can enter into the bacterial cells [29], possibly by disturbing the cell permeability and respiratory functions [30]. Ag^{+} ions suppress the bacteria by affecting the sulphur group of the biomolecules [31] and it has been suggested that the nanoparticles cause denaturation to the cell wall protein and other proteins avert prevent the replication process [9, 29, 32, 33]. In the present study, it was observed that there was gradual decrease of CFU with the reference to positive control plates. As negative control a total reduction of *Vibrio* sp. was achieved at 300 µl concentration of AgNps (Fig. 5). In the present study, plant based AgNps in *O. sanctum* showed maximum inhibitory activity against the *Vibrio* sp isolated from broiler chicken. This study suggests green synthesised nanoparticles have been effectively used as an alternative to antibiotic of bacteriocides against broiler chicken production.

5. ACKNOWLEDGEMENTS

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