Characterization and Biological Evaluation of Silver Nanoparticles Synthesized Using *Ifloga spicata* (forssk.) Crude Extract

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Abstract

Silver has been used in olden days to treat several diseases caused by pathogenic bacteria. This work deals with synthesis, characterization, antimicrobial, cytotoxic and antioxidant properties of the crude metabolic extract of plant *Ifloga spicata* silver nanoparticals. The synthesized silver nanoparticles were investigated using different analytical techniques such as UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, Scanning electron microscope (SEM), and X-ray diffraction spectrometry (XRD). Based on the finding of this study, the green synthesis of AgNPs is highly recommended to be used for treatment of various human diseases, because of their high potency and less side effect and eco-friendly properties. Due to small size, the green AgNPs can also be used for targeted drug delivery.

Keywords: *Ifloga spicata*, plant-based silver nanoparticles, cytotoxicity, antimicrobial potential

1. Introduction

Nanotechnology is one of the fasted growing areas in the modern material sciences. Nanoparticles vary in size and range from 1nm to 100nm and display various shapes and superior properties.[1] For formulating biological molecules for delivery, nanotechnology applications are highly suitable due to exclusive properties such as specific surface properties of such systems.[2-4]Among the various nanoparticles available, AgNPs shows unique characteristics such as various shape and size, electrical, magnetic and optical properties, etc. Different chemical and physical methods are used for the fabrication of silver nanoparticle.[5] Silver has strong antimicrobial potential.[6] Thus, using silver in various forms and AgNPs, have extensive advantages in medicine.[7] Silver also plays a role in prevention of infectious microbail growth in medical instruments and implants coated with silver saturated polymers.[8] Recently in textile industries for sporting wear, silver embedded fabrics are used.[9] Besides this, silver nanoparticles acting as antimicrobial agent and have low toxicity in human.[8] They can also use in treatment of open wounds healing and chronic ulcers. There are many different approaches used for synthesis of nanoparticles including biological, physical and chemical methods.[10-12] Chemical and physical methods used for nanoparticles synthesis is time consuming, expensive and requires costly instruments. Researchers are trying to develop such type of method for nanoparticles synthesis which have low cost, fast, environmentally friendly and give higher yield.[13, 14] Among the various available methods, biological method is given preference to be used for synthesis of nanoparticle due to their low cost and environmentally friendly nature.[15] To the best of our knowledge, there is no report available on the synthesis of silver nanoparticle using *Ifloga spicata* (forssk.) crude extract. The present study focusses on synthesis of silver nanoparticles under various experimental conditions using *Ifloga spicata* (forssk.) crude extract. The synthesised nanoparticles were characterized using various analytical techniques such as UV-Visible spectroscopy, Scanning electronic microscopy (SEM), Fourier transform infrared (FTIR), and X-ray diffraction analysis (XRD). The antimicrobial, antioxidant and cytotoxic potential of synthesised AgNPs were also investigated.

2. Experimental

2.1 Materials and methods

2.1.1 *Chemicals*

All analytical reagents used in the study were of analytical grade and were purchased from Merck, Pakistan.

2.1.2 Plant collection and Extraction

Ifloga spicata (Forssk Sch.Bip) plant was collected from the site of University of Science and Technology Bannu, Main Campus, Khyber Pakhtunkhwa, Pakistan. The plant was identified by a well-known taxonomist Dr. Fiazan Ullah Khan working as Assistant Professor in the Department of Botany, University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan. After collection the plant material, it was washed several times with distilled water to remove the dust and sand from the plant surface. After that, the plant was shade dried and later grounded into fine powder. The powder of plant was dipped in 80% aqueous methanol for a week. After proper filtration, the crude extract was dried by rotary evaporator. This crude methanolic extract was stored under control condition for further analysis.

2.1.2 Synthesis of Silver Nanoparticles

Plant based silver nanoparticles were prepared by previously well reported method.[5] Briefly, 10 mM $AgNO_3$ solution was prepared in 50 mL de-ionized water. The 0.01M $AgNO_3$ solution was diluted 10 times (1 ml $AgNO_3$ + 9 ml of de-ionized water) and its pH was adjusted using 0.1 N NaOH solution. The 1 ml of plant crude extract dissolved in methanol was added to $AgNO_3$ solution and shaken in an orbital shaker for 24 h until color of solution changed to brownish. The change in color of solution indicated the synthesis of silver nanoparticles.

2.1.3 Characterization of Silver Nanoparticles

AgNPs syntheses were confirmed using U-Visible spectroscopy within the range from 200 nm to 800 nm. The active functional groups available on the surface of nanoparticles were detected using FT-IR spectroscopy. The dried extract and AgNPs extract were placed then on the crystal surface of FT-IR (Alpha FT-IR broker model) spectrophotometer and spectra were recorder at range of 4000 cm^{-1} to 500 cm^{-1}. Surface morphology of the synthesised nanoparticle were investigated using SEM (MIRA3 TESCAN model) analysis. X-Ray Diffraction (Model D-8 advance Germany) was used for the determination of AgNPs size and structure.

2.2. In vitro Antioxidant Essays

2.2.1 DPPH Scavenging Activity

The DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was weighed and mixed in 50 ml methanol. Its absorbance was calculated at 0.765nm (<1). DPPH free radical scavenging prospective of the crude extract, AgNPs and ascorbic acid was determined by means of a revised process from Brand-Williams *et al*., (1995) method.[16]

The various solutions (25µg/ml, 50µg/ml, 75µg/m and 100µg/ml) of plant extract, ascorbic acid and AgNPs were prepared in deionized water separately. Each of the solution (100 µl) was mixed with 900 µl DPPH solution separately and placed in a dark place for incubation for 30 mints after this shake test very well. The absorbance of stable DPPH was noted at 517 nm for this activity. We use beam spectrophotometer water as reference.

2.2.2 Hydrogen Peroxide Scavenging Activity

For Hydrogen Peroxide activity, stock solution of plant crude extract, ascorbic acid and AgNPs were taken in several concentrations (20µg/ml, 40µg/ml, 80µg/m and 100µg/ml). A 0.2 ml of sample was mixed with 0.6 ml H_2O_2 and 0.4ml phosphate buffer. The tubes were shake well and after shacking we note absorbance 0.81 after 15 min at 230nm was noted.

2.2.3 ABTS free radical activity

Same volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution were mixed to make stock solution and incubated in the dark for 24hours at 37C to create a colored solution comprising $ABTS^{\bullet+}$ radicals. The stock solution and 50% methanol were mixed to prepare working solution for an initial absorbance of about 0.936 (± 0.02) at 745nm, with control temperature set at 30°C. Stock solution of plant extract, the different concentrations (25µg/ml, 50µg/ml, 75µg/m and 100µg/ml) were taken. 0.2 ml solution from all running solution was dissolve 0.8 ml ABTS solution having 836 absorbance values. After 6 min of mixing the fall in absorbance was measured respectively. ABTS Experiment was completed in two times. Ascorbic acid was used as positive control.

2.2.4 Antimicrobial Screening of AgNPs

The antimicrobial activity of AgNPs was checked against four different bacterial strains. Two bacterial strains, *E. coli*, *K .pneumonia* were gram positive, while *Acetobactor* and *S. aures* were gram negative. Revata *et al.,* 2013 method was used for determination of antimicrobial potential of green synthesized AgNPs. Nutrient Agar Medium was equipped by mixing 7gm of nutrient agar with 250 ml distilled water having pH 07, autoclaved and cooled at 45C°. Approximately 5 wells were digged in plates by using a sterile cork borer (3-6mm). The 1mg/ml in de-ionized water stock solution of AgNPs was prepared which was again diluted to the different concentrations. (20, 40, 80 and 100 µg/ml) .The antibiotic erythromycin was used ad standard and while DMSO was used as negative control. The plates were incubated for 24 hours at $37C^0$ and the results were noted .

2.2.5 Cytotoxic Brine shrimp assay

For preparation of media 2.8g of sea salt was dissolved in 100ml (2.8g/100ml) distilled water and was placed on compelling stirrer for 120mins. The tray used for hatching is four sided which have too much small holes into divider the media is place in the tray and then in one portion we put the shrimps and enclosed with aluminum foil and put this in incubator for 24 hours. After incubation the larvae are moved from dark side to that portion of the tray where light is available. Six test tubes were taken and labeled as i.e control, 10µg/ml, 20µg/ml,50µg/ml, 100µg/ml and 200µg/ml. In this 6 test tube we put 5ml of media (sea salt). Now 10 larvae are transformed with the help of dropper. Without control test tube we put $AgNO_3$ solution respectively in remaining 5 test tubes. After this every test tube is filled strongly by cotton swab and keep warm at 28c° for one day. After 24 hours development I checked the active rate of brine shrimps with the help of 3x magnifying glass.

3. Result and Discussion

3.1 Synthesis of AgNPs

To confirm the formation of AgNPs using plant extract, UV-Vis spectra was recorded in the range from 200nm to 800nm. The color of the silver solution upon addition of extract immediately changed to yellow, indicating the formation of AgNPs. Figure 2 shows the spectra recorded for the synthesised silver nanoparticles. The position of peak at position 450nm confirm the synthesis of AgNPs. The color alteration noted by UV–vis spectroscopy, showed Ag capping

ability with hydroxyl group present in plant extract. The result has been shown in the following (figure 1).

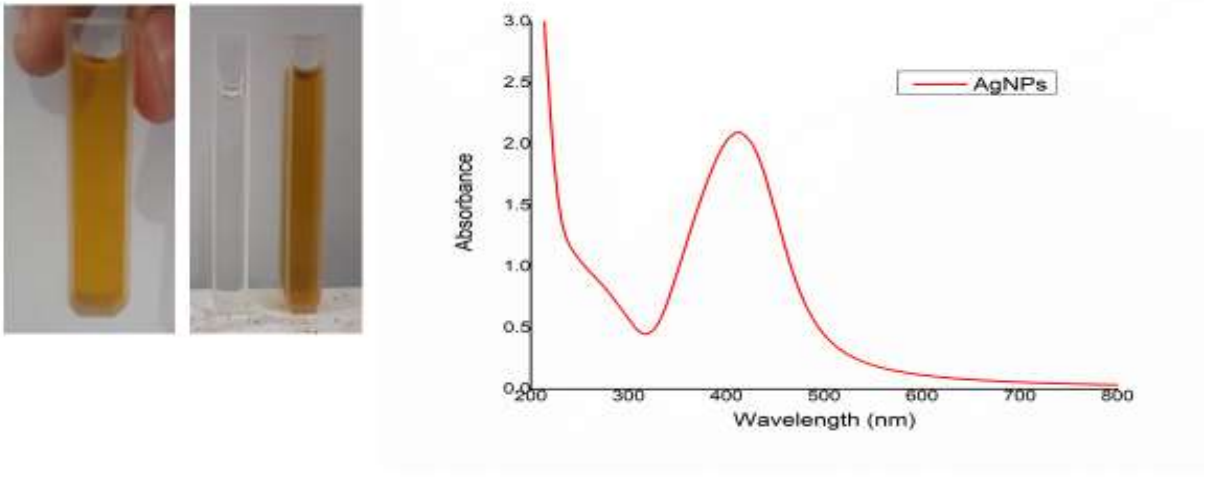

Fig 1: Calorimetric change of solution and UV-Visible spectrum of the synthesized AgNPS from extract of *I. spicata* plant**.**

3.2 Effect of pH on the synthesis of AgNPs

The formation of AgNPS was observed at a range i.e. pH 7, 8, 9, 10, 11 and 12. At pH 11 high absorbance was noted because the crude *Ifloga spicata* plant extract was stabilized and reduced to AgNPs therefore the pH 11 is selected for AgNPs from plant crude extract.

3.3 Extract amount effects on the synthesis of AgNPs

The plant extract amount concentration was evaluated in the range from 0.5 to2mL. Fig 3 represent that with increase the amount from.5 to 1mL, the intensity of absorption increases. When further the amount increase the intensity of absorbance then decrease, showing the stability and reduction of Ag ions are almost completed at I ml extract concentration.Therefor the 1ml amount is considered suitable concentration for AgNPs..

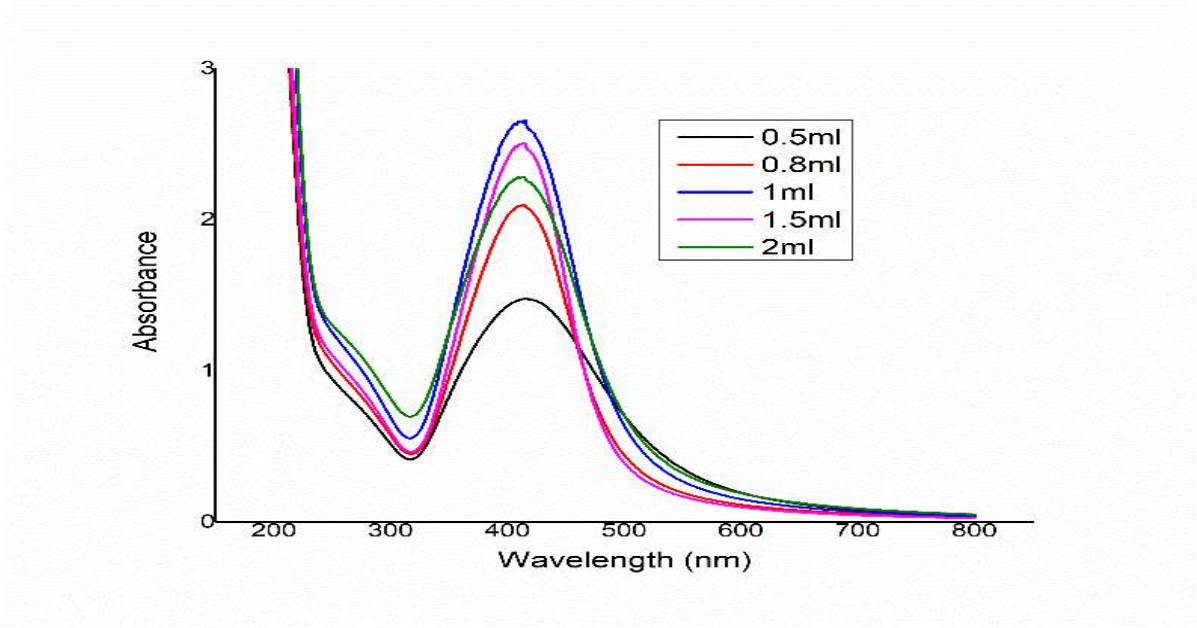

Fig 2 : Effect extracts amount on the synthesis of AgNPs.

3.4 FTIR of the synthesized AgNPs

FT-IR analysis is important to investigate the presence of important biomolecules in the plant extract. The IR spectra at 3289.10cm^{-1}show the stretching of –OH group in the plant extract with AgNps. Similarly, the peak found at 1603.13cm^{-1}, 1403.29cm^{-1}, 1259.19cm^{-1}, 1053.95cm^{-1} showing stretching, c=c bond streaching and CH3 bending modes respectively.

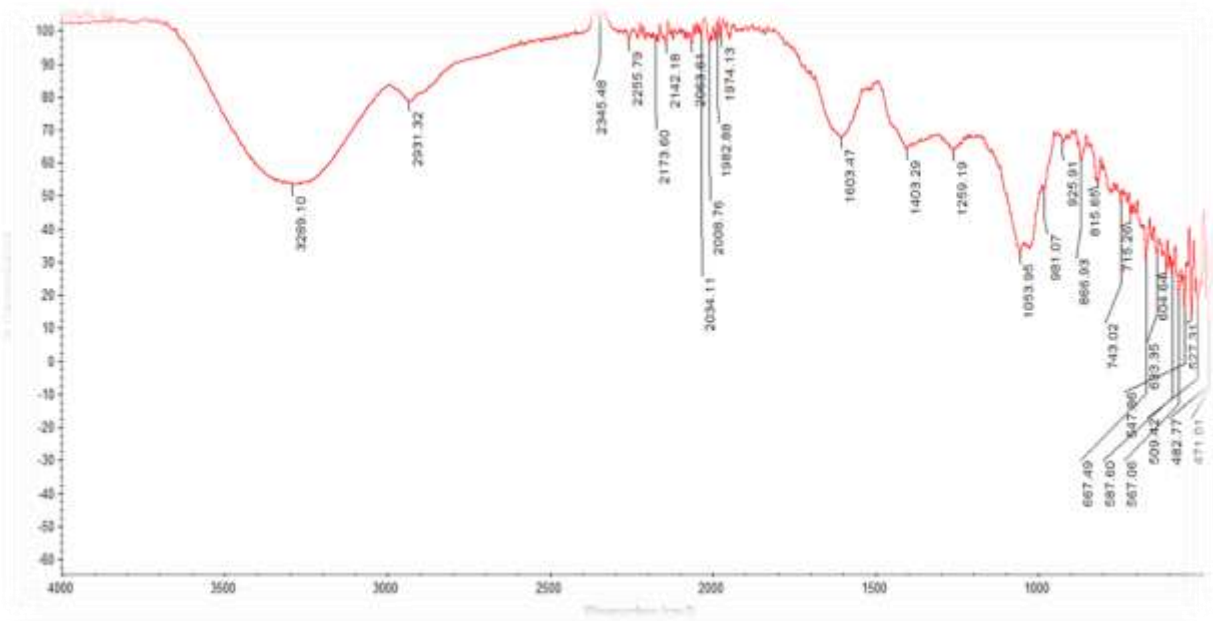

Fig 3: FT-IR spectra for *I. spicata* plant extract before AgNPs synthesis.

Fig 5 below show is the R spectra of AgNPs showing the decrease in wavelength due to stretching capability of important functional groups.The comparative data showed that AgNPs are synthesized.

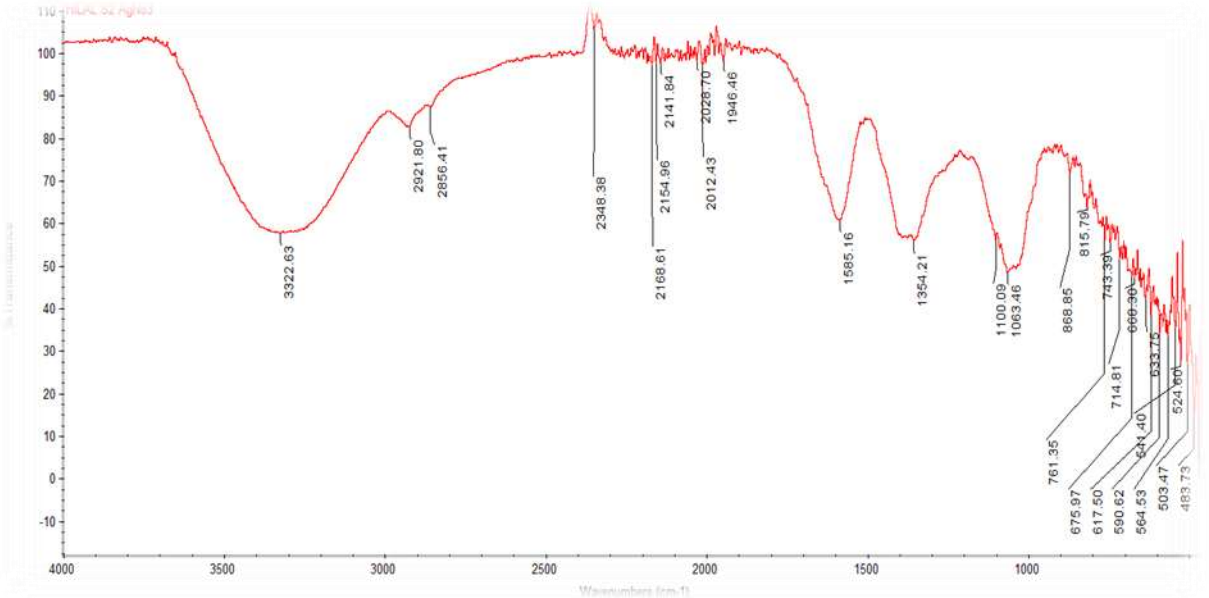

Fig:4 FTIR spectra of AgNPs synthesized from *I. spicata* extract.

3.5 SEM Analysis

SEM study was carried out to determine physical nature and morphology of synthesized AgNPs. The scanned electron micrograph of synthesized silver nanoparticles is given in Fig 6. showed the size 1µm of the AgNPs.

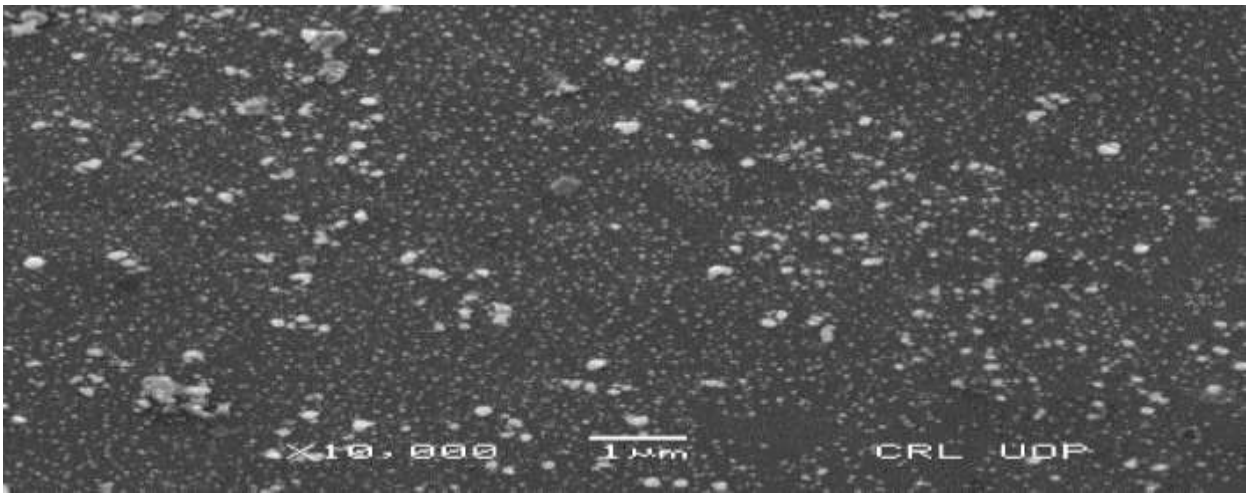

Fig 5: SEM analysis of AgNPs.

XRD

X-ray diffraction study was carried out to confirm the crystalline nature of the AgNPs. The XRD blueprint, (Fig. 7), reveal numbers of Bragg reflections at 2θ values of 38.21(111), 346.29(200), 64.64(220) and 77.55(311) sets of lattice plane. These planes demonstration based on the face-centered cubic structure of silver. The XRD pattern thus showing the crystaliine structure of the AgNPs.

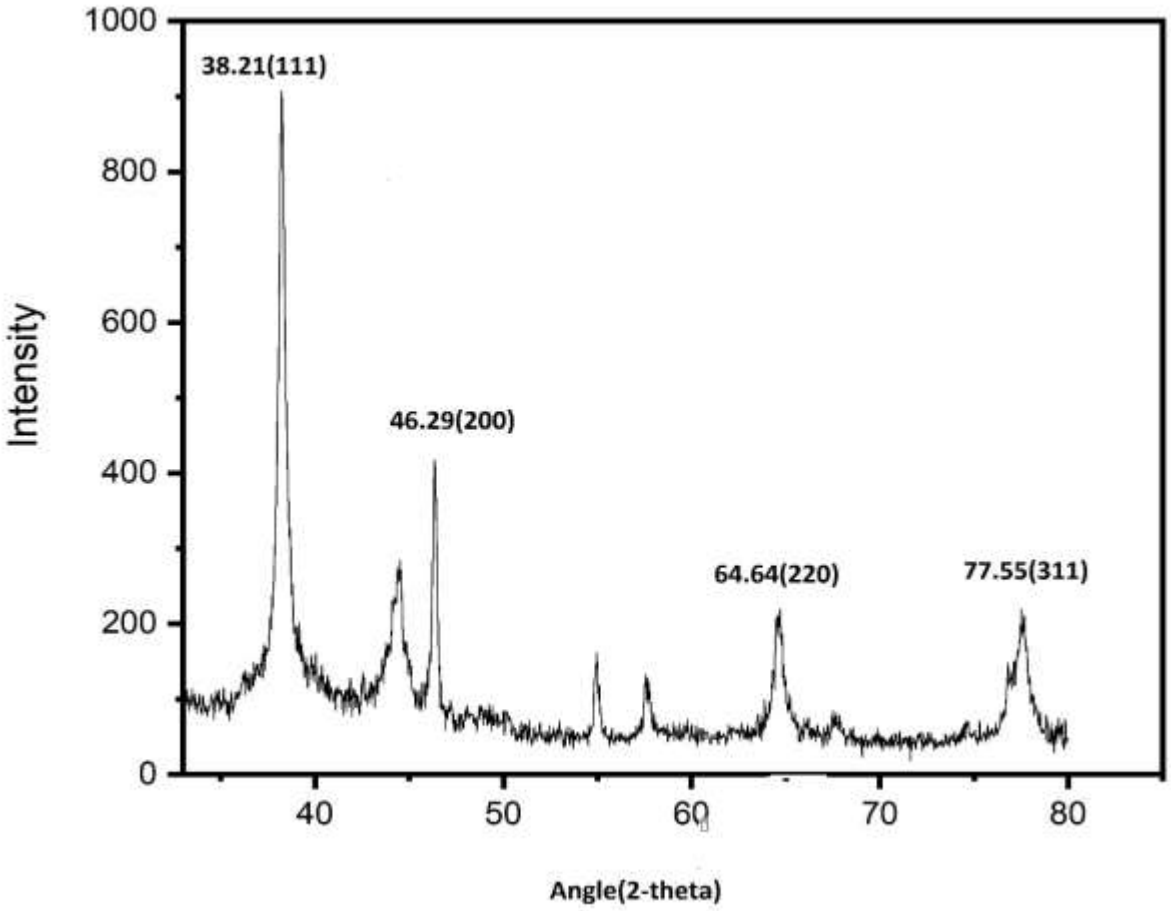

Fig: 6 XRD analysis of AgNPs prepared of *I. spicata*.

3.5 DPPH Scavenging Activity

The synthesized AgNPs showed potent antioxidant property as compared to plant extract. In this assay various concentrations of crude extract AgNPs were used. The maximum DPPH scavenging potential was shown by AgNPs at 50µg/ml as compare to crude plant extract. (Fig 8) showing concentration effect of both AgNPs and crude extract .

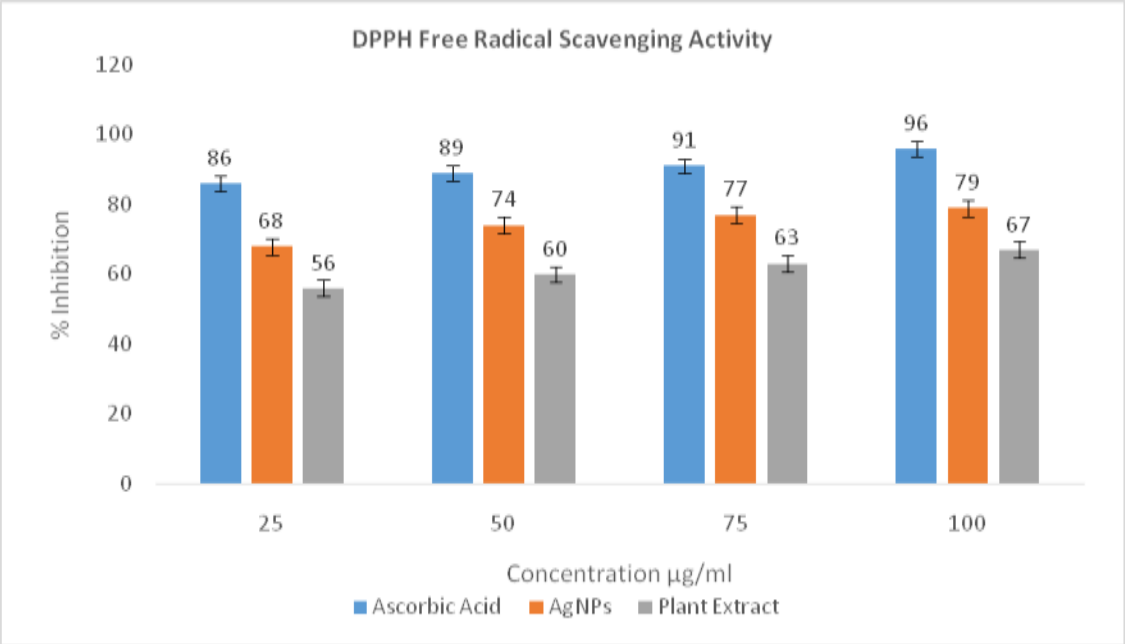

Fig: 7 DPPH free radical scavenging activity of synthesized AgNPs and *I. spicata* extract.

3.6 Hydrogen peroxide scavenging activity

Plant extract and AgNPs have free radicals scavenging property. In this assay four different concentrations of AgNPs and crude extract were used. The increase in concentration increase the scavenging ability of both AgNPs and crude extract. However the AgNPs sowing more scavenging effect as compare to crude plant extract of the same plant *I. spicata*.Fig 9 showing that at concentration 80 both plant and AgNPs maximum scavenging activity.

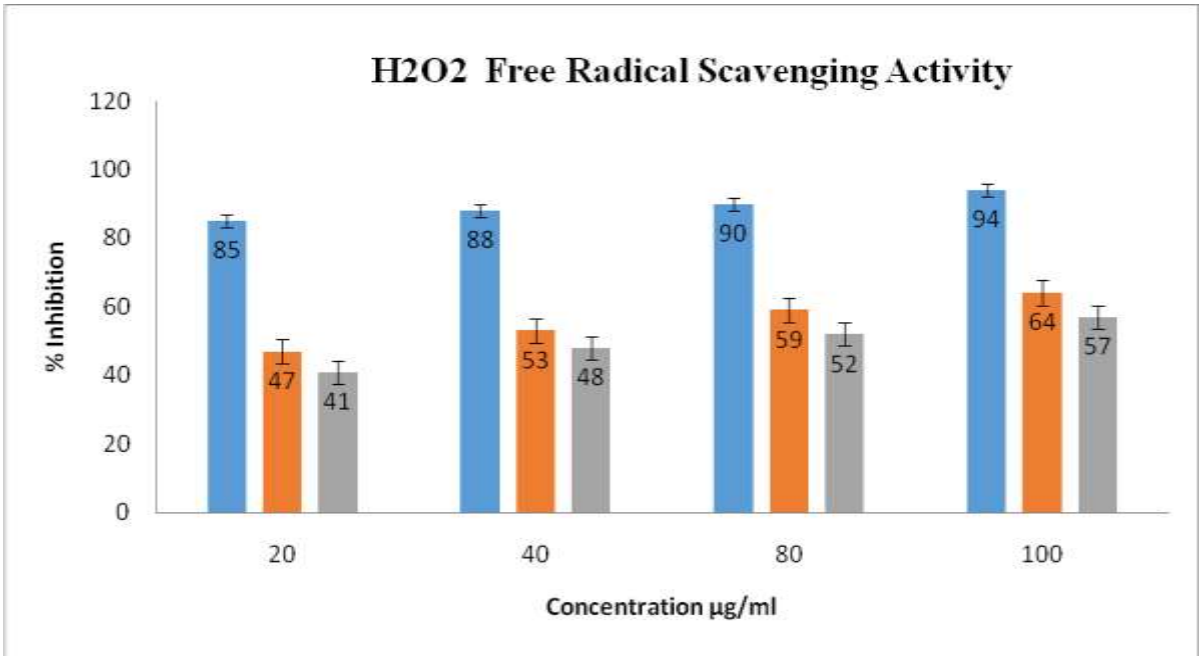

Fig. 8 H_2O_2 free radical scavenging of AgNPs and *I. spicata* plant extract.

3.7 ABTS Free Radical Scavenging Activity

In this assay ABTS free radical scavenging potential was shown by both plant based silver nanoparticles and crude extract. In comparative study the AgNPs exhibit maximum scavenging potential as crude extract of the same plant. Different concentrations were cheeked,25µg/ml,5025µg/ml,7525µg/ml and 10025µg/ml. The most potent inhibitory effect was shown by AgNPs at 50 and 75µg/ml respectively.

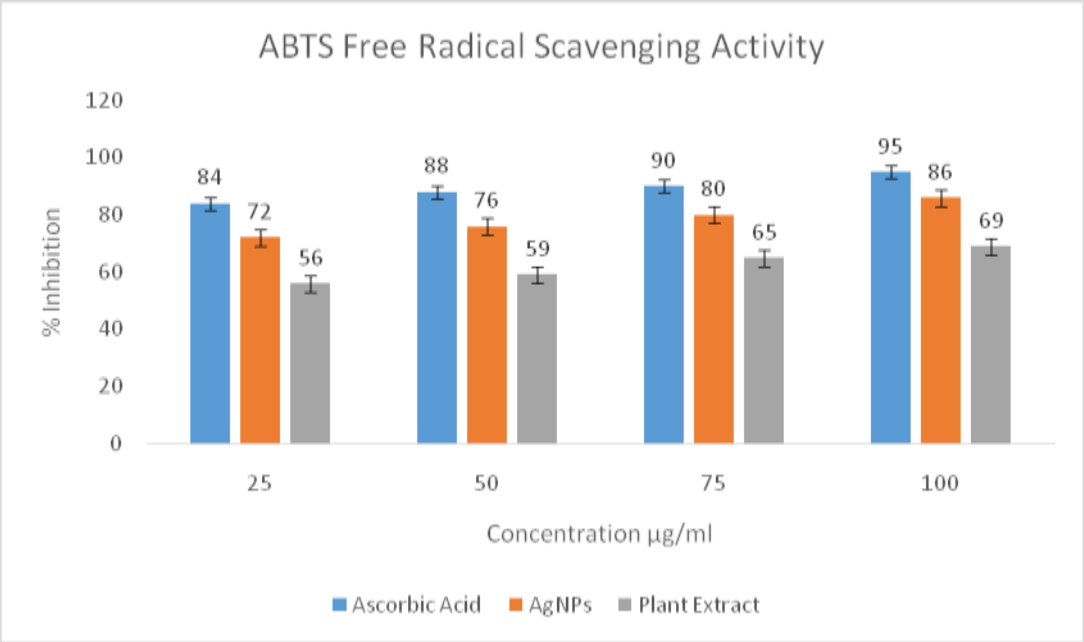

Fig: 9 ABTS free radical scavenging activity of AgNPs and *I. spicata* plant extract.

3.8 Antibacterial Activity (mm)

Table 1 shown antibacterial activity of plant extract of *I. spiccata* and synthesized AgNPs against four different bacterial strains name *Klebsiella pneumoniae* , *Escherichia coli* , *Acetobactor orientalis* and *Staphylococus aureus*. Different concentration of both AgNPs and crude extract were used. However it is reported that both AgNPs and crude extract exhibit maximum inhibitory potential at 20 µg/mL against *A. orientalis, S. aureus* and *E. coli* .The zone of inhibition is measured in (mm).

Concentrations	*K. pneumonia*	*E. coli*	*A. orientalis*	*S. aureus*
Erythromycine 100 µg/mL	47±0.78	39.5±0.89	44.3±0.35	44±0.76
I .spicata 20µg/mL	21.5±0.70	27.5±0.23	17.6±0.55	20.8±0.76
AgNPs 20 µg/mL	13.5±0.70	19.8±0.43	24.6±0.34	26.6±0.14
I. spicata 40 µg/mL	6.64±0.95	13.7±1.27	15±0.49	19.8±0.65
AgNPs 40 µg/mL	14.7±0.73	16±9.56	18±0.76	22.1±0.23

I .spicata 80 µg/mL	4.2±0.34	10.5±1.22	16.6±0.84	17.5±0.50
AgNPs 80 µg/mL	12.5±0.37	14±1.16	12.3±0.97	21.6±0.45
I .spicata 100 µg/mL	7.8±1.26	11.8±0.67	19.3±0.96	21.6±0.22
AgNPs100 µg/mL	28±0.46	21.8±1.46	17.5±0.06	12.8±0.84

Table: 1 Antibacterial activities of plant extract and synthesized AgNPs of *I. spicata*.

3.9 Cytotoxicity screening of Ifloga spicata synthesized AgNPs

The brine shrimp assay was performed for cytotoxic analysis of AgNPs .The data were collected after 24 hours. The results of the current study show that *I. spicata* AgNPs posses' significant cytotoxicity potential at a concentration 10µg(Fig11).

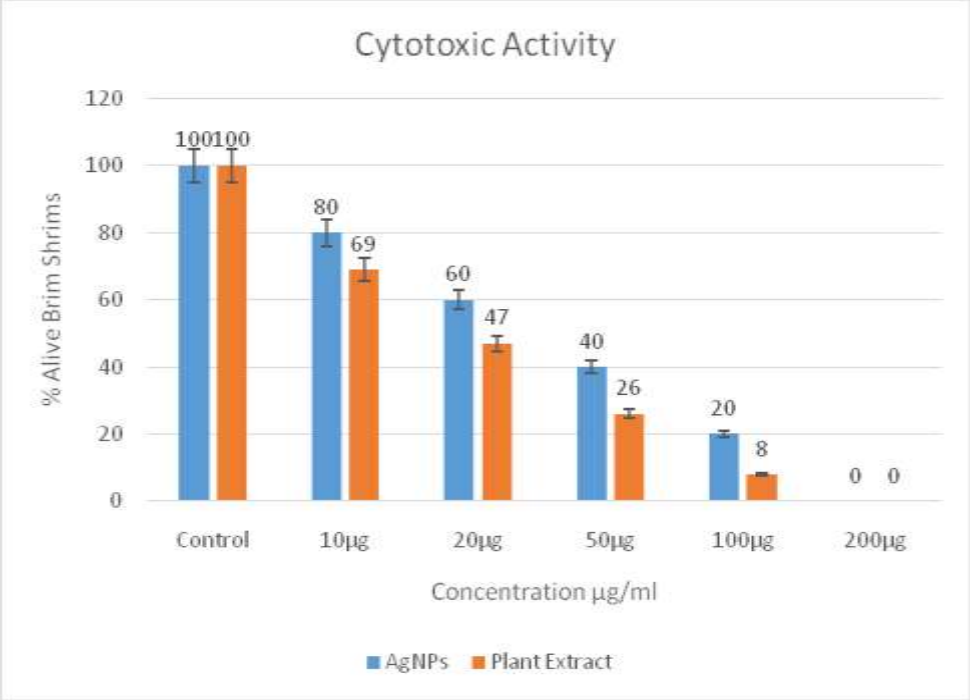

Fig:10 Cytotoxic effect of synthesized AgNPs .

4. Discussions

In current study plant based AgNPs have been synthesized. The Ft-IR study showed that the synthesized AgNPs from *Ifloga spicata* plant extract contains various functional groups. AgNPs

were characterized by using UV-Visible spectroscopy, (FTIR), (EDS), and (TEM). The nanoparticles sizes were spherical determined by SEM study. Similar reports were obtained by other scientists as well as in our lab.

Antibacterial activities of the synthesized AgNPs were also investigated against *Escherichia coli*. The antibacterial properties and silver release profiles were evaluated after interacting with phosphate-buffered saline or with serum *in vitro.*

The present reported that the nano particles prepared have significant cytoxic effect and will be useful in future for anticancer studies.

AgNPs prepared showed good inhibitory potential and significantly removed the free radicals.

5. Conclusion

Nanotechnology provides innovative approach to test and develop new synthesized drugs formulation based on biosynthesis nanopartical with different biological potentials such antioxidant and antimicrobial potentials. The physical parameters such as size and shape are important to enhance the antimicrobial potentials.

The plant-based silver nanoparticals is eco-friendly with low cast and more potent antioxidants and anticancer potentials.

Acknowledgments

The author is thankful to University of Science and Technology Bannu K.P Pakistan to provide research opportunity.

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