VOL. 11, NO. 19, OCTOBER 2016 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

# INVESTIGATION OF USING SIDR LEAVE EXTRACTS IN NANO-SILVER PREPARATION

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**ABSTRACT**

This investigation represents the effect of using Ziziphus spina extract as reducing agent for the conversion of silver nitrate to nano size silver particles. Use of Ziziphus spina extract as natural reducing agent in the synthesis of nano silver is an environmentally friendly process in contrary of using chemical reducing agent. The prepared nano silver particles size was tested High resonance transmission electron microscope (HR-TEM) while the morphology was tested using X-ray diffraction (XRD). 30nm spherical shape nano silver of successful antibacterial activity against E-coli and staphylococcus aureus was prepared which has larger inhibition zone than that of using 1 molar silver nitrate alone i.e. the use of nano silver increased the inhibition zone of E-coli and staphylococcus aureus. So the synthesis of nano silver leads towards chemical use as antibacterial agent.

**Keywords:** nano silver, ziziphus spina, reduction, antibacterial assay.

## 1. INTRODUCTION

The humankind always is looking for the improvement of the properties of materials and optimizing its daily use in life applications such as medicine, agriculture, industry, and information technology (as reported by Roco 2003). Currently researchers trying to achieve these requirements through nano technology due to its scientific revolution wrought by unique characteristics were obtained for nano –sized materials. One of the wide expected future application elements is nano silver because its higher thermal and electrical with excellent catalytic activity, chemical stability, and biocide activities against wide range of positive and negative gram bacteria and virus (Silva GA, 2004; Wilson *et al.* 2002, Walter et al 2004, and Sudhakar, 2009). There are many ways to produce silver nanometer such as the use of fumigation by plasma ray, or infrared or ultraviolet rays (Fei *et al.* 1997) or using lasers ablation (Chen Y.H. and S. Yeh. 2002), while there are traditional chemical reduction in aqueous solutions where the driving force for the silver ion reduction to nano silver metal may be photochemical (Huang *et al.* 1996), or ultrasonic (Li *et al* 2000), or radiolytic (Henglein, 2000) or electrochemical (Yu *et al.* 1997).

From the environmental point of view plant extract as reducing and capping agent in wet chemical reduction method for nano size silver particles preparation is the greenest technique to be used (LIPI *et al* 2014). Nano factory (microorganisms) as presented by Natarajan *et al* 2010 is another environmentally save silver nano particles preparation technique. Nano factories technique is time consuming which make the advantage for pant extract method to be used. Several studies show the use of different plant extract as reducing and capping agent. Among these extracts Neem leaves (Asmita *et al* 2014), Aloe Vera (Sun *et al* 2002), Bryophyllum (Baishya *et al* 2012), Alternanthera (Baishya *et al* 2013), and tea extract (LIPI *et al* 2014) etc. were used.

In the present investigation nano silver is prepared using the chemical reduction method using Ziziphus spina leaves extract. Ziziphus spina-christi is one of the most important natural plants which have many therapeutic uses, including the treatment of headaches, fever and facilitator of the abdomen and purifies the intestines, skin and strengthens, treatment of arthritis Fasciola and painful, treatment of malignant tumors, malaria and illness HIV, as well as its use in the treatment of stomach pain (the narrator, 1988) on the other hand it is highly effective against fungi and bacteria growth ((Sharma *et al.* 1998).

The bactericidal effect of different concentrations of the leave extract of Ziziphus spina was tested against S. aureus, E. coli Shigella spp and P. aeruginosa at. 40 µL of the extract show the maximum zone of inhibition, while weak antibacterial activity against Gram-negative bacteria was obtained which may be attributed to the presence of their cell wall, lippolysaccharide (Wiriyachitra *et al.* 1984). The seed oil has a major role as antidiarrhoec and antihemorrhagic agent due to the presence of tannins as reported by Hussain *et al.* 2010, and Junior A, Zanil C., 2000. Tannins can be toxic to bacteria and, preventing growth and protease activity as presented by Asquith TN, Butler LG 1986. The interaction of berberine and harmane with DNA of positive gram and negative gram bacteria may show the mechanism of action of highly aromatic planar quaternary alkaloids. Alkaloids also interfere with cell division (Sadipo *et al.* 1991). Saponins are a special class of glycosides which have soapy characteristics.

Ziziphus spina-christi containing many compounds in its chemical composition such as Steroids; β-sitosterol, β- D- glucoside, and condensed tannins. Also there are free sugars such as fructose, raffinose, sucrose, glucose, galactose, rhamnose (Kamil, 1994; Ghazanfar, 1999). Figure-1 shows the Ziziphus spina-christi tree, fruits, branches and leaves. The hydroxyl groups present in these sugars structures will be the responsible for the reduction of silver ion to silver nano particles.


**Figure-1.** Ziziphus spina plant (Fruits and Branches).

## 2. MATERIALS AND METHODS

### 2.1 Materials

Silver nitrate and distilled water (all of high laboratory grade) were used for the preparations of this study. Ziziphus spina leaves extract was used as reducing and capping agent in the formula which acts as the stabilizer for synthesized nano sized silver.

### 2.2 Experimental procedure

#### 2.2.1 Preparation of fenugreek extract

50 grams of Ziziphus spina leaves were washed, dried and grinded then boiled in alkaline water (pH9) using 1molar NaOH solution. The mixture was thoroughly agitated for 2hrs using a magnetic stirrer. The mixture was then filtered to obtain the light brown liquor. The liquor was centrifuged to remove the impurities.

#### 2.2.2 Nano sized silver particles preparation

A sample of nano silver was prepared by mixing 1 ml of Ziziphus spina extract with 49ml distilled water then raising the temperature of the reaction medium gradually to the desired temperature, then add silver nitrate solution drop wise. The reaction mixture was kept under constant rate of stirring (150rpm) for the experiment duration. Once the addition of silver nitrate is completed the reaction medium acquires a clear yellow color then converted to dark brown indicating the formation of silver nanoparticles. The propagation of the reaction was controlled by UV–Vis absorption; aliquots from the reaction bulk were withdrawn at different time intervals and evaluated. The color of the mixture was observed within a few minutes after heating to $60^0$C then it was kept overnight in dark for stabilization and the change in color was noted on the next day. Glass bottles with nano silver solutions were tightly closed to prevent external contamination and evaporation. Testing of the complete conversion of silver nitrate to nano size was detected using sodium chloride solution which gives white precipitate in case of presence of silver nitrate (nabila *et al.* 2014).

### 2.3 Testing and evaluation

#### 2.3.1 UV-VIS spectra

Ultra violet–visible spectra (UV-Vis) have been used to prove to the formation of nano silver particles because they exhibit an intense absorption peak due to the surface Plasmon excitation (it describes the collective excitation of conduction electrons in a metal). The UV–Vis spectra of silver nanoparticles embedded in Ziziphus spina extract were recorded by means of a 50 ANALYTIKA JENA Spectrophotometer from 190 to 600nm. A solution containing silver nitrate dissolved in de-ionized water was used as a blank.

#### 2.3.2 Transmission electron microscope Test (TEM)

Shape and size of the obtained nano sized silver particles were characterized by means of a JEOL-JEM-1200 Transmission Electron Microscope (TEM). The TEM sample was prepared by adding a drop of the nano silver solution on a 400 mesh copper grid coated by an amorphous carbon film and let the sample for drying in open air at room temperature. The average diameter of the silver nanoparticles was determined from the diameter of 100 nanoparticles found in several chosen areas in enlarged microphotographs. Also the same apparatus is used for the examination of the morphology and X-ray diffraction test of the obtained particles.

#### 2.3.3 XRD analysis

The structure of the as synthesized silver nanoparticle was investigated by X ray Diffraction test. The sample was casted on a glass plate and the analysis was made at the voltage of 40 kV and current of 40 mA. The source used was copper Kα line. Based on the XRD result, the average particle size has been estimated by using Debye-Scherer equation as reported in Khan et al 2011.

$$D=\frac{0.9\,\lambda}{W\,\cos\theta}$$

Where 'λ' is wave length of X-Ray (0.1541 nm), 'W' is FWHM (full width at half maximum), 'θ' is the diffraction angle and 'D' is particle diameter (size).

#### 2.3.4 Antibacterial analysis

The Antibacterial activity of the extracts was carried out by inhibition zone test. Bacterial strains obtained from Hi Media were revived in LB broth (Hi Media) by incubating overnight at 37ºC. Wattman filter papers of 5mm diameter were sterilized by autoclaving at 15lb/inch$^2$ for 15 minutes. The sterile paper were impregnated with equal volume (100µg/ml) of Ziziphus spina extracts and silver nanoparticles, and compared with 1mM silver nitrate solution. The round paper containing each of 100µl samples were aseptically placed on plates containing Muller Hinton Agar medium (Merck,

Germany) after being sprayed with each of the test pathogens. The plates were incubated at 37 ºC for 48 hours and the zone of inhibition was measured (in mm diameter). Inhibition zones with diameter less than 8 mm were considered as low antibacterial activity. Two bacteria strains were tested using the obtained nano silver prepared by the Ziziphus spina leaves extract. Those two strains are E-coli and staphylococcus aureus.

## 3. RESULTS AND DISCUSSIONS

### 3.1 Synthesis of silver nano-particles

During the preparation of the nano silver particles using the mixture of silver nitrate as metal source and Ziziphus spina leaves extract as reducing and capping agent, there was change in color from yellow to brown in the synthesized sample. This indicates the reduction of $Ag^{+}$ to $Ag^{0}$. This change in color is carried out due to the excitation of surface Plasmon vibrations with the silver nanoparticles (Nabila H Hussien et al 2014, and Nabila H Hussien et al 2014).

### 3.2 UV-Vis Spectroscopy analysis

Size and shape of produced nanoparticles in aqueous solutions are detected using UV-Vis spectroscopy at 400 nm wave length. Strong absorption gives use to the surface plasma resonance (SPR) at resonant electrons. This absorption is affected by nanoparticle sizes, surrounding environment and dielectric medicine. Figure-2 illustrates UV-Vis pattern of nanosilver produced while Figure-3 shows the plant extract and nanosilver solution.

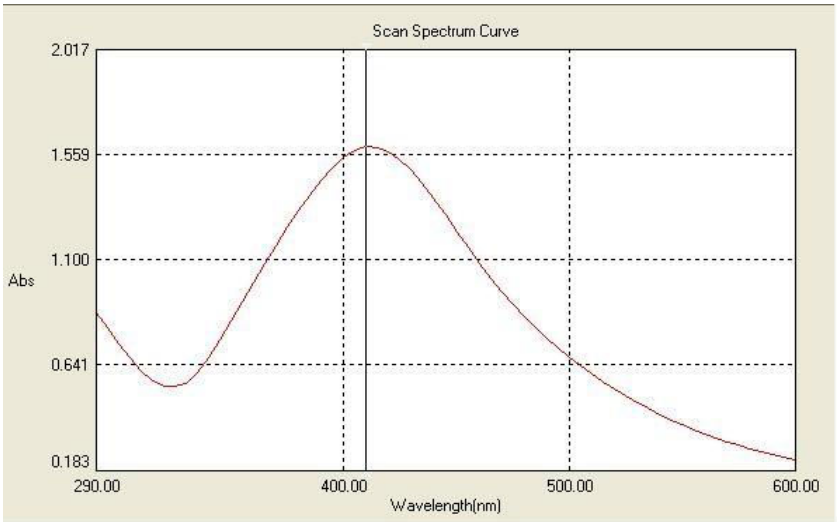


**Figure-2.** UV-Vis spectrum of the nano-silver particles obtained by reaction of Ziziphus spina extract and silver nitrate

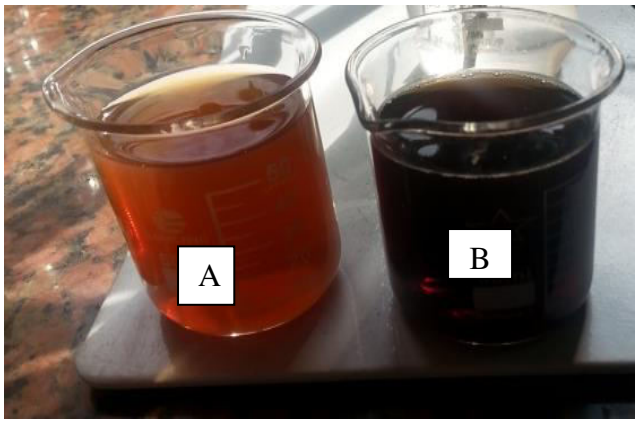


**Figure-3.** (A) Ziziphus spina extract and (B) Obtained Nano-silver.

### 3.3 XRD analysis

Results of XRD approved the crystalline structure of nanosilver produced and it is clear that the 2 θ values pattern ranged from 30º to 80º and the main peaks are at 38.18 º , 64.06 º and 75.01 º which match to miller indices (III), (200) and (311). Average particle size is found to be around 30 nm. Figure-4 illustrates scanning advances electronic diffraction (SAIED) results of obtained nanosilver produced from reaction of Ag ($NO_3$) and Ziziphus spina (sidr) extract.

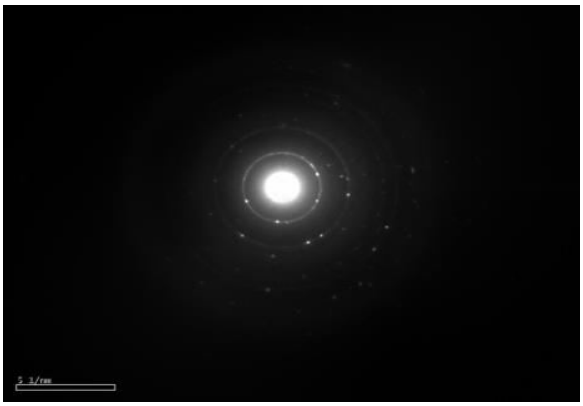

**Figure-4.** The SAIED pattern of the obtained Nano-silver particles

### 3.4 High resolutions transmission electron microscope analysis

Images of nano-silver obtained from reaction of $Ag(NO_3)$ and sidr leaves extract are shown in Figure-5. It is clear that most particles are spherical with smooth surface morphology while the average diameter about 30 nm and lattice planes of the crystal with d-spacing 0.2 nm. The image also depicts uniformity in sizes and shapes of nanosilver.

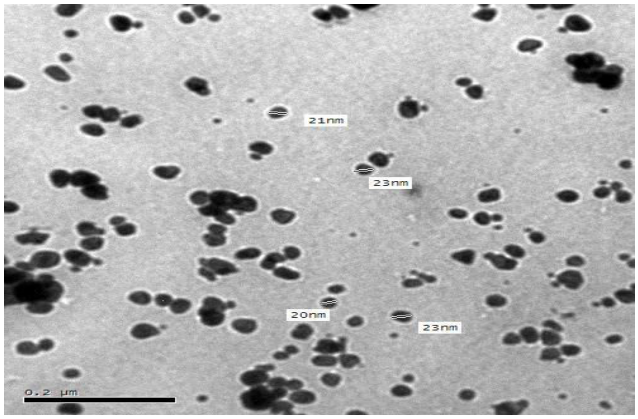


**Figure-5.** HR-TEM images of the obtained Nano-silver particles

### 3.5 Antibacterial assay

Antibacterial effect of prepared nanosilver was tested on E-coli and staphylococcus aureus so it was found that using 25µg of nanosilver, small inhibition zone for both tested strains were noted. Increasing nanosilver dose to 50µg the inhibition zones increased from 8nm to 14 nm and 16 nm for E- coli and staphylococcus respectively. This can be explained as follows: nanoparticles were attached at the surface of the bacteria and ruptured protein cell wall so it controls bacterial power due to presence of free radicals at the surface of nanoparticles which are responsible from antibacterial function (Ales, 2006). Figure-7 illustrates antibacterial results of prepared nanosilver. Antibacterial activity of sidr leaves extract explains anonymous claim on medical use of nanosilver from sidr leaves extract.

The histogram of the obtained nanosilver particles formed by the reduction of silver nitrate with Ziziphus spina leaves extract is illustrated in Figure-6 from which It is clear that the highest percent particle size present is 30nm (about 33%).

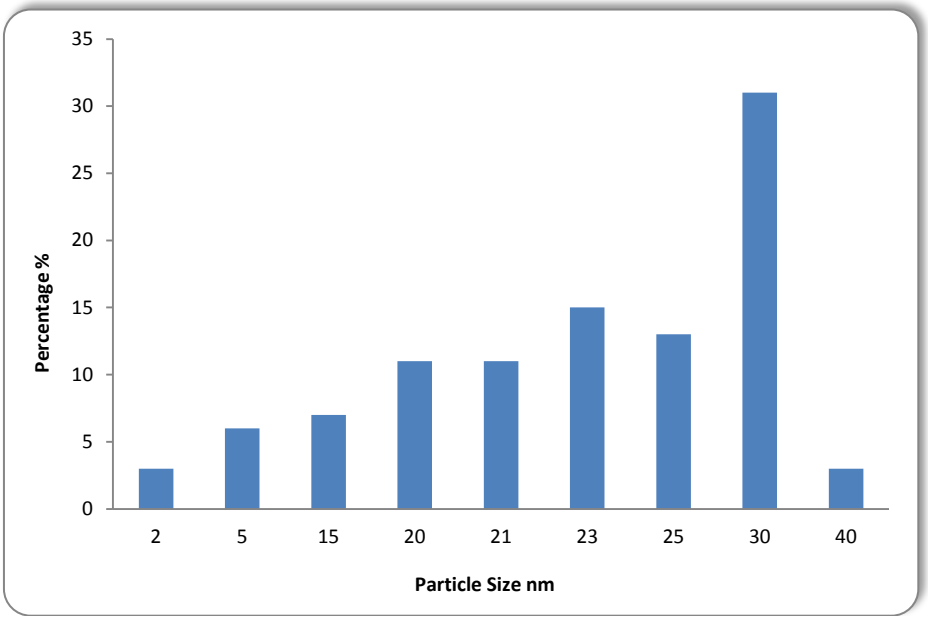


**Figure-6.** The histogram of the obtained nano-silver particles

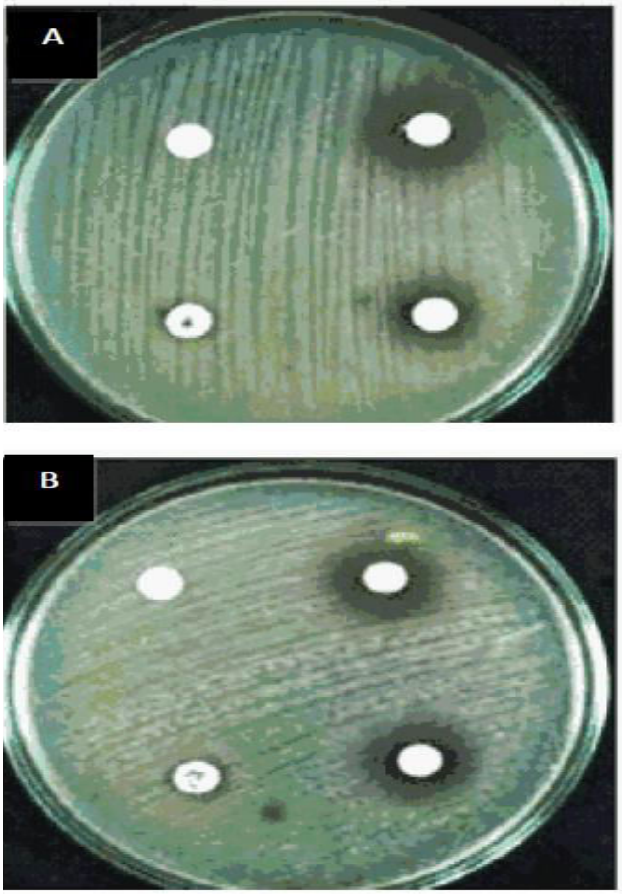


**Figure-7.** Antibacterial activity of nano:
(A) using 25mg of nano-silver, and
(B) using 50mg of nano-silver.

## 4. CONCLUSIONS

The conclusion of this study revealed that:

- Ecofriendly synthetic of silver nano particles is a low cost method at ambient conditions.
- It is shown that Ziziphus spina extract is the easy, economic and environmentally friendly process for nano particles preparation.
- Ziziphus spina represents a considerable improvement in the synthesis of a nano silver because it is used as reducing, capping agent and better control for size and shape.
- Silver nano particles are effective as antibacterial agent of the E-coli and staphylococcus aureus.

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