Research Article

Potential of Silver Nanoparticles Using *Melia azedarach L.* Against Some Bacterial Pathogens.

A. Josephine Anbarasi, *M. Chitra.

PG and Research Department of Biochemistry, S. T. E. T Women's College, Mannargudi – 614001, Tiruvarur (Dt), Tamil Nadu, India.

ABSTRACT

Nanoscience will leave no field untouched by its ground breaking technical innovations; the agricultural sector is no exception. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. A number of plants are being currently investigated for their role in the synthesis of nanoparticles. Gold nanoparticles with a size range of 2- 20 nm have been synthesized using the live alfa alfa plants The antibacterial effects of Ag salts have been noticed since antiquity and Ag is currently used to control bacterial growth in a variety of applications, including dental work, catheters, and burn wounds. Different biological methods are gaining recognition for the production of silver nanoparticles (Ag-NPs) due to their multiple applications. The use of plants in the synthesis of nanoparticles emerges as a cost effective and eco-friendly approach. One of the most important applications of Ag –NPs is their use as an anti-bacterial agent. *Melia azedarach* L. was selected in this study for producing silver nanoparticles that are tested against *E coli, Bacillus subtilis* and *Enterobacter aerogenes*. It was found that *Melia azedarach* nanoparticles display strong potential for the synthesis of silver nanoparticles as antibacterial agents by rapid reduction of silver ions. The silver nanoparticles of *Melia azedarach* showed better antibacterial activity against *E coli, Bacillus subtilis, Enterobacter aerogenes*.

Keywords: Nanoscience, silver nanoparticles, *melia azedarach* anti bacterial activity.

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***Address for correspondence:**
Dr. M. Chitra
PG and Research Department of Biochemistry, S. T. E. T Women's College, Mannargudi – 614001, Tiruvarur (Dt), Tamil Nadu, India.
E-mail: mschitra21@yahoo.com

INTRODUCTION

The use of nanoparticles is gaining impetus in the present century as they posses defined chemical, optical and mechanical properties. Among them, the metallic nanoparticles are most promising as they contain remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance against metal ions, antibiotics, and the development of resistant strains [1,2]. The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides [3]. Silver has long been known to exhibit a strong toxicity to a wide range of 116 micro-organisms [4]. For this reason silver-based compounds have been used extensively in many bactericidal applications [5, 6]. Silver compounds have also been used in the medical field to treat burns and a variety of infections [7]. Several salts of silver and their derivatives are commercially employed as antimicrobial agents [8]. Commendable efforts have been made to explore this property using electron microscopy, which has revealed size dependent interaction of silver nanoparticles with bacteria [9].

It is well known that Ag ions and Ag-based compounds are highly toxic to microorganisms, showing strong biocidal effects on as many as 12 species of bacteria

including E. coli [10]. Recently, Mecking and co-workers showed that hybrids of Ag nanoparticles with amphiphilic hyperbranched macromolecules exhibited effective antimicrobial surface coating agents [11]. In this study a *Melia azedarach* was selected to synthesis silver nanoparticles and its antibacterial activity.The stem-bark and root-bark contain in the alkaloid azaridine (margosine), Sterol and tannins. The leaves have the alkaloid, paraisine and the flavonoid, rutin. The seeds are rich in fatty oil consisting of stearic, palmitic, oleic acid and linoleic acids. It has insecticidal activity [12], antiviral activity [13], spermicidal activity [14], antifertility activity [15].

MATERIALS AND METHODS

Fresh and young leaf samples of *Melia azedarach* L. were collected, washed thoroughly with sterile double distilled water (DDW) and surface sterilized with 0.1% HgCl2 for2-3 min under the hood of laminar air flow. Twenty grams of sterilized leaves samples were taken and cut into small pieces. Finely cut leaf was placed in a 500ml Erlenmeyer flask containing 100ml of sterile DDW. After that the mixture was boiled for 5 min and filtered. The extract was stored at 4oC. 5ml of leaf was added to 100ml of 1mM AgNO3 (99.99%) aqueous solution in a conical flask of 250 ml at room temperature. The flask was there after put into shaker (150 rpm) at 30oC and reaction was carried out for a period of 48h.

Antibacterial activity was determined, using the agar well diffusion assay method. The bacterial test organisms such as, *E coli, Bacillus subtilis, Enterobacter aerogenes* were grown in nutrient broth 24h. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter. [16]. The bactericidal activity of silver nanoparticles was checked by determining the MIC [17].

Time dependent antibacterial activity:

The silver nanoparticles were suspended in millipore water to conduct the time-dependent antibacterial study. *E. coli, Bacillus substilis, Enterobacter aerogenes* cells were treated with 2.0 ml of each concentration (0, 10, 20, 30, µg ml-1) of silver nanoparticles as well as with varying time intervals for each concentration (0, 1, 3, 6 and 12 h). Before using the silver nanoparticles,the suspension was homogenized using ultrasonicator. Each treated bacterial culture was serially diluted till 106 dilution factor and 100 µl from each culture was homogeneously spread in NA agar plates. All plates were incubated at 37°C for 24 h and the number of colonies grown on agar plate was counted.

Growth pattern:

Growth pattern of *E. coli, Bacillus substilis, Enterobacter aerogenes* was studied with 0, 10, 20 and 30, µg ml-1 concentration of homogenized silver nanoparticles. *E. coli, Bacillus substilis, Enterobacter aerogenes* cells were treated with varying concentrations of silver nanoparticles as mentioned above and inoculated in 250 ml of Erlenmeyer flask. All the flasks were put on rotary shaker (180 rpm) at 37°C. Untreated culture flask was used as control. Optical density was measured after every hour (upto 16 h) using UV– Visible spectrophotometer at 600 nm.

Zone of inhibition:

Zone of inhibition test was performed in NA agar plates supplemented with 0, 10, 20 and 30, µg ml-1 of silver nanoparticles. For this, 20 ml NA agar was poured in well rinsed, autoclaved petri plates, 1.0 ml of active bacterial culture was homogeneously spread in the agar plates and paper disc containing different concentration of Ag nanoparticles were placed in agar medium. The plates were incubated at 37°C for 24 h. The zone size was determined by measuring the diameter of the zone.

RESULTS AND DISCUSSION

Toxicity studies of silver nanoparticles on human pathogen opens a door for a new range of antibacterial agents. The growth of the *E. coli, Bacillus substilis, Enterobacter aerogenes* cells are inhibited at a concentration of 10,20 and 30 µg ml-1 of *Melia azedarach* silver nanoparticles. Both Ag nanoparticles and AgNO3 inhibited the growth of *E. coli* cells at the different concentration but the rate of inhibition appears to be slow with increasing concentration of AgNO3 compared to silver nanoparticles. The duration of treatment markedly affected the *E. coli, Bacillus substilis, Enterobacter aerogenes* population.

When treatment duration increased from 1 to 12 h, 10 and 20µg ml-1 concentrations *Melia azedarach* AgNO3 compared to silver nanoparticles causes better inhibition on microorganisms. On the other hand 30µgml-1 of silver nanoparticles cause 100 per cent growth inhibition but at the same concentration of AgNO3 inhibit only 80 percent of growth during initial phase of treatment.

Nanoparticles caused a growth delay of *E.coli*, *Bacillus substilis, Enterobacter aerogenes* at lower concentration where growth was stopped altogether that was observed in silver nanoparticles. The growth rate of bacteria increased steadily with the increase in time at all concentrations, the growth was slightly affected in 10 and 20µgml-1 of AgNO3 but greater reduction was observed under different concentration of silver nanoparticles. The silver nanoparticles caused 100 per cent growth reduction when treated with 30 µg ml-1 concentrations. Zone of inhibition test was done for identification of degree of inhibition by different concentration of silver nanoparticles. It was found that 30 µg ml-1 concentrations was able to inhibit bacterial growth and create a zone of 1.8, 1.9 and 1.7cm by SNPs. The increasing concentration of Ag nanoparticle showed a consistent increase in the zone size and reached the maximum diameter in silver nanoparticles, respectively at 30 µg ml-1 concentrations.

The silver nanoparticles at 30 µg ml– 1 concentration showed 100 per cent growth reduction, whereas in AgNO3 much less effect was observed. The mechanism of inhibitory action of silver ions on microorganism shows that upon Ag+ treatment, DNA loses its replication ability and expression of ribosomal subunit proteins, as well as other cellular proteins and enzymes essential to ATP production, becomes inactivated [18]. It has also been hypothesized that Ag+ primarily affects the function of membrane bound enzymes, in the respiratory chain. It is well known that Ag ions and Ag-based compounds have strong antibacterial effects and many investigators are interested in using other inorganic nanoparticles as antibacterial agents [19]. These inorganic nanoparticles have a distinct advantage over conventional chemical antibacterial agents. The most important problem caused by the chemical antibacterial agents is multidrug resistance. Generally, the antibacterial mechanism of chemical agents depends on the specific binding with surface and metabolism of agents into the microorganism [20].

Various microorganisms have evolved drug resistance over many generations. The mechanism of the inhibitory effects of Ag ions on microorganisms is partially known. Some studies have reported that the positive charge on the Ag ion is crucial for its antimicrobial activity through the electrostatic attraction between negative charged cell membrane of microorganism and positive charged nanoparticles [21]. In contrast, Sondi and Salopek-Sondi [22] reported that the antibacterial activity of silver nanoparticles on Gram-negative bacteria was dependent on the concentration of Ag nanoparticle, and was closely associated with the formation of 'pits' in the cell wall of bacteria. Then, Ag nanoparticles accumulated in the bacterial membrane caused the permeability, resulting in cell death. In these study of antibacterial property of SNPs against *E-coli, Bacillus substilis and Enterobacter aerogenes* had been investigated. It was showed that the antibacterial activity of SNPs was size dependent. Silver nanoparticles mainly in the range of 1-10nm attach to the surface of cell membrane and drastically disturb its proper function like respiration and permeability of micro organism.

Table 1: Zone of inhibition of micro organisms.

Treatments	Zone of inhibition (cm)		
	E-Coli	Bacillus Substilis	E.aerogenes
AB	0.7	0.7	0.7
NN	0.1	0.1	0.1
SNPs	1.8	1.9	1.7

AB – Antibiotics as control (chloramphenicol – 100 ug/ml), NN – Plant extract of *M.azedarach*
SNPs – Silver nanoparticles of Melia azedarach L.

Table 2: MIC of micro organisms.

Concentration (µg/ml)	Minimum inhibitory Concentration (cm)		
	E. coli	*Bacillus substilis*	*E. aerogenes*
10	3.8	3.2	2.5
20	4.5	3.8	2.8
30	36	33	30

CONCLUSION

It concluded that the bio-reduction of silver ions through *Melia azedarach* extracts was carried out and it was tested for its antibacterial activity. The results indicated that *Melia azedarach* silver nanoparticles have good antibacterial activity against different microorganisms such as *E.coli, Bacillus substillis*, *Enterobacter aerogenes*, and it was confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the preparation of drugs used against bacterial diseases. A detailed study of unraveling the underlying mechanism behind the high antibacterial activity of Ag nanoparticles should be carried out in future.

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