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Original Article

ANTIMICROBIAL, ANTIOXIDENT AND CYTOTOXIC EVALUATION OF IFLOGA SPICATA (Forssk) Sch.Bip. BIOSYNTHESIZED SILVER NANOPARTICLES

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ABSTRACT:

The study aimed to determine pharmacological and phytochemical potential of different solvent fractions of Ifloga spicata. Antimicrobial potential, free radical scavenging activity 1, 1-diphenyl-2-picrylhydrazyl (DPPH), hydrogen peroxide scavenging assay, and cytotoxic effects of plant samples on human blood lymphocytes under H_2O_2 induced stress was performed. Ifloga spicata ethyl acetate fraction markedly showed higher antibacterial potential against P. aeruginosa (ATCC 27853) and E.coli (ATCC 25922) bacterias. While higher antifungal potential was shown by the chloroform fraction against all three different strains A. fumigates (ATCC 204305), A. flavus (ATCC 9643) and A. niger (ATCC 16404) as compared to others fractions of the sample. DPPH maximum antioxidant potential was noted for methanolic extract followed by ethyl acetate with order of Methanol>Ethyl acetate>Chloroform>n-Hexane. Similarly, the hydrogen peroxide effect was significantly decreased by ethyl acetate fraction. In blood lymphocytes the raise in ROS and TBARS level due to stress given by H_2O_2 was also recovered by different extracts of I. spicata. The decrease level of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) was also restored to its normal by all extract of the Ifloga Spicata.

Keywords: Ifloga spicata, Antimicrobial, Reactive oxygen species, Human blood lymphocytes, Hydrogen peroxide.

INTRODUCTION

Herbal medicines have been practiced since ancient period and still raising in demand of the modern era, because multiple drugs resistance has been developed against microbial diseases due to irregular use of the synthetic drugs. In order to overcome the resistance side effects of the synthetic drugs scientists are trying to use herbal medicine against microbial infections. Medicinal plants contain certain bioactive ingredients which are used for various therapeutic purposes. In the developing countries approximately 60% to 90% peoples are using herbal plants for prevention of various diseases(1-2). The development of newly safe antimicrobial drug is one of the ever raising demands. Pathogenic bacteria have adverse effects on human and livestock all around world (3). Therefore, to minimize the adverse effect of these pathogenic microbes scientists are trying to identify and isolate antimicrobial agents of plant origin (4). Fruits, seeds, flower, leaves and stem possess various bioactive agents with antibacterial, phytochemicals and antioxidant potential (5-6). During metabolism Hydrogen peroxide is produced. In normal metabolism hydrogen peroxide (H_2O_2) is converted into water through peroxiredoxins, catalases and peroxidases and glutathione (7). But when this normal metabolism is disturbed then hydrogen peroxide (H_2O_2) directly form bond with transition metal and thus form hydroxyl radicals by Fenton reaction. These radicals then attack on DNA molecule sugar component and damages a single DNA molecule stand (8). To overcome the adverse effect of these agents medicinal plants are used as anticancer, anti-inflammatory agents and as management for digestive problems (9). Naturally there are different varieties of medicinal plants in Pakistan (10-11). Based on ethno medicinal value Ifloga spicata is used in present study. I. spicata belonging to family Asteraceae, is an annual herb having height of 5-10 cm usually branched at the base. The stems and branches of the plant make dense globular to cylindrical inflorescence either at base or in the upper portion. It usually prefers to grow in desserts. Ifloga spicata

is mostly distributed in Canary Island, southern Spain to N. Africa through Middle East to Afghanistan, Pakistan and India. Flowering period is February-September (12). These plants are readily available and have potential to be used as therapeutic options. However, there is limited literature available. Therefore, this study was aimed to determine antimicrobial, antioxidant and cytotoxic potential of I. spicata extracts on human blood lymphocytes exposed to H_2O_2 oxidative stress.

METHODS:

Plant Collection:

The plant I. spicata was collected from Bannu District Khyber Pakhtonkhwa, Pakistan. Dr. Faizan Ullah Khan Assistant professor Department of Botany University of Science and Technology Bannu identified the plant. It was assigned with a voucher No (Is-I5) and was stored in Deportment of Botany University of Science and Technology Bannu.

Extraction and final product preparation:

Plant material was first washed with tap water followed by distilled water. The plant material was dried under shadow condition, converted into fine powder. 5 Kg powder of I. spicata was dissolved in 80% aqueous methanol and was placed for several days. After complete dissolution, the methanolic solution of I. spicata was filtered through Whatman filter paper. The filtration process was repeated for several times and was placed for evaporation. Using rotary evaporator (Buchi Rota vapor R-200) at 45°C to evaporate the methanolic contents. The extract was placed in the laboratory under control conditions for further analysis.

Fractionation:

The dried material was dissolved in 80% aqueous methanol and portioned with hexane to remove the fatty materials. Remaining residue was dissolved in water and fractionation was carried out with chloroform, ethyl acetate in increasing order polarity. All the five extracts were dried using rotary evaporator. For further analysis all extracts were stored under control condition.

Antimicrobial Potential:

For antibacterial potential of fraction (3 mg/ml DMSO) of each was checked against bacterial species S. aureus (ATCC 29213), P. aeruginosa (ATCC 27853) and E.coli (ATCC 25922) by agar well diffusion method (14). The antibiotics Cefixime and Roxithromycin (1 mg/ml DMSO) were taken into consideration as a positive control. A 100 µl of each extract or antibiotics was poured into separate wells made in agar plates inoculated with cultured bacteria. After incubation of plates at 37°C for 24 hours period, clear zones of inhibition surrounding the wells were measured in mm.

Antifungal activity:

For antifungal activity 67µl of different extracts (12 mg/ml DMSO) were mixed with melted dextrose agar in test tubes. Test tubes were kept in a slating position and inoculated with fungi Aspergillus flavus (ATCC 9643), Aspergillus niger (ATCC 16404) and Aspergillus fumigatus (ATCC 204305) separately. Positive control had antifungal agent Terbinafine. After seven days of incubation at 28°C inhibition in linear growth of fungi in each test tube was determined.

$$\% \text{ inhibition growth} = (dc-dt/dc)\ x100$$

Negative control group was represented by c whereas sample growth is represented by t.

DPPH and Hyderogen Peroxide Free Radical Scavenging Activity:

The DPPH free radical scavenging assay was performed accordingly with that of Bibi et al., (2011) (15) whereas Hydrogen Peroxide Scavenging activity was done by the procedure given by Ruche, 1998 (16).

Cytotoxicity

In the initial step lymphocytes were isolated from blood samples in a saline phosphate buffer (pH 7.4) solution collected from healthy persons with an average age of 25 years old. The samples of blood were rotated in a centrifuge machine and after discarding supernatant the pellets were added with 3 ml of ficoll-hypaque solution. After rotating the samples again in a centrifuge machine (200 x g) lymphocytes were appeared above the ficoll-hypaque layer and were collected in a saline phosphate buffer solution. The isolated lymphocytes were then diluted with culturing medium (RPMI-1640, thermoscientific). A 10 µl of the culture medium was further added with trypan blue stain (0.2%) and taken to a haemocytometer equipped with a light microscope. After separating the stained (dead) and alive (non-stained) lymphocytes it was observed that more than 85% of the cells were in a living state. The culture media having lymphocytes was further added with more culture media (1×108 cells /ml) either pure or having 1, 10, 100 µg/ml of the extracts in separate containers and placed in an incubator at 37°C for complete

two hours. After centrifugation (200×g) for 15 minutes the pellet was collected and preserved in PBS (1×106) at -20°C. The pellet having lymphocytes was used for biochemical evaluation. The antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were extracted and determined. The method of Marklund ,1974 was followd for SOD determination by determining optical density of enzyme extract at 470 nm using a spectrophotometer (17). The SOD activity unit was demonstrated as mU/106 cells.

For catalase action dichromate/acetic acid reagent (1:3) was initially prepared by Sinha, et al (1972) (18). The H_2O_2 (0.2 M) was dissolved in 0.01 M phosphate buffer. Reaction mixture was comprised of 0.01 M phosphate buffer, tissue homogenate (100µl) and 2M H_2O_2 (400µl). After adding 2 ml dichromate/acetic acid reagent samples were incubated and analyzed for absorbance at 530 nm. The catalase activity was measured µM of H_2O_2 consumed/min/mg protein.

Using method of Carlberg and Mannervik,1975 peroxidase activity was measured. Homogenete (0.1 ml) was set containing guaiacol (100 µl), H_2O_2 (300 µl) and 50 mM phosphate buffer. Change in color after incubation of samples for 60 seconds optical density was measured at 470 nm. Unit of POD was as a change occurring in absorbance of samples as 0.01unit/minute.

Estimation of TBARS was made according to Li et al.,2010 by measuring absorbance of samples at 535 nm (19). The TBARS content unit was nano-moles per 106 cells.

The content of reactive oxygen species was estimated according to procedure of Hayashi et al., (2007) (20). A suspension of cells (5 µl) or pure H_2O_2 (taken as standard) was poured in a well plate containing sodium acetate buffer (pH 4.7). Mixtures of solutions were incubated at 37° C for 5 minutes and then added with DEPPD and FeSO4 (mixed in ratio of 1:12) solution (100 µl) and again incubated at 37° C for one minute. Samples absorbance was measured at 505 nm for a duration of three minutes at an interval f 3 minutes. The ROS concentration was reported as Units/106 cells.

Statistical analyses

Using two-way ANOVA data of cytotoxicity were analyzed. The data of anti-microbial activity were analyzed by ONE WAY ANOVA.

RESULTS:

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 synthesized silver nanoparticles. The position of peak at position 450nm confirmed 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 Figure 1.

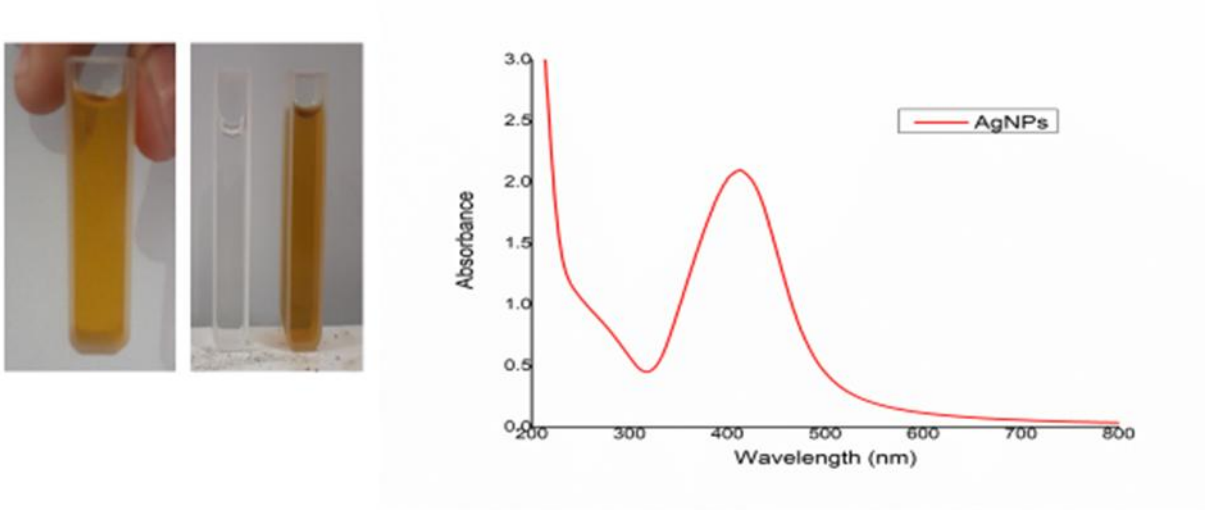

Figure 1. Calorimetric change of solution and UV-Visible spectrum of the synthesized AgNPS from extract of I. spicata plant.

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.

Extract amount effects on the synthesis of AgNPs

The plant extract amount concentration was evaluated in the range from 0.5 to 2ml. Figure 3 shows that with increase the quantity from 0.5 to 1ml, the intensity of absorption increases. When further the amount increase the intensity of absorbance then declines, showing the stability and reduction of Ag ions are almost completed at 1 ml extract concentration. Therefore, the 1ml amount is considered suitable concentration for AgNPs.

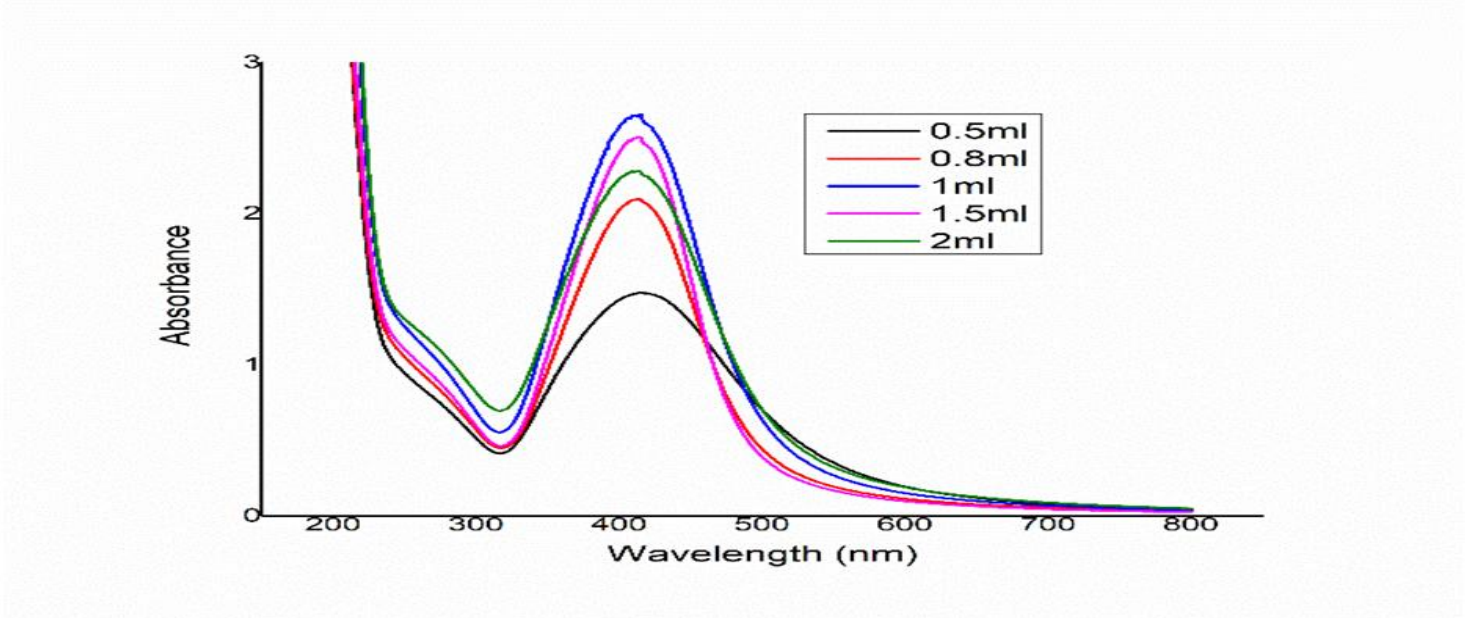

Figure 2. Effect extracts amount on the synthesis of AgNPs

FTIR of the synthesized AgNPs

FTIR analysis is important to investigate the presence of important biomolecules in the plant extract. The IR spectra at 3289.10 〖cm〗 ^(_1)show the stretching of –OH group in the plant extract with AgNps. Similarly, the peak found at 1603.13 〖cm〗 ^(_1), 1403.29 〖cm〗 ^(_1), 1259.19 〖cm〗 ^(_1), 1053.95 〖cm〗 ^(_1) showing stretching, c=c bond stretching and CH3 bending modes respectively.

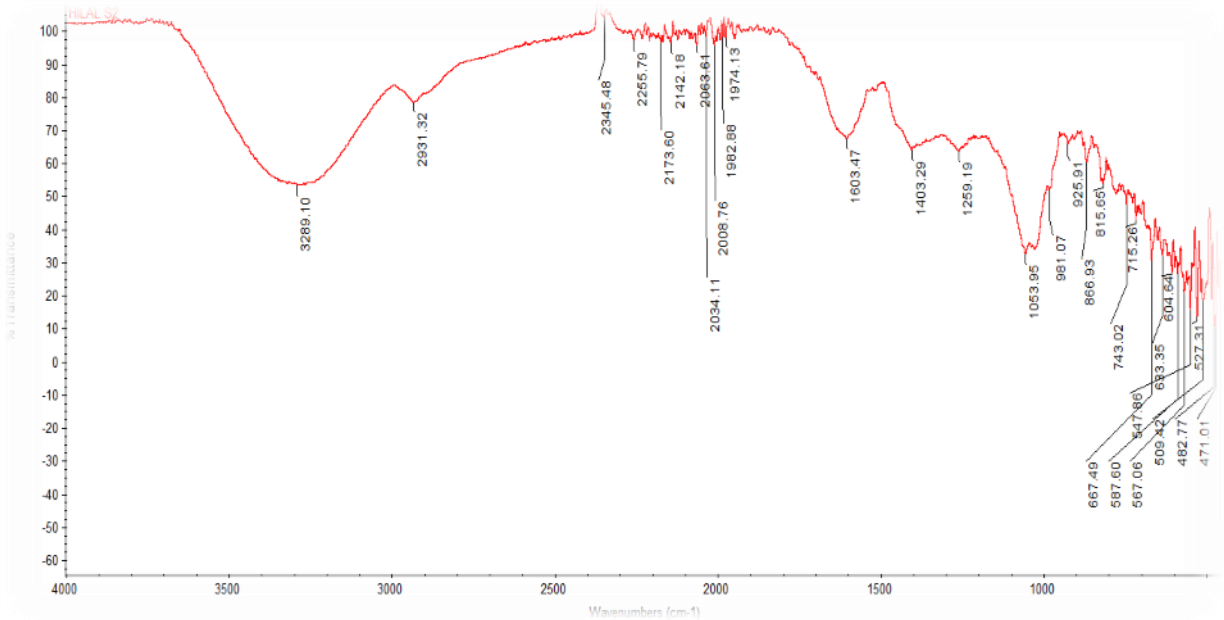

Figure 3. FTIR spectra for I. spicata plant extract before AgNPs synthesis.

Figure 4 shows 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.

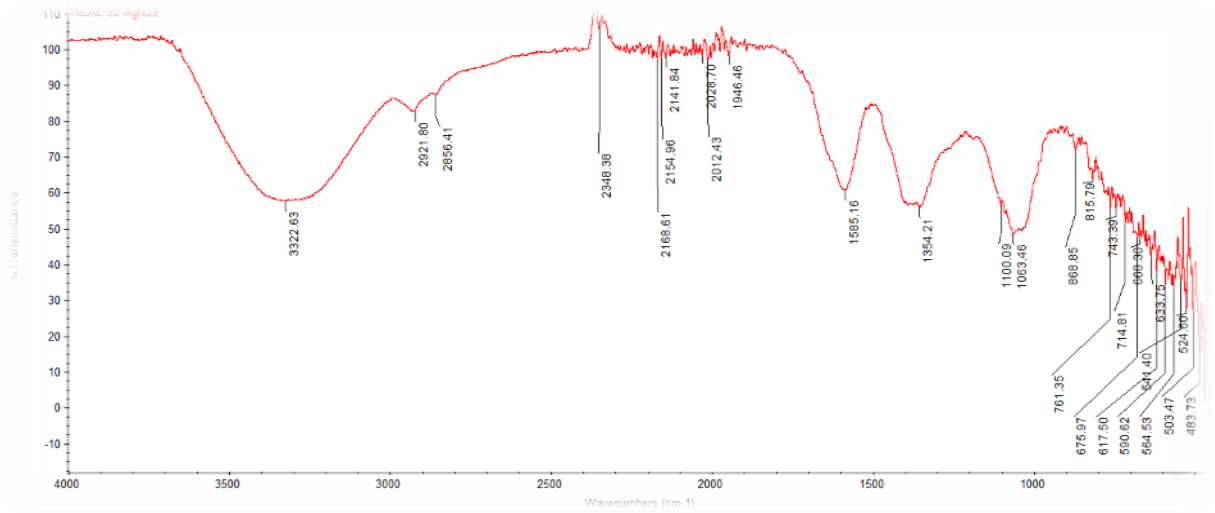

Figure 4. FTIR spectra of AgNPs synthesized from I. spicata extract.

Scan Electron Microscope (SEM)Analysis

The SEM study was carried out to determine physical nature and morphology of synthesized AgNPs. The scanned electron micrograph of synthesized silver nanoparticles are presented in Figure 5, showing 1µm size of the AgNPs.

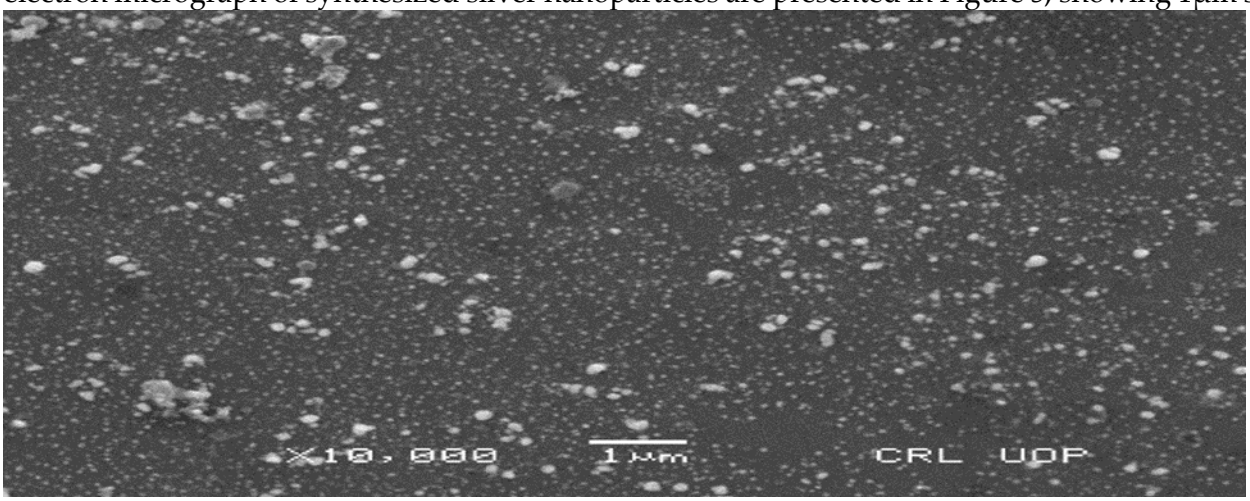

Figure 5. SEM analysis of AgNPs

X-Ray Diffraction (XRD)

X-ray diffraction study was carried out to confirm the crystalline nature of the AgNPs. The XRD blueprint, (Figure 6), 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 crystalline structure of the AgNPs.

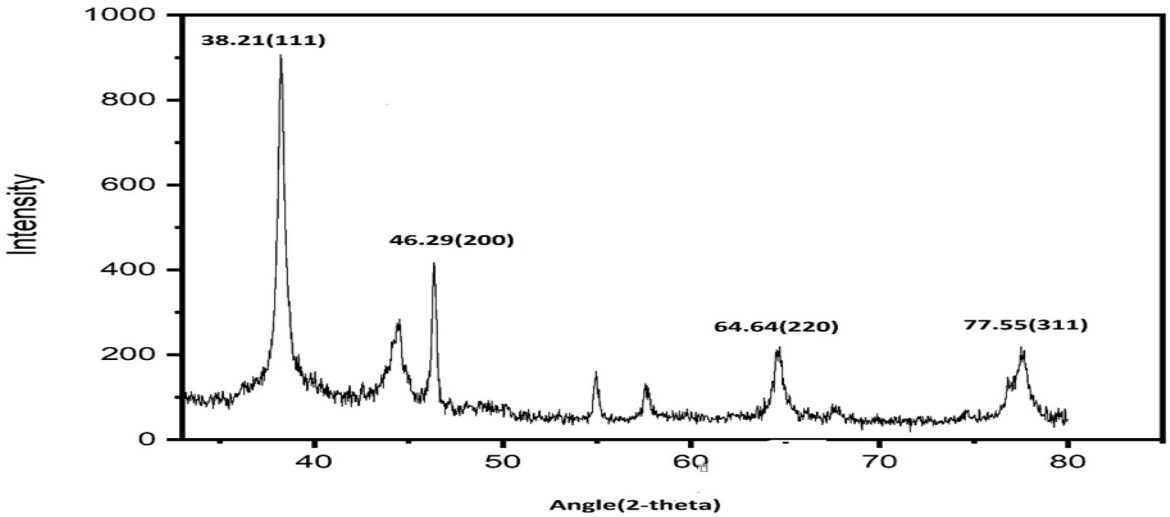

Figure 6. X-Ray Diffraction (XRD) analysis of AgNPs prepared of I. spicata.

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. Figure 7 showing concentration effect of both AgNPs and crude extract.

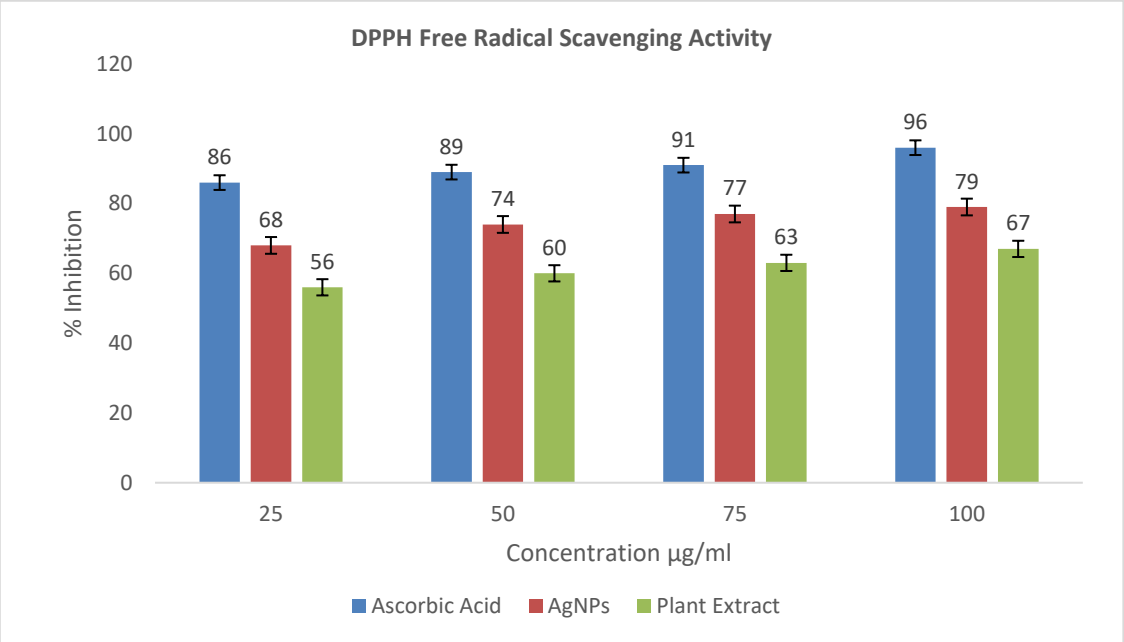

Figure 7. DPPH free radical scavenging activity of synthesized AgNPs and I. spicata extract.

Hydrogen peroxide scavenging activity

Plant extract and AgNPs have free radicals scavenging property. In this assay four different concentrations of AgNPs and crude extracts were used. The increase in concentration increase the scavenging ability of both AgNPs and crude extract. However, the AgNPs showing more scavenging effect as compared to crude plant extract of the same plant I. spicata. Figure 8 showing that at concentration 80 both plant and AgNPs maximum scavenging activity.

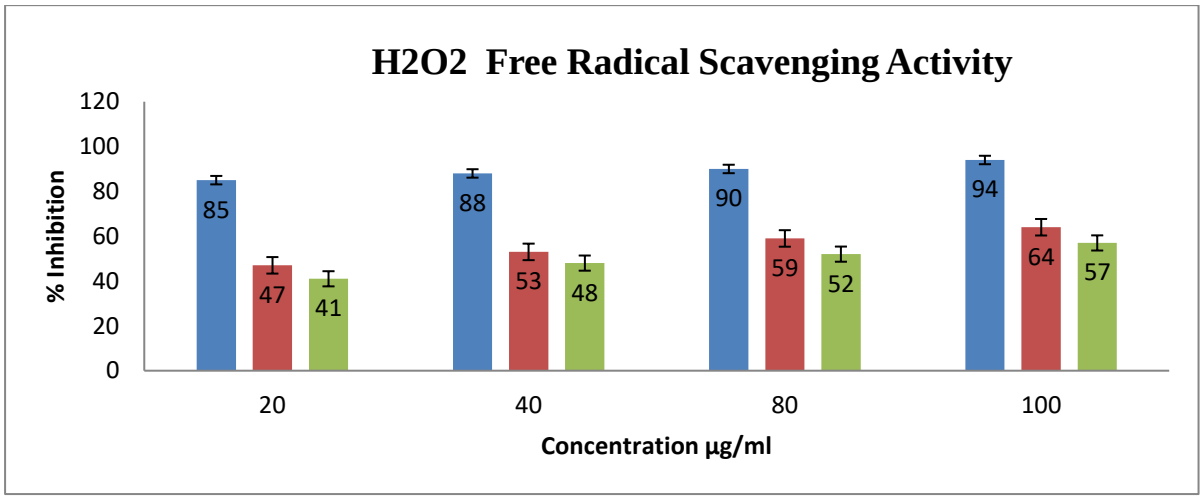

Figure 8. H_2O_2 free radical scavenging of AgNPs and I. spicata plant extract.

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 including 25µg/ml, 50µg/ml,75µg/ml and 100µg/ml. The most potent inhibitory effect was shown by AgNPs at 50 and 75µg/ml respectively.

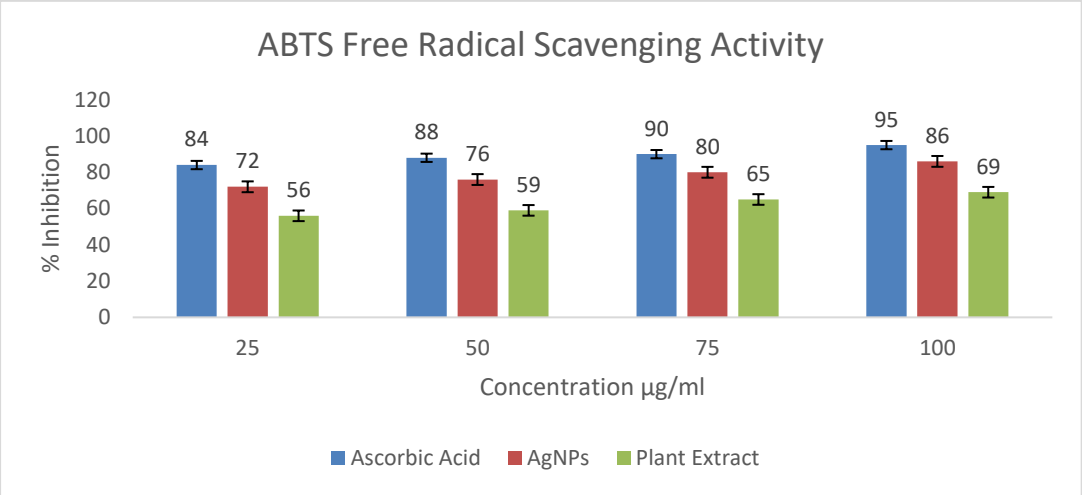

Figure 9. ABTS free radical scavenging activity of AgNPs and I. spicata plant extract.

Antibacterial Activity (mm)

Table 1 shows antibacterial activity of plant extract of I. spiccata and synthesized AgNPs against four different bacterial strains namely Klebsiella pneumoniae, Escherichia coli , Acetobactor orientalis and Staphylococus aureus. Different concentrations 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).

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

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

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 (Figure 10).

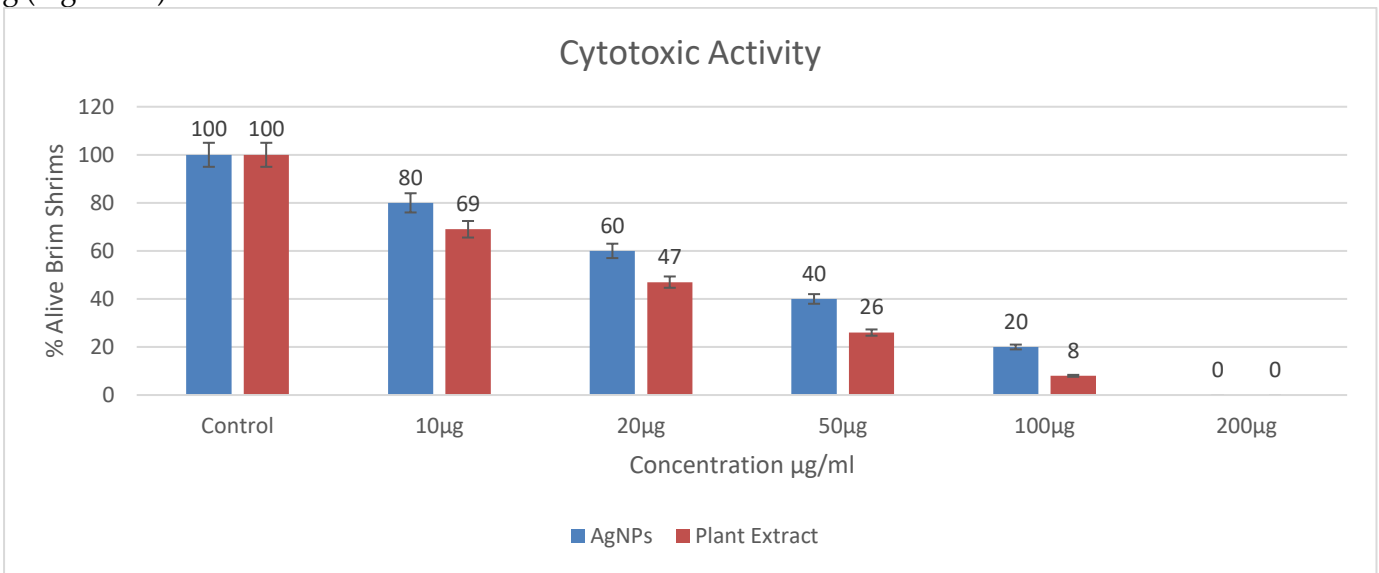

Figure 10. Cytotoxic effect of synthesized AgNPs

Effect of Plant based AgNPs on ROS content of lymphocytes

Under normal conditions extract of plant based AgNPs has no adverse effect on ROS contents in blood lymphocytes. The ROS level in blood lymphocyte was increased due to H_2O_2 stress. The elevated ROS level due to H_2O_2 treatment was considerably recovered by different fractions of I.spicata based AgNPs. Most effective treatments were found at 5ug/ml and 50 ug/ml different concentrations. The fractions ethyl acetate and n-hexane was significantly recovered ROS level ($p>0.05$).

Effect of Ifloga spicata on Antioxidant Enzymes Activity of Blood Lymphocytes

Due to H_2O_2 stress the blood lymphocytes SOD, CAT and POD activities were significantly deceased as compared to normal control ($p<0.05$). Under normal conditions the plant based AgNPs having no side effect on the SOD, CAT and POD activities of blood lymphocytes. However, the various fractions of plant based AgNPs at 5 µg/ml and 50 µg/ml significantly increased antioxidant enzymes activity (SOD, CAT and POD) potential. Despite the other fractions, methanol and aqueous fractions were significantly more effective in recovering the above enzymes activities as shown in Tables 2-4.

Effect of Plant based AgNPs on TBARS of blood lymphocytes

The blood lymphocytes were observed with higher TBARS activity (nano-moles per 106 cells) when treated with H_2O_2 as compared to control group. However, the different fractions of plant based AgNPs recovered the TBARS contents. 5 ug/ml and 50 ug/ml were the most effective concentration at which blood lymphocytes recovered maximum TBARS contents. As shown in Table 5 the two AgNPs fractions, ethyl acetate and chloroform were more significantly recovered the induced TBARS oxidative stress ($p<0.05$).

Table 1. Showing the effect of plant based AgNPs on ROS content (Units / 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress.

Treatment	Fractions					Mean
	E1(Methanol	E(Ethylacetate)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	0 .2220 ±0.03g-h	0 .2560±0.04g	0 .2560±0.04g	0 .2560 ±0.03g	0 .2560 ±0.03g	0 .256±0.02de
100µM Hydrogen per oxide	0 .3950 ±0.6a	0 .3950 ±0.03a	0.3950 ±0.017a	0 .3950 ±0.06a	0 .3950±0.02a	0 .3950±0.017a
AgNPs 0.5 µg/ml	0 .2433±0.4 g	0 .486±0.06e	0 .2643±0.05f	0 .2399±0.01g	0 .2650±0.10f	0 .2582±0.032d
AgNPs 5 µg/ml	0 .2401±0.16 h	0 .2529 ±0.04e	0 .2337 ±0.04h-j	0 .2379±0.3g	0 .2360±0.02g	0 .2393±0.06de
AgNPs 50 µg/ml	0 .2111±0.2 i	0 .2423±0.02g	0 .2357 ±0.03g-j	0 .2189±0.05i	0 .2327±0.05h	0 .2281±0.04 e
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	0 .3753 ± 0.014ab	0 .3889±0.024ab	0 .3679±0.07 ab	0 .3639±0.02ab	0 .3559±0.04a	0 .3694 ±0.02b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	0 .2837±.0.2 e	0 .36457±0.03ab	0 .3475±0.02a-d	0 .3124±0.022d	0 .3449 ±0.02b	0 .3298±0.012c
100µM Hydrogen per oxide + AgNPs 50 µg/ml	0 .2733 ±0.21e	0 .3795±0.03ab	0 .3621 ±0.03a-c	0 .3148 ±0.4c	0 .3495 ±0.5ab	0 .3351 ±0.14c
Mean	0 .2835±0.02 c	0 .3185 ±0.03a	0 .2964 ±0.05ab	0 .2899 ±0.08b	0 .2932±0.04ab	

± showing standard error value.

Table 2. Showing the effect of Plant based AgNPs on CAT activity (milli Units / 10^6 cells) of human blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	3.9211±0.14b-e	3.9211± 0.16b-e	3.8100±0.13b-e	3.9211±0.14b-e	3.91211 ±0.14b-e	3.9211 ±0.12a
100µM Hydrogen per oxide	1.8571±0.12n	1.8571±0.13n	1.8571±0.112n	1.8571±0.15n	1.8571 ±0.12n	1.8571 ±0.114c
AgNPs 0.5 µg/ml	4.4671±0.305ab	3.86110±0.16b-e	3.4671 ±0.14e-h	3.7811±0.17b-e	3.8183±0.14b-e	3.8829 ±0.16a
AgNPs 5 µg/ml	4.3411±0.12abc	3.6611±0.25d-f	3.7291±0.16c-e	3.969±0.13b-e	3.9870 ±0.05b-e	3.9430 ±0.16a
AgNPs 50 µg/ml	4.7991±0.13a	3.6450±0.12d-e	4.2371±0.15a-d	3.8269 ±0.4b-e	3.9531±0.14b-e	4.0143 ±0.14a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	2.9131 ±0.16 f-j	1.8990 ±0.0121n	1.9420 ±0.14n	2.5581±0.12j-m	2.7821±0.13i-m	2.499 ±0.13b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	3.5711 ±0.14d-g	2.1199±0.10mn	2.15620±0.15nm	2.3911±0.15j-m	2.82540 ±0.17-l	2.6070 ±0.14b
100µM Hydrogen per oxide + AgNPs 50 µg/ml	3.4270 ±0.11e-i	2.08940 ±0.011mn	218980 ±0.11k-n	2.3920±0.02j-m	2.8523 ±0.03j-k	2.6455 ±0.12b
Mean	3.6998 ±0.13a	2.8449 ±0.17c	2.9885±0.12c	3.0197 ±0.12bc	3.2899 ±0.011b	

± showing standard error value.

Table 3. Showing the effect of Plant based AgNPs on POD activity (nmol / 10^6cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethyl acetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	5.9930 ±0.123ab	5.9931±0.32ab	5.9931± 0.14ab	5.9931±0.34ab	5.9931 ±0.04ab	5.9931 ±0.43b
100µM Hydrogen per oxide	3.9391 ±0.14de	3.9391±0.12de	3.9391 ±0.23de	3.9291±0.22de	3.9391 ±0.32de	3.9391 ±0.13cd
AgNPs 0.5 µg/ml	6.1371 ±0.134ab	4.7571±0.4cd	6.999± 0.11a	6.9999±0.15a	6.3285 ±0.15ab	6.2445 ±0.16ab
AgNPs 5 µg/ml	5.9511 ±0.144ab	6.288 ±0.31ab	6.0645± 0.10ab	6.2711 ±0.32ab	6.645 ±0.34a	6.2943±0.51ab
AgNPs 50 µg/ml	7.1493 ±0.12a	6.6911 ±0.44a	6.4485±0.12a	6.3911±0.22ab	6.8150 ±0.16a	6.724±0.125a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	3. 3841 ±0.13de	3.4995±0.22e	3.49999± 0.15e	3.481±0.23e	3.3291 ±0.23e	3.4329±0.12d
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	3.7351±0.16de	4.3373 ±0.14c-e	3.6791± 0.14de	3.9389 ±0.13c-e	4.3751 ±0.35c-e	3.9555 ±0.22cd
100µM Hydrogen per oxide + AgNPs 50 µg/ml	4.3631 ±0.23c-e	3.4460 ±0.02de	3.7890± 0.15de	3.5937±0.12de	5.1871±0.432bc	3.9882 ±0.13c
Mean	5.1526 ±0.06ab	4.8862±0.001b	4.9678 ±0.13ab	4.9938±0.34ab	5.3569 ±0.33a	

± showing standard error value.

Table 4. Showing the effect of Plant based AgNPs on SOD activity (milli Units / 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	10.355 ±0.34d	10.355±0.33de	10.355 ±0.12d	10.355 ±0.32d	10.355 ±0.47d	10.355 ±0.23b
100µM Hydrogen per oxide	5.147 ±0.43k	5.147 ±043k	5.047±0.14k	5.147 ±0.25k	5.047±022k	5.147±144 e
AgNPs 0.5 µg/ml	11.759±0.43a-d	11.235± 0.11a-e	10.263±0.17e	10.221 ±0.34ef	10.720 ±0.32b-e	10.838 ±0.13ab
AgNPs 5 µg/ml	11.395±0.44a-e	10.663±0.5cde	11.267 ±0.66a-e	10.981±0.14b-e	12.193±0.01a b	11.299 ±0.35a
AgNPs 50 µg/ml	11.859±0.54ab	11.588 ±0.47a-e	10.769±0.7b-e	8.6779 ±0.44g	12.585 ±0.10a	11.018±067a
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	7.409±0.55ijk	7.027±0.33ijk	5.503±0.34jk	5.907±0.7i-k	7.403 ±0.77g-i	6.312±0.53d
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	10.688 ±0.55g	9.327 ±0.34h	8.663±0.55i-j	7.495±0.63k	6.881±0.44ij	7.969±0.39c
100µM Hydrogen per oxide + AgNPs 50 µg/ml	8.6587 ±0.44gh	6.373 ±0.32ij	6.223 ±0.01i-j	5.977±0.33i-k	8.746±0.66fg	7.1216 ±0.2c
Mean	9.2577±0.12a	8.4868±0.33b	8.258 ±0.33bc	7.8563±0.26c	9.2564±0.33a	

± showing standard error value.

Table 5. Showing the effect of Plant based AuNPs on TBARS (nano-moles/ 10^6 cells) of blood lymphocytes induced H_2O_2 oxidative stress

Treatment	Fractions					Mean
	E1(Methanol)	E(Ethylacetat)	E3(*n*-hexane)	E4(Chloroform)	E5(Aqueous)	
Control	0 .7971±0.012f	0 .7971 ±0.013f	0 .7971 ±0.22f	0 .7971 ±0.11-j	0 .7971 ±0.13f	0 .7971 ±0.2a
100µM Hydrogen per oxide	1 .0255±0.11a	1 .0255±0.10a	1 . 0255±0.11a	1 . 0255±0.12aA	1 . 0255±0.014a a	1 . 0255 ±0.11a
AgNPs 0.5 µg/ml	0 .6463±0.03k-l	0 .7421±0.22h-j	0 .7633 ±0.23h-j	0 .73470.23h-l	0 .7629 ±0.0031h	0 .7299 ±0.011e
AgNPs 5 µg/ml	0 .5951±0.003lm	0 .6199 ±0.41i-	0 .7945±0.11g	0 .7617 ±0.08h	0 .6899±0.11i	0 .7142 ±0.04e
AgNPs 50 µg/ml	0 .5763 ±0.01m	0 .5985 I±0.02i-m	0 .6790 ±0.30j-m	0 .7520±0.20h-k	0. 6690 ±0.001j	0 .6749 ±0.04e
100µM Hydrogen per oxide + AgNPs 0.5 µg/ml	0 .9145±0.04abc	0 .9185±0.57abc	0 .9849±0.01ab	0.8853±0.004bcd	0 .7575±0.02d-h	0 .8981 ±0.12b
H_2O_2 (100µ100µM Hydrogen per oxide + AgNPs 5 µg/ml	0 .8417±0.012c-f	0 .9213 ±0.012abc	0 .8943±0.62abcd	0 .8593±0.016cd	0 .7651 ±0.013d-h	0 .8565 ±0.16bc
100µM Hydrogen per oxide + AgNPs 50 µg/ml	0 .8360 ±0.017e	0 .9544 ±0.017cd	0 .9230 ±0.11c	0 .9700±0.013ab	0 .8278±0.33e	0 .9202 ±0.23c
Mean	0 .8168±0.14b	0 .8849 ±0.015a	0 .8980±0.11a	0 .894 ±0.11a	0 .881±0.11b	

± showing standard error value

DISCUSSION

The development of easy, reliable and eco-friendly methods helps to increase interest in the synthesis and application of nanoparticles that are beneficial for mankind. Speedy biosynthesis of stable gold, silver and bi-

metallic Ag/Au core shell nanoparticles with 20 g of Azadirachta indica leaf biomass and 1mM aqueous $AgNO_3$, with a 90% reduction of the metal ions within 4 hrs. The dissimilarity in the rates of bio-reduction observed may be due to the differences in the activities of the enzymes present in the A. indica and I. herbstii aqueous leaf extracts. S. Ankanna et al.,2010 also reported the reduction of silver ion into silver particles when mixed to plant extracts could be followed by a color change (21). The silver nanoparticles exhibited a dark yellowish-brown color in the aqueous solution due to the surface plasmon resonance phenomenon.

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 size were spherical determined by SEM study. 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 study showed promising results directing towards therapeutic potential of the studied plant extracts, however further studies will be required to confirm targeted used and drug development.

CONCLUSION

Nanotechnology provides innovative approach to test and develop new synthesized drugs formulation based on biosynthesis nanoparticles 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 nanoparticles are eco-friendly with low cost and more potent antioxidants and anticancer potentials.

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Conflict of Interest

Authors declare no conflict of interest.

Ethical consideration

The study was approved by the local Ethical Review Committee.

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