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Antimicrobial activity of silver Nanoparticles using leaves extract of *Alstonia scholaris*

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Abstract
The present study is an attempt to test the antibacterial efficacy of SNPs produced by using the leaf extract of *Alstonia scholaris*, which have been using in traditional medicine without any validation. Plant extract of *Alstonia scholaris* was prepared. Then silver nanoparticle was prepared using $AgNO_3$. The antimicrobial activity of the nanoparticles was demonstrated against both gram positive and gram negative bacteria. The study indicates that gram positive bacteria are relatively resistant to the bactericidal action of silver nanoparticles than gram negative bacteria. Thus present study confirms the use of *Alstonia scholaris* plant for biosynthesis of silver nanoparticles and its potential use against microbes.

Keywords: Nanoparticle, SNPs, *Bacillus subtilis, Staphylococcus aureus*

1. Introduction
Nowadays Nanotechnology has become one of the most promising technology of all areas of science. Nanotechnology is used to bring better impact in the areas of drug development. This is extensively useful in diverse areas of health science such as diagnostics, cancer treatment and targeted drug delivery system. The term metal nanoparticle is used to describe nanosized metals within the size range of 1-100 nm. In the present scenario, pharmaceutical and biomedical sectors are facing the problems regarding continuous increase in the multidrug-resistant (MDR) human pathogenic microbes. Antibiotic resistance ultimately causes the emergence and re-emergence of multidrug-resistant (MDR) pathogens and parasites [1]. It is increasingly difficult to treat infectious diseases due to the development of bacterial resistance against antibiotics. Therefore it is necessary to formulate new methods of treatment. It has been reported that silver nanoparticles (SNPs) are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [2]. In small concentrations, silver is safe for human cells, but lethal for microorganisms [3]. Even though many people know that silver has superior antibacterial and antibiotic characteristics, there has been limitation on the application in real life because of darkening at high temperature and high cost. Nanotechnology solved all these problems at the same time. Therefore, nanosilver is now considered as one of the most viable alternatives to antibiotics because it seems to have high potential to solve the problem of antibiotic resistance, which is often observed in several mutant bacterial strains [4-6]. Silver nanoparticles will combine with the cell walls of pathogenic bacteria and then directly get inside the bacteria and quickly combine with sulphydryl (-SH) of oxygenic metabolic enzyme to deactivate them, to block cellulas respiration and metabolism and suffocate the bacteria. Other possibility to the bactericidal action of silver nanoparticles is the release of silver ions from the nanoparticles [7].
Alstonia scholaris is an evergreen tropical tree in the family of Apocynaceae. It is native to the Indian subcontinent, Indomalaya, Malesia, and Australasia. It is a moderate to large sized tree grows upto 30 meters in height. The leathery simple leaves are elliptical, ovate, linear or lanceolate and wedge-shaped at the base. It is known from Ayurveda that *Alstonia scholaris* is useful as an astringent herb for the management of diseased condition of skin disorder, malaria, urticaria, chronic dysentery, diarrhea etc. [8]. This work is aimed to study the antibacterial efficacy of SNPs produced by the leaf extract of *Alstonia scholaris.*

2. Material and Methods
2.1 Preparation of plant extract
Fresh leaves of *Alstonia scholaris* were collected. The leaves were washed thoroughly with distilled water and airdried. After that leaves were kept in the hot air oven at 60^0C for 2-3 hours these leaves were ground to get the fine powder. 30.0 g of dried powder was boiled in 100 ml of phosphate buffer, pH 8.0 for 30 minutes.

After cooling at room temperature, it was centrifuged at6000 rpm for 10 minutes and filtered The filtrate was stored at 4 °C for further experiments as reducing as well as stabilizing agents in the synthesis of AgNPs.

2.2 Preparation of silver nanoparticles using Alstonia scholaris extract

For the synthesis of silver nanoparticles 1mM aqueous solution of silver nitrate (AgNO3) was prepared. 10 ml of *Alstonia scholaris* leaves extract was added into 90 ml of aqueous solution of 1 mM Silver nitrate and incubated in the dark, overnight at room temperature. After overnight incubation in the dark brownish yellow colour solution was formed, which indicated the formation of silver nanoparticles. The reduction of pure Ag2+ ions were monitored by measuring the UV-Vis spectrum of the reduction media at 5 hours after diluting a small aliquot of the sample in distilled water by using systronic 118 UV-Vis Spectrophotometer.

2.3 UV-visible spectrum analysis

Equal amount of sample aliquot and distilled water (1ml each) were mixed in a 10 mm-optical-path-length quartz cuvettes, and the UV-vis spectrum analysis of the reaction mixture was carried out to detect the reduction of pure Ag+ ions. The concentration of AgNPs produced was measured using a Systronics UV double beam spectrophotometer, at a resolution of 1 nm, between 200 and 800 nm (Fig. 1).

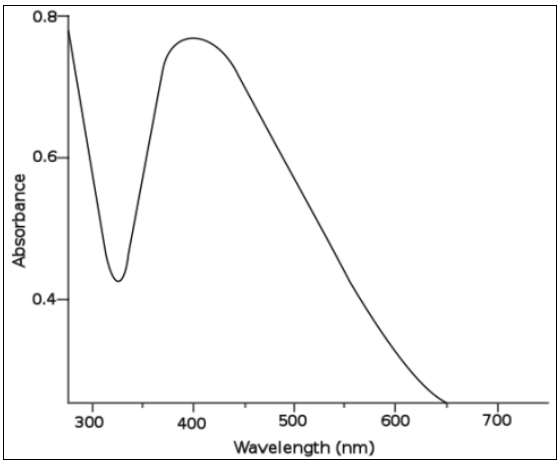

Fig 1: Absorption peak of silver nano particles using *Alstonia scholaris* under UV- Vis absorption spectroscopy.

2.4 Antimicrobial Sensitivity Assay

The antibacterial activities of SNPs were carried out by Cup plate method. Cup plate method is one of the official methods in IP, where the test samples diffuse from the cup through an agar layer in a Petri dish or plate to such an extent that the growth of added microorganisms is restricted entirely to a circular area or zone around the cavity containing the solution of an antibiotic substance [9]. The agar plates were kept for incubation at 37^0C for 24 hours. The antimicrobial activity for control, SNPs and silver nitrate is expressed as zone diameter in millimeters, which is measured by a scale. The experiments were repeated thrice and mean values of zone diameter were presented. The experiments of antimicrobial activity were carried out against pure culture of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris.*

3. Results and discussion

The green synthesis of silver nanoparticles through plant extracts were carried out. It is evident that reduction of silver ion into silver particles during the reaction with plant extracts could be followed by characteristic color change [10]. It is well known that silver nanoparticles appear as yellowish - brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [11]. The presence of yellowish-brown colour in the reaction flasks indicates the formation of silver nanoparticles (SNPs) [12] (Fig-1). Characterization of biosynthesized nano silver particles was carried out using UV-Vis Spectroscopy. UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been determined as a function of time. Absorption spectra of nano silver particles formed in the reaction media has absorbance peak at 434 nm, and from the peak it is clear that the particles are polydispersed because there is indication of widening of peak (Fig.1).

Toxicity studies on pathogen opens a door for nanotechnology applications in medicine. Biological synthesis of metal NPs is a traditional method and the use of plant extracts has a new awareness for the control of disease, besides being safe and no phytotoxic effects [13]. The biologically synthesized silver nanoparticles using medicinal plants were found to be highly toxic against different pathogenic bacteria of selected species. The anti-microbial activities of biosynthesized silver nanoparticles against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris* were investigated. Both silver ions and silver nanoparticles were known to have excellent anti-microbial activities [14]. The result obtained showed the silver nanoparticle to be effective against the tested bacterial isolates at 1mM concentration (Table 1). The antimicrobial activity of the nanoparticles was demonstrated against both gram positive and gram negative bacteria. The study also confirms that gram positive bacteria are relatively resistant to the bactericidal action than gram negative bacteria. The inhibition zone produced by gram–positive bacteria *S. aureus* and Bacilus subtilis are 8mm. and 6mm respectively as compared to gram-negative bacteria *E. coli* (10mm) and *Proteus vulgaris (13mm).* There are two explanations as to why gram-positive bacteria are less susceptible to Ag^+ than gram-negative bacteria. The first involves the charge of peptidoglycan molecules in the bacterial cell wall. Gram-positive bacteria have more peptidoglycan than gram-negative bacteria because of their thicker cell walls, and because peptidoglycan is negatively charged and silver ions are positively charged, more silver may get trapped by peptidoglycan in gram-positive bacteria than in gram-negative bacteria [15]. The decreased susceptibility of gram-positive bacteria can also simply be explained by the fact that the cell wall of gram-positive bacteria is thicker than that of gram-negative bacteria. This result is in line with those found by [16]. However, due to the rapid emergence of mutant strain and antibiotic resistance the silver nanoparticles will be the best alternative.

Table 1: The antimicrobial activity of silver nanoparticle synthesis using leaf extract of *Alstonia scholaris*.

Zone of inhibition in millimeter			
Microorganisms	**Silver nanoparticle**	**Positive control**	**Negative control**
Bacillus subtilis	6	20	6.5
Bacillus subtilis	6	20	6.5
Staphylococcus aureus	8	21	7.0
Escherichia coli	10	22	7.5
Proteus vulgaris	13	21.5	7.0

Positive control: Streptomycin & Negative control: Silver nitrate

4. Conclusion

The present study of biosynthesis of silver nanoparticles used *Alstonia scholaris* leaf extract as reducing and capping agent. The method is cost effective and environmental friendly. The synthesis of silver nanoparticles were confirmed by the change of colour of plant extracts. These environmentally benign silver nanoparticles were further confirmed by using UV-Vis spectroscopy. The antimicrobial activity of these nanoparticles was well demonstrated against both gram positive and gram negative bacteria. The study also confirms that gram positive bacteria are relatively resistant to the bactericidal action than gram negative bacteria. The approach of use of *Alstonia scholaris* extract for biosynthesis of nanoparticles is novel and can be adopted to biosynthetic approaches of other metal nanoparticles. Thus present study confirms the use of *Alstonia scholaris* plant for biosynthesis of silver nanoparticles and its potential use against microorganisms. Though the effectiveness of *Alstonia scholaris* as reducing agent for biosynthesis of silver nanoparticles awaits further study.

5. References

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