



# Green Synthesis and Antibacterial Properties of Silver Nanoparticles Using Sudanese *Azadirachta indica* Leaves Extract

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**Abstract:** Nanoparticles have emerged as a key player in modern medicine and involved in drug delivery, it can be manufactured using various approaches including chemical, physical, and biological methods. Nanoparticles are generally characterized by their size, morphology and surface charge using advanced microscopic techniques such as SEM and TEM. This study was aimed for synthesis and characterization of silver nanoparticle and to detect their antibacterial activity using *Azadirachta indica* (Neem) leaves extract. The results showed a visible change of colour after the addition of Neem leaf extract to AgNO<sub>3</sub> solution to dark brown which indicates the formation of silver nanoparticle. UV spectrophotometer showed absorbance peak in range of 390-700 nm, the result revealed the highest peak showed by concentration 1 ml at 428.80 nm. SEM and XRD analysis were used to characterize synthesized silver nanoparticles. The antibacterial activity against Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* and *Klebsiella spp* Microorganisms was carried out using disc diffusion method, the results showed that the highest activity was 14 mm against *S. aureus*. *A. indica* silver nanoparticles can be used as a new nano drug in formulation and delivery. The synthesized silver nanoparticles are extremely important in various areas of life and are widely used for the benefit of human being.

**Keywords:** Green Synthesis, Silver Nanoparticles, *Azadirachta indica*, SEM, XRD, Antibacterial Properties, Sudan

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## 1. Introduction

Nanoparticles have unique chemical, physical and biological properties, and are therefore used in various fields such as business, therapies, electronics, cosmetics; chemical catalysis and drug delivery [1]. Metal nanoparticles have attracted a lot of attention in recent years due to their unique properties that differ from bulk materials. These particles have gained importance in recent years due to their wide application in a number of processes such as agriculture,

cosmetics, health care, delivery of drugs or genes, medical devices, biosensors and chemical catalysis [2]. AgNPs have the ability to work as therapies with diverse clinical and pharmacy properties. It can be used in wide applications, including anti-cancer or bactericidal agents during surgery. In this way, green synthesis of AgNPs as new therapeutic powers will be important in various biomedical applications [3]. Green method of NP synthesis is used with plant extracts,

bacteria, actinomycetes, fungi, and enzymes due to their environmentally friendly nature and bio-compatibility [4, 5]. The main compounds present in plant extracts are glycosides, alkaloids, phenols, amines and terpenoids that convert silver ions into nanoparticles of silver [4, 6]. Thus, the leaves, bark, flowers and seed extract from plants containing this chemical compounds are used as a source for reducing factors. *Azadirachta indica* belongs to family Meliaceae, it is an important medicinal plant in Sudan. Traditionally, Sudanese have used *A. indica* leaf to treat many diseases, and many researchers have reported pharmacological studies of *A. indica* including; anti-malarial activity, wound, molluscicidal, antimicrobial activity...etc [7]. The present study reports the synthesis and characterization of silver nanoparticle from *A. indica* leaves extract and their antimicrobial activity.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.1. Plant Material

*Azadirachta indica* leaves used in this study was collected directly from the field from the University of Medical Sciences and Technology, Khartoum, Sudan. The specimen has been deposited in Pharmacognosy department.

#### 2.1.2. Chemicals

Silver nitrate ( $\text{AgNO}_3$ ; 99.8%) was purchased from Sigma-Aldrich Co. (St Louis, MO, USA). All general reagents used in this study were purchased from British Drug Houses (BDH), England. Sigma Chemical Company, USA.

#### 2.1.3. Bacterial Strains

Isolated Gram positive *Staphylococcus aureus* and Gram negative *Escherichia coli* and *Klebsiella spp* were used in this study. The tested organisms were obtained from Department of Microbiology, Medical Laboratory Sciences, University of Medical Sciences and Technology.

### 2.2. Methods

#### 2.2.1. Preparation of Plant Extract

About 20 grams of fresh clean leaves of *Azadirachta indica* were chopped into small pieces. The sample was soaked in deionized water (100 ml) and boiled in water bath for 45 minutes, the aqueous solution obtained after reflux was filtered by whatman paper number 4.

#### 2.2.2. Synthesis of Silver Nanoparticles

For the synthesis of the silver nano particles, a certain volume of the Neem leaves extract (1, 2, 3, 4 and 5 ml) was added to the  $\text{AgNO}_3$  solution and the volume was adjusted to 10 ml with 1mM  $\text{AgNO}_3$  prepared as starting material for synthesis of silver nanoparticles in a test tubes, then the reaction mixture in a test tubes were incubated at room temperature for 2 to 4 hours. Blank solutions were prepared in the same manner without addition of silver nitrate solution. Separation of nanoparticles pellet was done by centrifugation (at 6000 rpm for 20min) of reaction mixture

and wash of pellet by deionized water and acetone (1:1). This process was repeated 3 times in order to get a pure silver nanoparticle pellet, then allowed to dry at room temperature and stored in clean dry container for further analysis [8].

### 2.2.3. Characterization of Silver Nanoparticles

#### (i). Ultraviolet (UV)/Vis Spectroscopy

The formation of silver nanoparticles was evaluated using UV spectrophotometer (Shimadzu). All reaction mixture solutions were scanned in a range of 390-800 nm. The nanoparticle formation was ascertained by recording UV-visible spectra (double beam spectrophotometer 2202-Systronics).

#### (ii). SEM analysis

SEM analysis was done using Scanning electron Microscopic (SEM) (TESCAN MIRA). Thin films of samples were prepared on a carbon coated copper grid by just dropping a very small amount of sample on the grid.

#### (iii). X-Ray Diffraction Analysis

The silver nanoparticle solution was centrifuged at 2500 rpm for 20 min. The pellet was washed three times with 20 ml of deionized water. The dried powder of silver nanoparticles was collected for the determination of formation of silver nanoparticles (XRD-7000s/7000L Shimadzu).

### 2.2.4. Antibacterial Assay

#### Disc diffusion method

The antibacterial assay was done on human pathogenic *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella spp* using standard disc diffusion method [9]. The bacterial stock suspensions were streaked on Mueller Hinton agar medium plates using sterile cotton swab. Sterile paper discs of 6 mm diameter saturated with different concentrations of Neem silver nanoparticles (20, 10 and 5 mg/ml) placed in each plate and incubated at room temperature for 24 hour. DMSO used as a control negative and Ciprofloxacin used as a control positive. The antibacterial activity was evaluated by measuring the zone of growth inhibition against the tested gram positive and gram negative bacteria.

## 3. Results and Discussion

### 3.1. Characterization of NPs

#### 3.1.1. Synthesis of Silver Nanoparticles

*Azadirachta indica* leaves were used to produce silver nanoparticles and the reduction of silver ions into silver particles during exposure to the plant extract was followed by colour change. In all experiments, addition of plant extract of *A. indica* into the tubes containing aqueous solution of silver nitrate led to the change in the colour of solution from colourless to yellowish to reddish brown (Figure 1) within reaction duration due to excitation of surface plasmon vibrations in silver nanoparticles [10].

**3.1.2. Analysis of Silver Nanoparticles by UV-visible Spectroscopy**

The UV absorption spectrum of silver nanoparticles from *A. indica* leaves extract of different concentrations showed in Table 1. The peak occurs at 428.80 nm ( $\lambda$  max) which corresponds to the best absorbance of silver nanoparticles.

Highest peak has been attributed to the excitation of surface plasmon resonance (SPR) of Ag NPs (Figure 2). Profile of the UV-Vis spectrum depends on the concentration of substrate and silver ions. The intensity of peak decrease with increase of concentration, the result revealed the highest peak showed by concentration 1 ml.

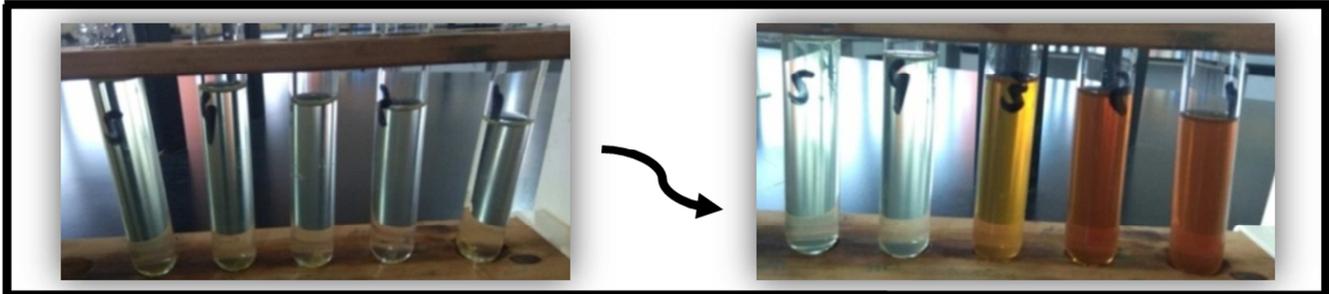


Figure 1. Colour Change in the five concentrations of *A. indica* extract with silver solution.

Table 1. UV spectrum of silver nanoparticles formed from *A. indica* leaves extract.

Concentration	1 ml	2 ml	3 ml	4 ml	5 ml
Wave length	428.80	424.00	416.20	395.40	397.40
Absorbance	1.165	1.002	0.751	0.542	0.529

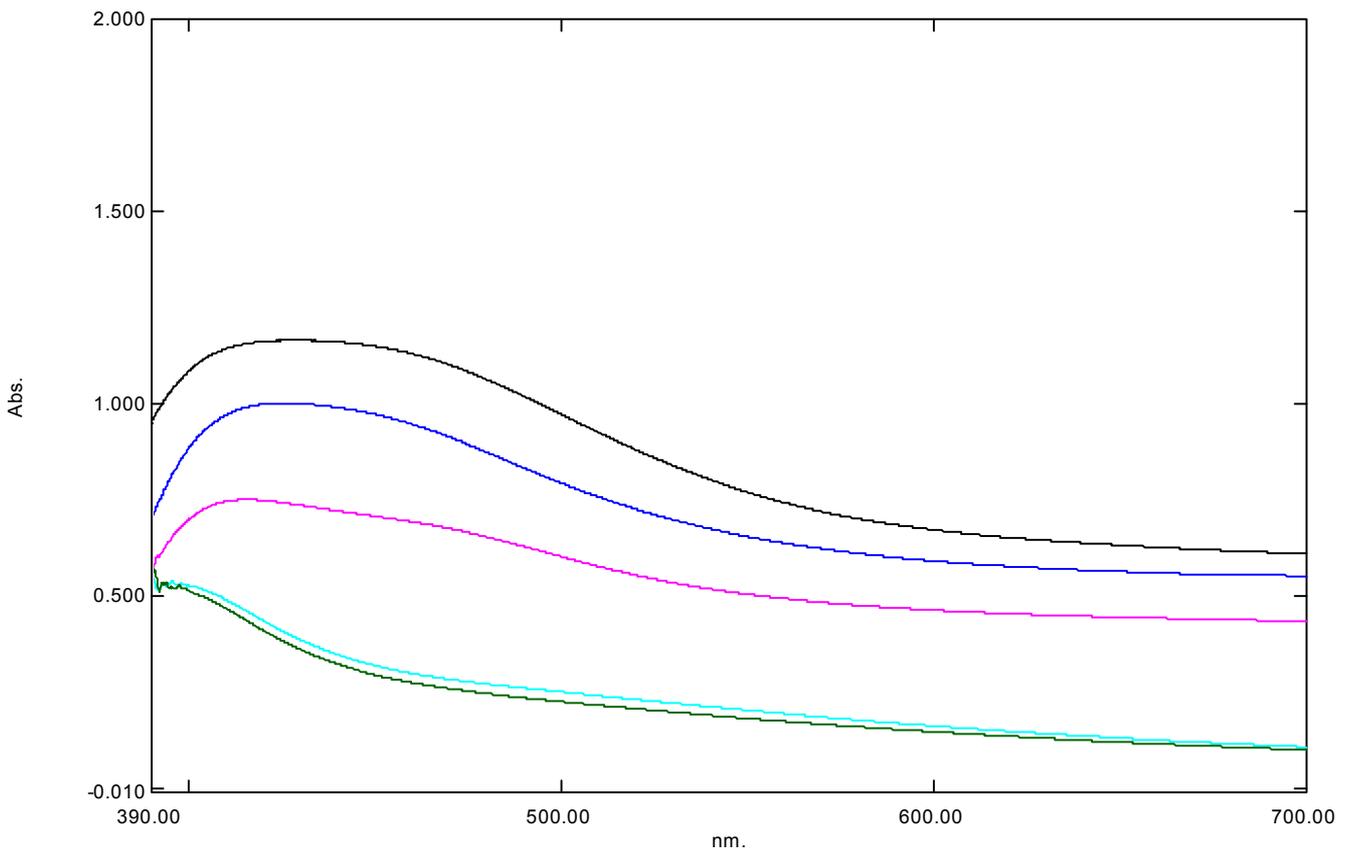


Figure 2. UV spectrum of silver nanoparticles formed from *A. indica* leaves extract.

**3.1.3. SEM Analysis**

The surface morphological and nanostructural studies using SEM are shown in Figure 3. The topology and size were also confirmed by SEM images showing the presence

of small and uniformly spherical shaped Ag NPs with smooth surface and very narrow distribution range of 13 nm, 17.61 nm and 27 nm.

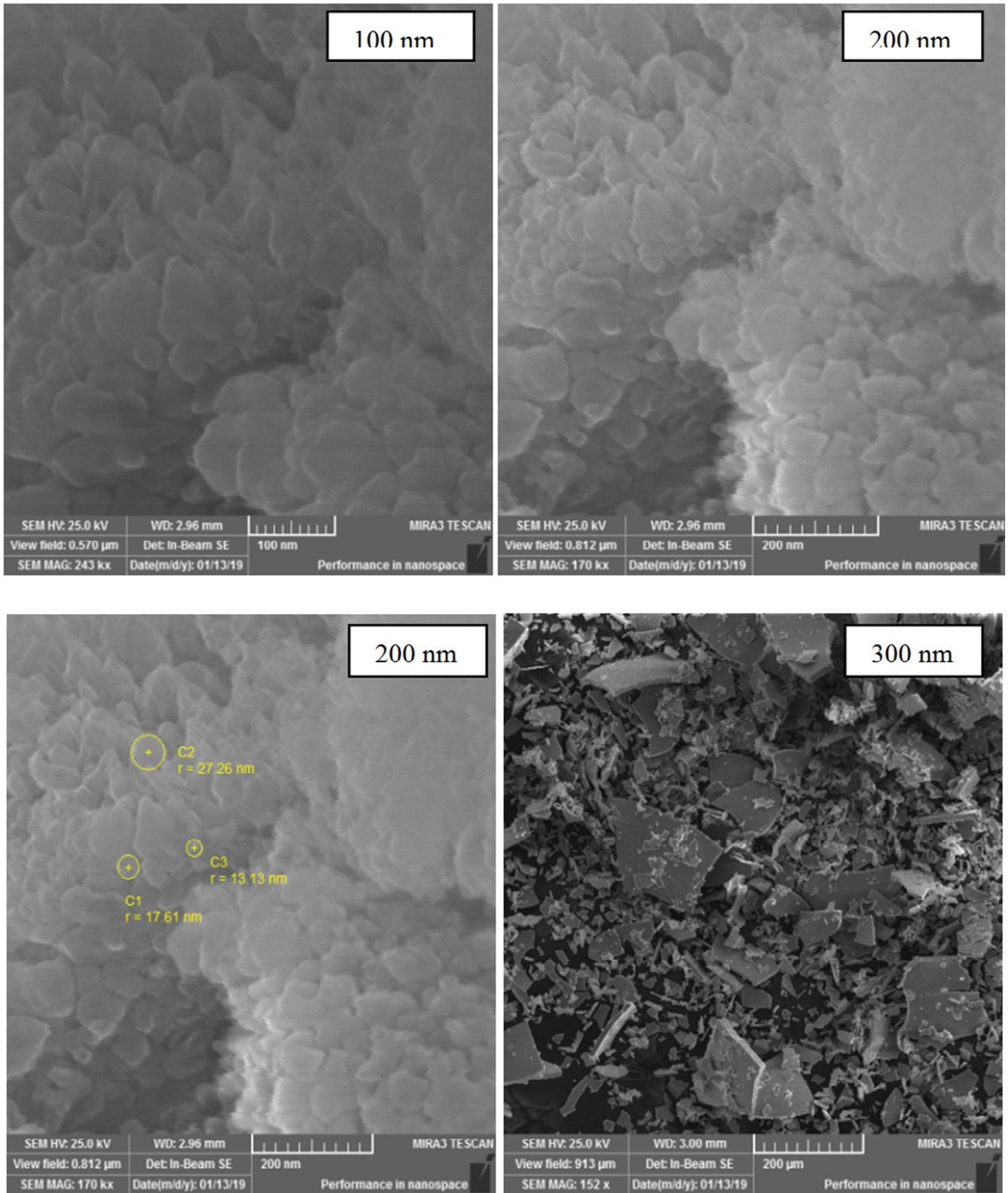


Figure 3. SEM analysis of silver nanoparticles.

### 3.1.4. XRD Analysis

X-ray diffraction (XRD) is a commonly used method to determine the crystal structures of nanoparticles. The average size was (44.99 nm) ranging between 10 and 80.01 nm (Figure 4).

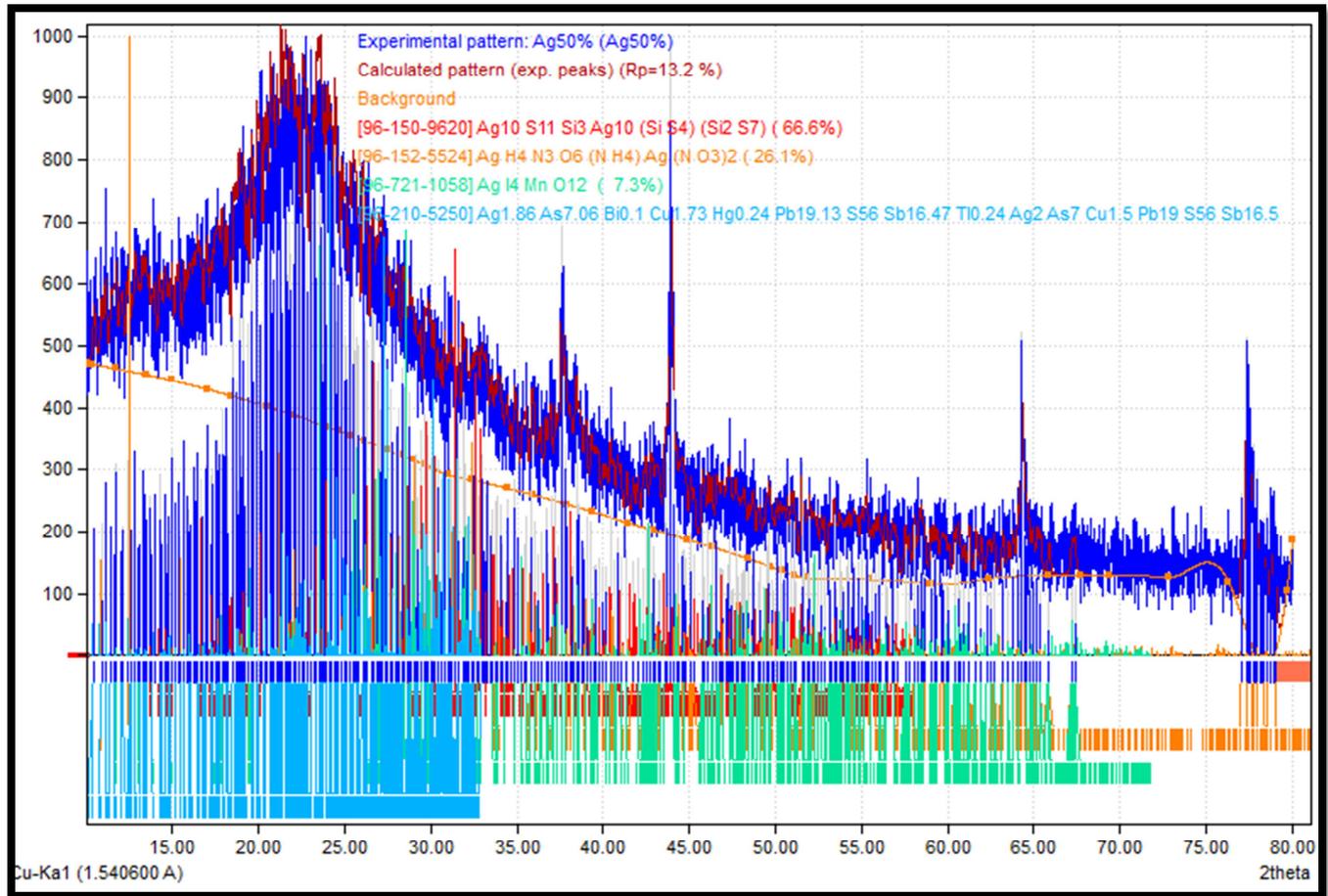


Figure 4. XRD analysis of silver nanoparticles.

### 3.2. Antibacterial Activity

Table 2. Antibacterial activity of silver nanoparticles against three bacteria species.

Concentration	Diameter of Inhibition Zone (mm)		
	<i>Klebsiella spp.</i>	<i>E. coli</i>	<i>S. aureus</i>
20 mg/ml	10	8	14
10 mg/ml	7	6	8
5 mg/ml	6	6	7
Ciprofloxacin	26	18	31

The results of antibacterial activity of silver nanoparticles with different concentrations against three microorganisms are presented in Table 2. The activity of silver nanoparticle was concentration dependant. The highest activity of silver nanoparticles showed against *S. aureus* (14mm). Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. Vijayakumar *et al* [11] reported silver nanoparticles synthesized Asteraceae have great susceptibility to different microbes. Other study showed that the synthesis of silver nanoparticles using plant extract gave good antimicrobial activity against *Escherichia coli*, *Proteus*

*mirabilis* and *Shigella flexneri*. Silver nanoparticles are reported to possess anti-fungal, anti-inflammatory, anti-viral, anti-angiogenesis and antiplatelet activity besides effective antimicrobial agent against various pathogenic microorganisms [12].

### 4. Conclusion

In this study, *Azadirachta indica* conjugated silver nanoparticles were synthesized using their leaves extract. The synthesized nanoparticles were characterized by using UV-Vis spectrum, SEM and XRD. The formation of NPs was observed by visible colour change from yellow to brown and confirmed by UV visible analysis. Crystalline nature of the nanoparticles is evident from sharp peaks in the XRD pattern. The AgNPs displayed antibacterial activity against *E. coli*, *Klebsiella spp* and *S. aureus*. The results showed that *A. indica* plays an important role in the reduction and stabilization of silver to silver nanoparticles and further investigations need to develop new formula.

### Conflict of Interests

Authors declare no conflict of interests.

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