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BIOSYNTHESIS OF SILVER NANOPARTICLES USING *Azadirachta indica* LEAF EXTRACT AND ASSESSMENT OF ITS ANTIBACTERIAL ACTIVITY ON SOME PATHOGENIC ENTERIC BACTERIA

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Abstract: **Silver is known for its antimicrobial activity, silver nanoparticles are gaining great importance due to their antimicrobial activities. "Green technology" is the use of various plant materials for the biosynthesis of nanoparticles, as it does not involve any harmful chemicals. Bioactive compounds such as flavonoids, terpenoids etc present in plant extracts have made them best material for the green synthesis of nanoparticles. In this study we have reported the synthesis of silver nanoparticles by reducing the silver ions present in the silver nitrate solution by the aqueous extract of *Azadirachta indica* leaf. Silver nanoparticles (AgNPs) were successfully synthesized using *A. indica* leaf extract and the formation and stability of the reduced silver nanoparticles in the colloidal solution were monitored using UV-Vis spectroscopy. The broad surface plasmon resonance (SPR) peak was at 437nm. The antibacterial effect of the synthesized AgNPs produced was studied using some enteric ptathogenic bacteria such as *Salmonella* Typhi, *Pseudomonas aeuruginosa* and *Escherichia coli*. From the disc diffusion results, the highest antibacterial activity of synthesized AgNPs was found against *S.* Typhi (14mm) and inhibitory zone of 12mm was recorded for *E. coli* and *P. aeruginosa* respectively. The synthesized AgNPs showed excellent antibacterial property compared to the $AgNO_3$ solution and *A. indica* leaf extract. It could be concluded that *A. indica* leaf extract can be used effectively in the production of antimicrobial AgNPs for commercial applications.**

Keywords: ***Azadirachta indica*, Antibacterial activity, Biosynthesis, Silver nanoparticles.**

1. INTRODUCTION

Resistance of microorganisms to common antibiotics has become a great burden to general healthcare facilities, especially in developing nations with little or improper medical resources (Marasini *et al*., 2015). The treatment of bacterial infection with antibiotics is a route which is rapidly becoming more and more difficult to sustain, due to increase in emergence and re-emergence of multidrug resistant pathogens (Taylor *et al.*, 2005). In pursuit of novel treatment, there is growing interest in the use of nanomaterials with antimicrobial potentials to combat the menace of pathogen cessation, since their large surface to volume ratio ensures a broad range of attack on bacterial surface. Microbes also find it difficult to acquire resistance toward nanoparticles as they target multiple bacterial components, contrary to the mechanistic action of conventional antibiotics (Dhanalekshmi *et al*., 2013).

Nanoparticles can be synthesized via different approaches namely chemical, physical, and biological (Shah *et al.*, 2013). In comparison with chemical and physical methods, biological synthesis has many advantages: it is simple, cost effective, non-hazardous, environmentally friendly and easily scaled up for large scale synthesis (Veerasamy *et al*., 2011). Also, processes employed for making nanoparticles using plant extracts are readily scalable and less expensive in comparison to the relatively expensive methods based on microbial processes and whole plants (Mittal *et al*., 2013), nanoparticles produced from plant extract possesses medicinal properties and could be used in drugs, targeted drug delivery and cosmetic industry (Saranyaadevi *et al*., 2014). One of the most promising nanoparticles which act as a highly effective antimicrobial agent is silver. Various investigations on silver nanoparticles have been done to study its antimicrobial activity, silver nanoparticle exhibited significant antibacterial activity against *Escherichia coli, Staphylococcus aureus* and antifungal activity against *Trichophyton, Trichosporon beigelii* and *Candida albicans* (Gajbhiye et *al*., 2009).

Plant extracts have enzymes and phytochemicals such as terpenoids, flavonoids and phenolic compound (Rao *et al*., 2013) which act as bioreductants as well as capping agent in the presence of metal salt for nanoparticles synthesis. This study deals with the synthesis of silver nanoparticles from neem leaf. Neem (*Azadirachta indica*) commonly called 'India Lilac' or 'Margosa', belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential. It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species (Sreeram *et al*., 2008). Various parts of the neem tree have been used as traditional Ayurvedic medicine in India, neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter. Neem oil finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and phthisis (Sreeram *et al*., 2008). Biosynthesis of silver nanoparticles has been performed using a number of plants, some of which include *Aloe vera* extract (Chandran *et al*., 2006), *Allium sativum* (Garlic) extract (Von White, 2012) *Svensonia hyderobadensis* (LingaRao and Savithramma, 2011), *Acalypha indica* (Vasireddy *et al*., 2012) and *Shoreatum buggaia* (Venkateswarlu *et al*., 2010). Considering the vast potentiality of plants as sources this work aims to investigate the use of *Azadirachta indica* leaf extract for biosynthesis of AgNPs and the synthesized AgNPs was evaluated for its antibacterial against on some selected pathogens. To the best of my knowledge, this research will represent the first reference to the use of *A. indica* leaf extract for green synthesis of silver nanoparticles in Nigeria.

2. MATERIALS AND METHODS

2.1 Collection of Plant materials

Fresh and healthy *Azadirachta indica* leaf was collected from a garden near Federal University of Technology, Minna. The plant material was taken to the Department of Biological Sciences, Federal University of Technology, Minna, for identification by a taxonomist, Mr Muhammad.

2.2 Medicinal Plant Extraction Preparation

The *Azadirachta indica* leaf was washed with distilled water, and then air dried for 7 days at ambient temperature. The plant material was ground to a fine powder using electrical blender. Five grams (5g) of the powdered leaf was soaked in 100ml distilled de-ionized water for 30 minutes under vigorous shaking in the water bath at 60˚C. The obtained leaf extract was filtered through Whatmann filter paper no.1 and was stored in refrigerator at 4ºC for further experiment (Devasenan *et al*., 2016).

2.3 Preparation of bacterial isolate

Already isolated and identified clinical bacterial isolates was collected from the Microbiology Lab, Federal University of Technology, Minna. The bacterial isolates used include *S.* Typhi, *E. coli*, and *P. aeruginosa*. The isolates was maintained on agar slants and refrigerated for further use.

2.4 Synthesis of Silver Nanoparticles

One (1) mM $AgNO_3$ solution was prepared and stored in amber colour bottle. One (1) ml of *Azadirachta indica* Leaf Extract (ALE) was added to 9ml 1 mM aqueous silver nitrate. Nine (9) mL of 10^{-3} M volume of silver nitrate solution

was considered as control. The resulting solution was kept under direct sunlight. Observation for gradual colour change was done and the result was recorded (Das *et al*., 2016).

2.5 Characterization of the Nanoparticles

The bioreduction of pure Ag^{+} to Ag^{o} ions was confirmed by subjecting 2mL of the synthesized AgNPs to UV-Visible spectrophotometer (Model- Shimadzu UV-1800, Japan) in the range of 190-800nm (Das *et al*., 2016).

2.6 Antibacterial Activity of the Synthesized TLE-AgNPs

The silver nanoparticles synthesized using *Azadirachta indica* was tested for antibacterial activity by standard agar well-diffusion method (Perez *et al*., 1990) against pathogenic enteric bacteria which include *S.* Typhi, *E. coli*, and *P. aeruginosa*. The pure culture of bacterial pathogens was subcultured on nutrient agar. 100µl of fresh overnight grown cultures of the respective bacteria was spread on Nutrient Agar containing Petri plates. Each strain was swabbed uniformly using sterile cotton swabs. Wells of 6mm diameter was made on nutrient agar using sterile cork borer and 100µL of the synthesized ALE-AgNPs, $AgNO_3$ solution and aqueous leaf extract (control) was loaded into the different wells. After incubation at 37°C for 24 hours, the zone of inhibition was measured (Kora *et al.*, 2009).

3. RESULTS & DISCUSSION

Silver nanoparticles with their unique chemical and physical properties are proving to be an alternative for the development of new antibacterial agents. Silver nanoparticles (AgNPs) have also found diverse applications in the form of wound dressings, coatings for medical devices and silver nanoparticle impregnated textile fabrics etc (Rai et al., 2009). A detailed study on the biosynthesis of silver nanoparticles and its antibacterial activity against some pathogenic enteric bacteria was conducted.

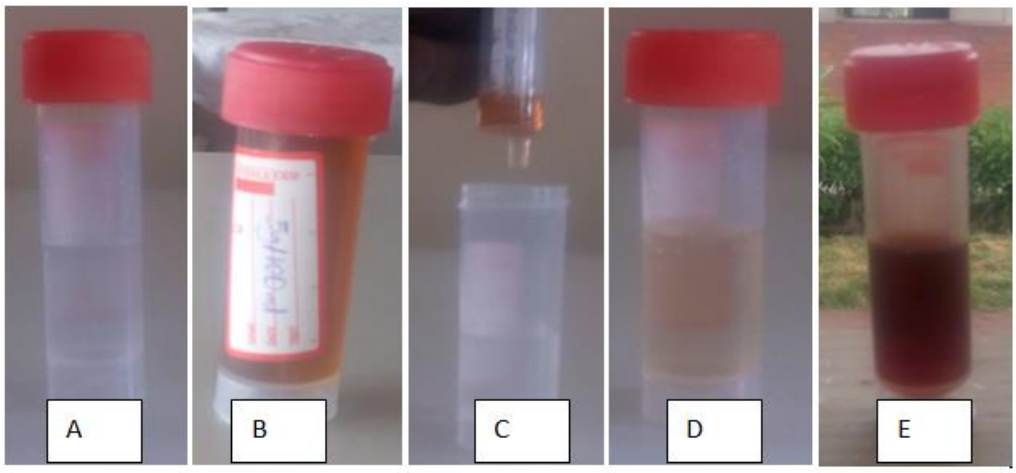

Plate 1: Biosynthesis of AgNPs using aqueous leaf extract of *Azadirachta indica*

Key:

A = 1 mM $AgNO_3$ solution

B = Plant extract

C = 1ml of the plant extract dispensed into 9ml $AgNO_3$ solution

D = 1 mM $AgNO_3$ solution + Plant extract at zero minute

E = Colloidal AgNPs after 20 minutes

Previous studies reported that AgNPs can be synthesized by plants such as *Capsicum annuum* (Bar *et al*., 2009), *Carica papaya* (Jha and Prasad, 2010), *Gliricidia sepium* (Raut *et al*., 2010), *Eucalyptus hybrid* (Dubey *et al*., 2009) and microorganisms such as *Aspergillus fumigatus* (Bhainsa and D'Souza, 2006), *Cladosporium cladosporioides* (Balaji *et al*., 2009), *Fusarium oxysporum* (Ahmad *et al*., 2003), *Pseudomonas aeruginosa* (Husseiny *et al*., 2007) and

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Rhodopseudomonas capsulate (He *et al*., 2007). In this study, aqueous silver ions were reduced to AgNPs after mixing with 5% *Azadirachta indica* leaf extract (1:9), a stable reddish brown colour was produced within 10 minutes under solar irradiation (Plate 1). This change in colour has been previously observed by several investigators (Saxena *et al*., 2010, Khandelwal *et al*., 2010). These authors suggested that the colour change appeared due to the surface Plasmon resonance of the deposited AgNPs (Noginov *et al*., 2006).

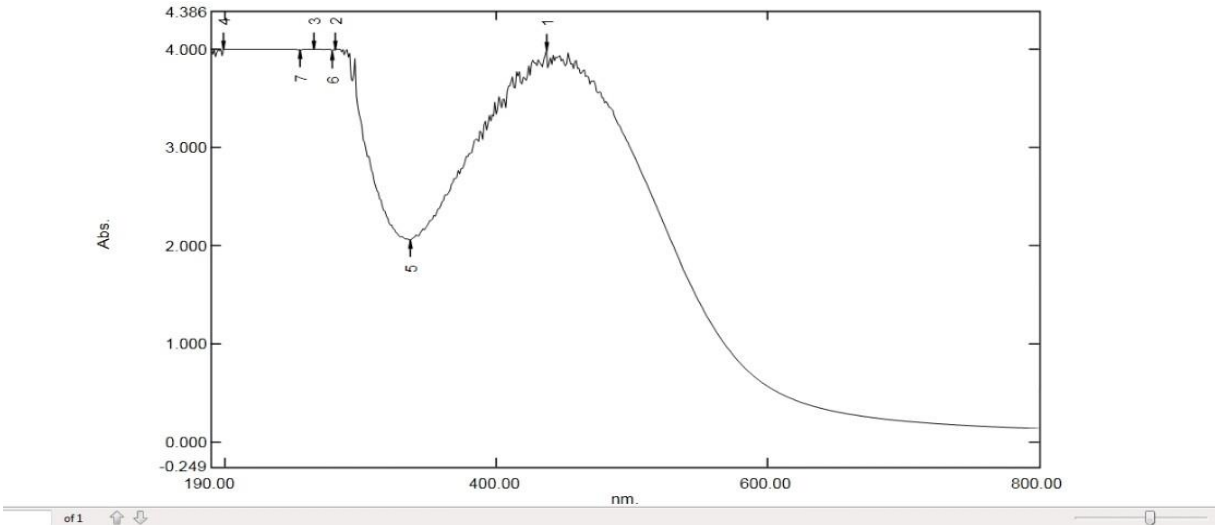

Figure 1: UV- vis spectra of silver nanoparticles synthesized by *Azadirachta indica* leaf extract.

The presence of nanoparticles was confirmed by subjecting 2mL of the synthesized AgNPs to UV-visible spectroscopic analysis in the range of 190-800nm (Figure 1). From this analysis, the absorbance peak was found at around 437nm. It is earlier reported that absorbance around 430nm for silver is a characteristic of these noble metal particles (Nestor *et al*., 2008). The antimicrobial activity of AgNPs was reported in a series of report (Rai *et al*., 2009, Jha *et al*., 2010, Khandelwal *et al*., 2010). The antibacterial activity of AgNPs produced from *A. indica* leaf extract was studied against some selected pathogenic enteric bacteria, using the well diffusion method. The diameter of inhibition zones (mm) around each well with silver nanoparticles solution is represented in Table 1. The highest antimicrobial activity of the synthesized AgNPs was found against *Salmonella* Tyhi (16mm), the diameters of the inhibition zones against *Escherichia coli* and *Pseudomonas aeruginosa* were found to be 12mm respectively. The synthesized AgNPs showed higher antimicrobial activity against the three enteric bacteria when compared to $AgNO_3$ and *A. indica* leaf extract (Control).

Table 1: Results of antibacterial activity test of the plant extract, AgNPs and $AgNO_3$.

Test organisms/ zone of inhibition(mm)	**Plant extract**	**$AgNO_3$**	**AgNPs**
***S.* Typhi**	10	12	14
E. coli	09	10	12
P. aeruginosa	09	11	12

A number of theories for antimicrobial actions of colloidal silver solution have been proposed. For example, alteration of permeability of cell membrane (Sondi, and Sondi, 2004), release of lipopolysaccharides and membrane proteins (Amro *et al*., 2000), generation of free radicals responsible for the damage of membrane (Kim *et al*., 2007), and dissipation of the proton motive force resulting in the collapse of the membrane potential (Chun-Nam *et al*., 2006), however; the exact mechanism has not been fully deciphered. Moreover, Tripathi et al. 2010 studied the effect of silver nano balls on *Escherichia coli*, *S. typhimurium*, *B. subtilis* and *P. aeruginosa* by colony forming unit (cfu) and growth curve at a concentration of 40µg/ml and showed a significant reduction of bacterial population and their growth pattern at the studied concentration. Overall, the results of this study indicated that the nano-sized silver produced by *A. indica* showed excellent antibacterial property and high antimicrobial activity compared to the ionic silver.

4. CONCLUSION

In this study, *Azadirachta indica* conjugated silver nanoparticles were synthesized using their leaves extract. The biosynthesized silver nanoparticles were proved to have excellent antimicrobial performance against pathogenic enteric bacteria, *S.* Tyhi, *P. aeruginosa* and *E. coli*, using *A. indica* leaves extract. Therefore, AgNPs producing *A. indica* may be potentially utilized for the economical production of AgNPs for many pharmaceutical applications.

Conflict of Interest

The author has declared no conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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