International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 4(6): November-December 2014

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 27-10-2014; Revised: 31-10-2014; Accepted: 01-11-2014

PLANT-MEDIATED SYNTHESIS OF SILVER NANOPARTICLES USING NEEM LEAF EXTRACTS AND ITS ANTIBACTERIAL ACTIVITY

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Keywords:

Silver nanoparticles, neem, Antimicrobial activity

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ABSTRACT

Synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures. The silver nanoparticles were synthesized by using important medicinal plants of *Azadirachta indica* (neem). Initially the silver nanoparticles characterized by using UV-visible spectrophotometer. The peak found at 420 – 440 nm indicates the silver nanoparticles synthesis. The antibacterial activity of silver nanoparticles against disease causing bacteria *B. subtilis*, *K. planticola*, *K. Pneumoniae, Staphylococcus sp and E. Coli* was analyzed, the good results shows the nanoparticles used for the medicinal field.

INTRODUCTION

Herbals are used for many of centuries by many cultures for their medicinal values. Herbal treatment is very popular because it is easily available, cheap and best and less toxic. *Azadirachta indica* (neem) is an herbal plant widely distributed in our subcontinent during all seasons. Various biological activities showed by neem compounds, neem extracts and a reasonable medicinal application along with their safety evaluation.Neem has been extensively used in ayurveda, unani and homoeopathic medicine. The Neem is still regarded as 'village dispensary' in India or a tree for solving global problems'. More than 135 compounds have been isolated from different parts of neem with the chemical and structural diversity. These compounds have been divided into two major classes: isoprenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C-secomeliacins such as nimbin, salanin and azadirachtin) and nonisoprenoids, which are proteins or amino acids and carbohydrates (polysaccharides), sulphur compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc (*Biswas et al., 2002*). Nimbidin, a major bitter component of seed kernels oil of *A. indica* demonstrated several biological activities. From crude components some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated. Almost all parts of the neem tree have been used as traditional Ayurvedic, unani and sidhha medicine in India. Neem oil, bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, constipation, blood purifier and also as a general health tonic. It also used for the treatment of rheumatism, chronic syphilitic sores and indolent ulcer. Neem oil used to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers, burning sensations and pthysis. Neem contained various compounds which showed various biological activities such as anti-inflammatory; Antiarthritic; Antipyretic; Hypoglycaemic; Antigastric ulcer; Spermicidal; Antifungal; Antibacterial; Diuretic; Antimalarial; Antitumour; Immunomodulatory etc. (Aditi et al., 2011)

Figure 1: Neem tree

The neem constituent belonging to chemically diverse classes have been divided into two major sections viz. I) isoprenoids, II) non-isoprinoids. The later category comprises glycerides, polysaccharides, sulphurones compounds, flavonoids and their glycosides, amino acids, aliphatic compounds. (Badam et al., 1999) Synthesis of nanoparticles using plants or parts of plants can prove advantageous over other biological processes by eliminating the elaborate processes of maintaining microbial cultures (Shankar *et al*., 2003). Preparation of silver nanoparticles has attracted particularly considerable attention due to their diverse properties and uses, like magnetic and optical polarizability, electrical conductivity, catalysis, antimicrobial and antibacterial activities (Sharma 2007; Shahverdi *et al.,* 2007), The silver nanoparticles were synthesized using *neem* leaves extract and evaluated its antimicrobial activity against *disease causing pathogens,Bacillus subtilis,Klebsiella planticola, Klesiella pneumoniae, Staphylococcus.*

MATERIALS AND METHODS

Synthesis and characterization of silver nanoparticles using *A. indica*

Plant materials and preparation of plant extracts

A. indica leaves were collected from Vellore in Tamilnadu, india. *A. indica* leaves were washed with tap water and shade the dried at room temperature. An electrical blender powdered the dried plant material. The powder 2g of the leaves was extracted with 100 ml of distilled water and 10 minutes heating in the boiling water bath. Then extracts were filtered with funnel in filter paper. The extract was used for further studies.

Synthesis of silver nanoparticles

The *A. indica* leaves extract is used for nanoparticals. *A. indica* leaves extract and 90 ml of distilled water and 1mM silver nitrate was added and then shaken well.

Visual observation

The treated with *A. indica* silver nitrate solution were observed for the change in colour comparison to as a visual method of detection of silver nanoparticles synthesis.

UV-visible spectrum

The reduction of silver ions from silver nitrate to silver nanoparticels was monitored by measuring the absorbance using UV-vis spectrophotometer. The absorbance was recorded from 370-510nm at resolution of 1nm for detection of phytosynthesized silver nanoparticles.

Antibacterial activity of synthesized silver nanoparticles

The antibacterial activity of synthesized silver nanoparticles was performed by agar well diffusion method against pathogenic bacteria are *Bacillus subtilis, Klebsiella planticola, Klesiella pneumoniae, Staphylococcus sp* and *E. coli.* Fresh overnight culture of each strain was swabbed uniformly onto the individuals plates containing sterile Luria Bertani Agar and 5 wells were made with the diameter of 6 mm. Then 25µl of purified silver nanoparticles, leaf extract, and silver nitrate solution were poured into each well and placed commercial antibiotic discs as control and incubate for 24 h at 37^0C. After incubation the different levels of zonation formed around the well and it was measured. This experiment was repeated in three times.

RESULT AND DISCUSSION

Characterization results of AgNPs synthesized by using neem leaf extract

Optical evidence

In the present study, the rind extract of the medicinal plant *neem* was taken for the synthesis of silver nanoparticle. Optical evidence is the preliminary study to conform the formation of AgNP synthesis. The main reason for synthesis of AgNPs was due to the presence of phytochemicals (Vanaja et al., 2013). The color identification is the preliminary analysis to confirm the formation of nanoparticles. The color changes from pale yellow to brown confirming the formation of silver nanoparticles in the reaction mixture is shown in Fig. 2. The color changes happened due to the excitation of surface Plasmon resonance in the obtained metal nanoparticles.

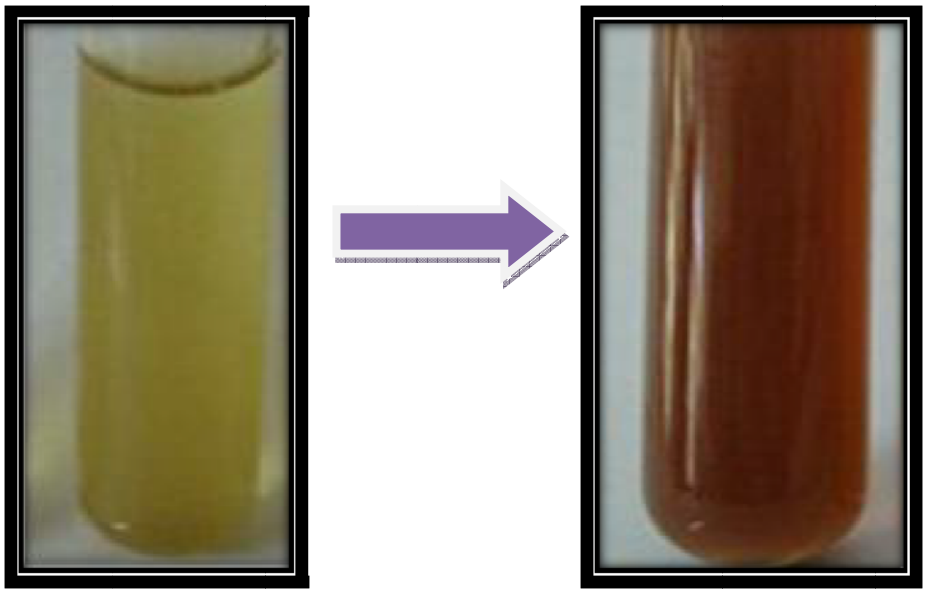

Figure 2: Synthesis of silver nanoparticles Initial and final colour changes

UV-Vis Spectrum

UV-visible spectroscopy is the primary method to indicate the bioreduction of silver metal ions from the aqueous silver nitrate solution to AgNPs. 1mM silver nitrate is used as a substrate for synthesis AgNPs by using leaf extract of *neem*. The synthesis is started within 10 min, and shows rapid yield of AgNPs is obtained. As the leaf extract is mixed with aqueous solution of the silver nitrate, it started to change the color from pale yellow to brown due to reduction of silver ions into AgNPs is shown in Fig. 3.

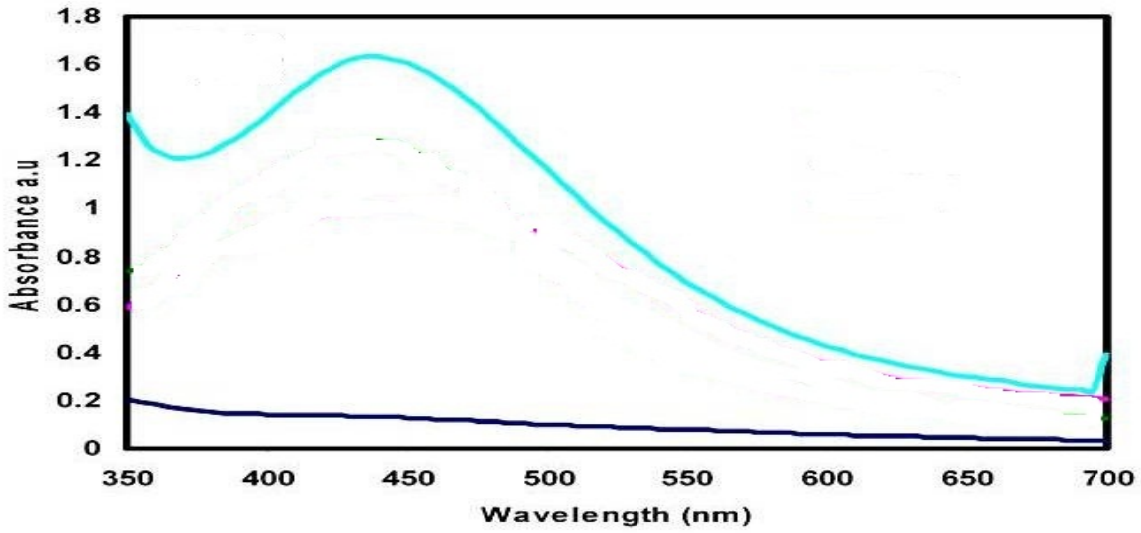

Figure 2 : UV-Spectrum of synthesized nanoparticles

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES

The antimicrobial activity of silver nanoparticles prepared by neem extract was examined against bacteria *B. subtilis*, *K. planticola*, *K. Pneumoniae, Staphylococcus sp and E. Coli* (Fig. 4). Herein, three different concentrations like 30 µl, 60 µl and 90 µl was used to check the antimicrobial activity of silver nanoparticles. In that, the 90 µl concentration shows strapping antimicrobial activity in all aforementioned bacteria. Among the bacteria, *K. pneumoniae* (Fig. 5) forms high zone of inhibition, which shows silver nanoparticles has high antimicrobial activity against *K. Pneumoniae* (Fig. 4. When compared with the standard drug Ampicillin our compound having good zone of inhibition against all type of disease causing pathogenic bacterial isolates.

Table : Antimicrobial activity of nanoparticles

Conc of AgNPs	*K. planticola*	*E. coli*	*Klebsiella pneumoniae*	*B. subtilis*	*Staphylococcus sp*
30 µL	12.17±0.167	12.00±0.000	11.33±0.667	09.17±0.441	09.00±0.000
60 µL	13.83±0.088	14.00±0.000	12.50±0.289	10.67±0.334	10.77±0.234
90 µL	14.27±0.145	15.33±0.272	14.17±0.167	11.50±0.289	11.80±0.200
Ampicillin	12.87±0.133	13.00±0.000	11.67±0.334	09.33±0.334	10.33±0.334

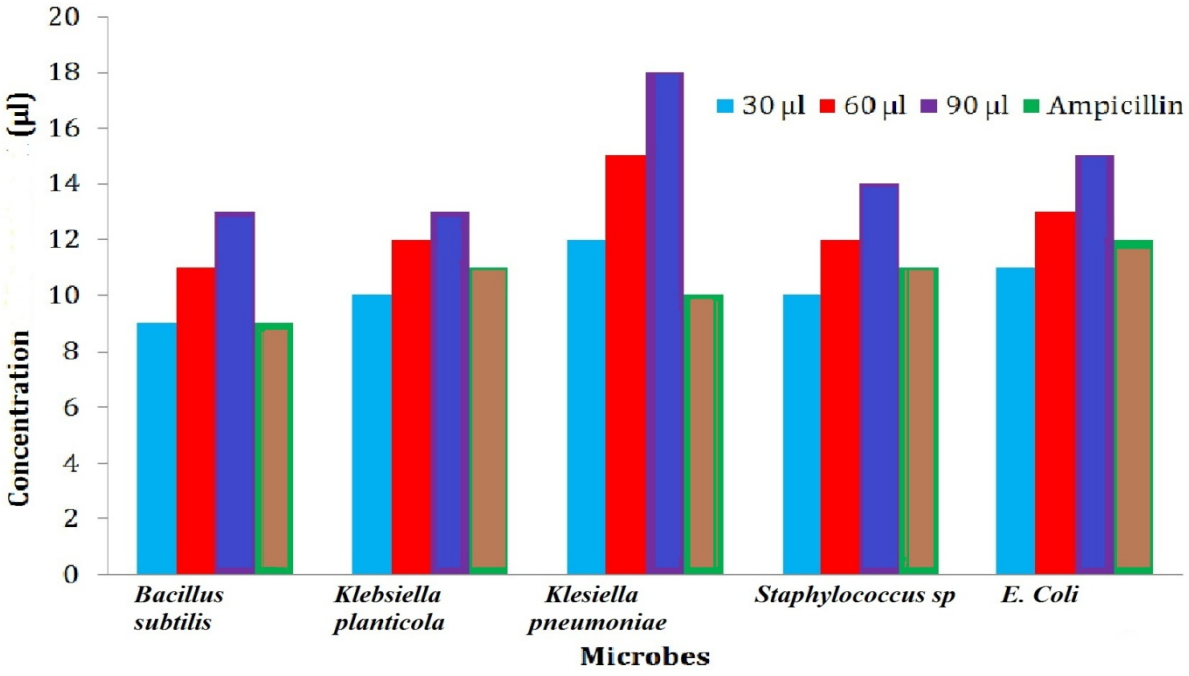

Figure 5: Antimicrobial activity of nanoparticles against different bacterial strains

CONCLUSION

The silver nanoparticles were synthesized by using important medicinal plants of neem. Initially the silver nanoparticles characterized by using UV-visible spectrophotometer. The peak found at 420 – 440 nm indicates the silver nanoparticles synthesis. The antibacterial activity of silver nanoparticles against disease causing bacteria *B. subtilis*, *K. planticola*, *K. Pneumoniae, Staphylococcus sp and E. Coli* were analyzed. Silver has been used as an antimicrobial agent for centuries; the recent resurgence in interest for this element particularly focuses on the increasing threat of antibiotic resistance, caused by the abuse of antibiotics. Nanoparticles are incorporated into the wound dressing, and the silver-enhanced wound dressings.

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