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Original Research Paper

Pharmacology

THERAPEUTIC ROLE OF AZADIRACHTA INDICA LEAF EXTRACT AGAINST UTI (URINARY TRACT INFECTION)

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ABSTRACT

Azadirachta indica is a multipurpose tree with multiple health benefits. Different parts of the plant are shown to exhibit antimicrobial effects against a wide variety of micro organisms. In the present study we investigate the antibacterial activity of Azadirachta indica extract against UTI (urinary tract infection) causing bacteria. Silver nanoparticles could be synthesized using the leaves of Azadirachta indica as reducing agent. The resulting silver nanoparticles were characterized using UV-Vis spectrophotometer, X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), and Fourier Transformations Infra Red (FTIR) spectroscopic analysis. The antibacterial activity was assayed by Agar well diffusion method using 20μl each of sterile Nutrient Agar (NA) (Hi-Media) and Potato-Dextrose Agar (PDA). The resulting silver nanoparticles exhibited best inhibitory activities against Escherichia coli and Proteus mirabilis which are main causing agents for UTI.

KEYWORDS : Azadirachta indica, antibacterial activity, Escherichia coli, Proteus mirabilis, urinary tract infection.

INTRODUCTION

Urinary tract infections (UTI) are infection from microbes in any part of urinary system i.e. in kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract – the bladder and the urethra. In the present study we deal urinary tract infections which are caused by bacterial outgrowth in interior walls of lower urinary tract. This type of UTI is caused by bacteria commonly found in the gastrointestinal tract (GI). Usually, women are more likely to get urinary tract infection due to the location of urethra, which is very close to anus where bacteria thrive. Also, the female urethra is shorter in length than that of man, making it easier for bacteria to enter and cause infection in the bladder.

Having a urinary tract infection can be very uncomfortable and pain. Symptoms and signs of UTI are
- Burning sensation when urination.
- Frequent urge to urinate even when the bladder is empty.
- Pain or heaviness in the lower abdominal area or in the lower back.
- Foul smelling of urine or appears cloudy or blood-tinged.
- Fever or chills.

From times immemorial, the use of medicinal plants for the treatment of various types of ailments was common practices in Manipur, the northeastern part of India. In the case of urinary tract infection, people have been using *Azadirachta indica* in different forms according to the nature or type of the local physician concerned.

Nanoparticle research is currently a great influencing area of intense scientific research community due to a wide variety of potential applications in biomedical, optical, and electronic fields. Out of all kinds of nanoparticles, silver nanoparticles have been attracting more and more attention owing to their intriguing electrical, thermal and optical properties. Metal nanoparticles can be synthesized by conventional chemical and physical methods[1-3]. However, most of the current chemical synthetic processes are regarded as having a relatively high environmental cost. Recently biosynthetic methods employing plant extracts have been emerged as environmentally sustainable alternatives to chemical synthetic procedures [4-6]. Biosynthesis of silver nanoparticles using medicinal plants extract is a very quite novel method which provides advancement over chemical and physical methods. It is a simple, rapid, low cost, eco friendly and a single step method and easily scaled up for large scale biosynthesis process. In the present study, we report the biosynthesis of silver nanoparticles by the reaction of aqueous solution of silver nitrate with the leaves extract of *Azadirachta indica* at room temperature. *Azadirachta indica* has known medicinal properties forming an important component of ayurvedic medicine and has been used since ages to treat several ailments.

MATERIALS AND METHOD

Preparation Of *Aqueous* Leaf Extract

Fresh leaves of *Azadirachta indica* were collected from the local areas and washed thrice in distilled water and dried on paper toweling. 20 gram each of the leaves were cut into fine pieces and boiled at 100°C with 100ml of sterile distilled water for about 15 minutes. The crude extract was filtered through a Whatman filter paper (No. 40) to prepare the aqueous leaf extract. 1mM aqueous solution of Silver nitrate ($AgNO_3$, AR grade) was prepared and used in the synthesis of silver nanoparticles. 10 ml each of the aqueous *Azadirachta indica* leaves extract was added to 190 ml of 1 mM aqueous $AgNO_3$ solution in a beaker separately and kept at room temperature for 48 hours for reduction.

Characterization Of Synthesized Silver Nanoparticles.

UV-Visible Spectrum analysis

The UV-Vis spectrum for the reaction solution of silver nanoparticles was measured with UV-Vis Spectrophotometer (Model: HR Ocean Optics 4000).

XRD Analysis

The XRD measurement was carried out for the identification of the crystallinity of silver nanoparticles using an XPert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu K radiation in a 2 configuration.

SEM Analysis

The morphology of the synthesized silver nanoparticles was characterized by the Scanning Electron Microscope (FEI Quanta 250).

FTIR Analysis

FTIR spectrum of the synthesized silver nanoparticles was recorded with a Shimadzu spectrometer (Model FTIR- 8400S).

Antibacterial Evaluation

The antibacterial activity was assayed by agar well diffusion method using 20 ml each of sterile Nutrient Agar (NA) (Hi-Media) for testing the bacterial activity [7].

RESULTS

UV-Vis spectrum analysis of the synthesized silver nanoparticles using *Azadirachta indica* leaf extract.

Figure 1a shows the appearance of a single but strong surface plasmon resonance band absorption peak centered at about

420 nm which indicates the formation of silver nanoparticles[8]. Plasmon bands are broad with an absorption tail in the longer wavelength. The cause of the infrared absorption is the stretching vibration within molecule and could be due to the presence of nitrogen, hydrogen, carbon and oxygen bonds.

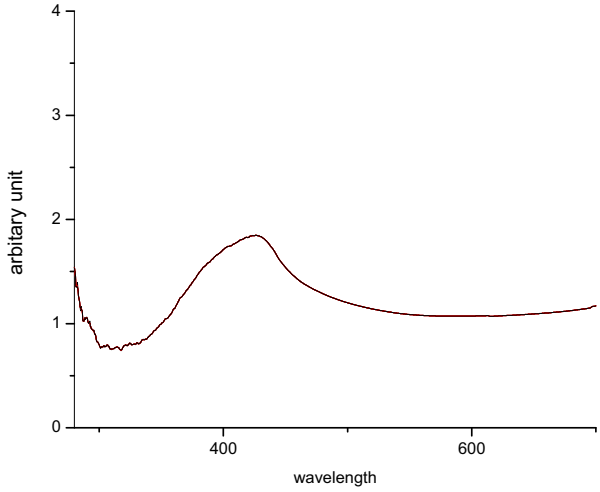

Fig.1a. UV-Vis Spectrum Of Silver Nanoparticles

XRD Spectrum Analysis Of Synthesized Silver Nanoparticles

The silver nanoparticles thus obtained were purified by repeated centrifugation at 5000 rpm for 10 minutes followed by re-dispersion in 10 ml of distilled water. Fig. 1b shows the XRD pattern of silver nanoparticles. The peaks at 2= 38.16°, 44.54°, 64.58° and 77.61° correspond to the (111), (200), (220) and (311) planes respectively of silver crystal. All the diffraction peaks can be indexed to the planes of face centred cubic structure of metallic silver ions respectively revealing that the synthesized silver nanoparticles are composed of pure crystalline silver. No impurities were detected from this pattern within the resolution limit of XRD. The crystallite size was found to be 38 nm and it was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Debye Scherrer formula.

$$D = 0.94\,\lambda / \beta \cos\theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X- ray wavelength, β is the Full Width at Half Maximum (FWHM) and θ is the diffraction angle.

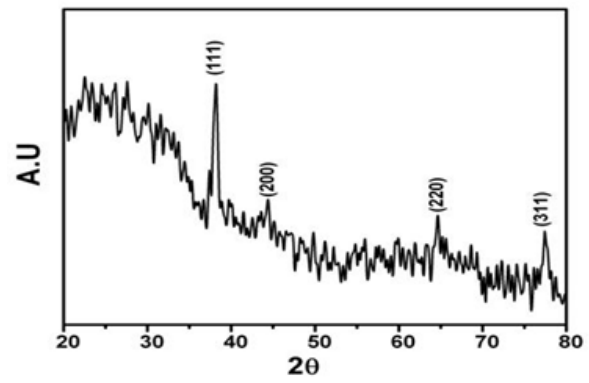

Fig. 1b. XRD Pattern Of Silver Nanoparticles.

SEM Analysis Of Synthesized Silver Nanoparticles

A typical SEM image of the synthesized silver nanowires is presented in Fig. 1c. It was clearly seen that the prepared samples consisted of an abundance of nanowires. The average diameter of the nanowires is about 40-60 nm on average but the length of the nanowires cannot be measured due to the interlacing of their ends.

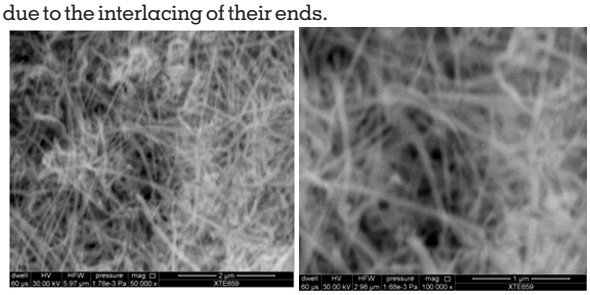

Fig1c. Shows The Typical SEM Images.

FTIR Spectrum Analysis Of Synthesized Silver Nanowires

For FTIR spectrum analysis, the silver nanowires synthesized using the *Azadirachta indica* leaves extract were centrifuged at 10000 rpm for 20 min to remove free proteins or other compounds present in the solution if any. The centrifuged and vacuum dried particles were made in a KBr pellet and the spectrum was recorded. FTIR measurements were carried out to identify the possible biomolecules responsible for the capping leading to the efficient stabilization of the silver nanowires. Fig.1d shows the FTIR spectrum of the silver nanowires synthesized using the leaf broth of *Azadirachta indica* . The medium intense band at 1069.33 cm-1 is assigned to the C-N stretching mode of amine group. The sharp band at 1631.35 cm-1 arises from C=O (amide I band). The absorption bands located at 3217.62 cm-1 and 3423.56 cm-1may be attributed to O-H stretching mode of alcohols and phenols. The presence of these active functional groups in leaf extract results in the swift reduction of silver ions to silver nanowires.

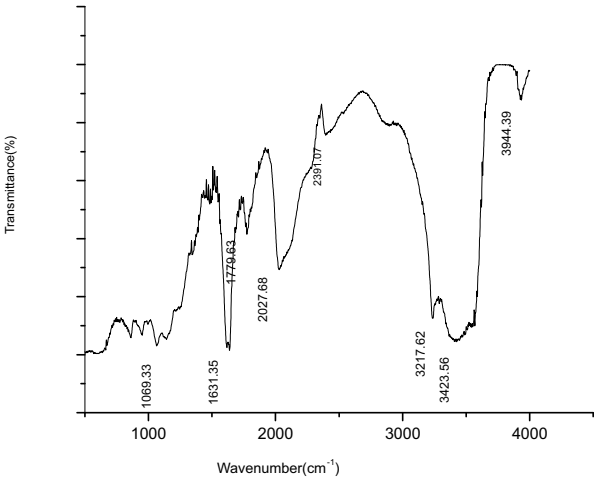

Fig.1d. FTIR Spectrum Of Synthesized Silver Nanoparticles

Antibacterial Activity Evaluation Of Synthesized Silver Nanoparticles

The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Sterile 6mm diameter cork borers were pierced in the agar at equidistant spots. 20 l of the diluted solution (16 g/ml) was deposited on the inoculated well and left for 10 min at room temperature for the compound diffusion. Ciprofloxacin (Hi- Media) was used as control. The plates inoculated with bacteria were incubated at 37°C for 24 hr. The experiment was repeated thrice and the average results were recorded. The antibacterial activity was determined by measuring the diameter of the inhibition zone (mm) around the well.

The susceptibility of microbial was determined by minimum inhibitory concentration determination method [9]. The minimum inhibitory concentrations (MICs) of the prepared

silver nanoparticles were determined by serial dilution against the micro-organisms. The minimum concentrations at which no visible growth was observed were defined as the MICs, which were expressed in mg/ml.

Table 1. Antibacterial Activity (zone Of Inhibition)

	Zone of inhibition (mm)									
	Proteus mirabilis		Klebsiella pneumoniae		Escherichia coli		Salmonella paratyphi		Pseudomonas aeruginosa	
	1	0.5	1	0.5	1	0.5	1	0.5	1	0.5
Azadirachta indica Silver nanowire	14	11	12	10	12	11	12	10	10	-
Ciprofloxacin (16μg/ml)	32		34		36		36		34	

Table 2. MIC For Antibacterial Activity

	BACTERIA	MIC
Azadirachta indica Silver nanowire	*Proteus mirabilis*	<0.0625
	Klebsiella pneumoniae	>0.0625
	Escherichia coli	<0.0625
	Salmonella paratyphi	=0.0625
	Pseudomonas aeruginosa	>0.125

The prepared *Azadirachta indica* silver nanowires showed the best inhibitory activities against *Proteus mirabilis* and *Escherichia coli* among the tested bacterial pathogens

DISCUSSION

The formation of silver nanoparticles was primarily confirmed by the colour changes from pale yellow to dark brown due to the Surface Plasmon Resonance (SPR) property of silver nanoparticles. SPR in nanometer-sized structures is called Localized Surface Plasmon Resonance(LSPR). LSPRs are the collective electron charge oscillations in metallic nanoparticles that are excited by incident light. For nanoparticles, LSPRs can give rise to intense colours of suspensions or sols containing the nanoparticles. Nanoparticles of noble metals exhibit strong absorption bands in the ultraviolet visible light regime that are not present in the bulk metal. In the present study a single but strong surface Plasmon resonance band absorption peak was observed at 420 nm which indicates the formation of silver nanoparticles. In the beginning of the biosynthesis, silver ions were reduced to metallic silver by certain components existing in the broth of fresh *Azadirachta indica* leaf extract solution. Biomass proteins perform multiple tasks as a reducing agent of silver ions and capping agent for nanoparticles. Hydroxide ions also played a key role in the production of nanoparticles. In human body as well as in plant system, free radicals are the main component which can easily damage cell due to the presence of free electrons.

Generally antioxidants can rapidly lose electron to a free radical and get paired. Phenolic compounds can neutralize free radicals and help to repair the damage occur in cell due to free radicals. Phenolics possess hydroxyl and carboxyl groups, able to bind to heavy metals. They may inactivate metal ions by chelating. Antioxidant action of phenolic compounds is because of their high tendency to chelate metals. The potent antioxidant capacity exhibited by the *Azadirachta indica* leaf extract may be due to the presence of phenolic compounds like gallic acid, syringic acid etc. These compounds have rapid electron loosing capacity and results in the formation of H^+ radical which reduces the size of the silver to nanosize. Furthermore, toxicity studies on pathogens open a new room for nanotechnology applications in medicine.

Biosynthesis of metal nanoparticles is a traditional method and opened a new awareness for the control of disease. In the present investigation, the biologically synthesized silver nanoparticles are found to be moderate toxic against the tested bacterial pathogens. The prepared *Azadirachta indica* silver nanowires showed the best inhibitory activities against *Proteus mirabilis* and *Escherichia coli* which are the most UTI causing bacteria's that live in the lower part of the digestive system of man.

CONCLUSION

Most urinary tract infections are caused by bacteria such as *Escherichia coli* and *Proteus mirabilis* that live in the digestive system of almost all the warm-blooded organisms including human being. Other causes of UTI include sexual intercourse, overconsumption of sugar, antibiotic overuse, pregnancy, hormonal imbalances and injury to the area.

According to Bergeron et al[10] *Escherichia coli* is responsible for more than 85% of all UTI. However, sometime other bacteria other than tested are also responsible for UTI. Opting for a natural remedy using *Azadirachta indica* extract is an excellent way to clear the bacteria from the urinary tract.

The present study confirmed a simple, efficient biological method at room temperature for the synthesis of silver nanowires with diameters in the range of 40-60nm using *Azadirachta indica* leaf extract and testing of their antibacterial activities. Biomolecules with carbonyl, hydroxyl and amine functional groups have the potential for metal ion reduction and for capping the newly formed nanoparticles. Antibacterial activities were assayed and exhibited the best inhibitory activities against *Escherichia coli* and *Proteus mirabilis* which are responsible for UTI.

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