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RESEARCH ARTICLE

OPTIMIZATION OF GREEN SYNTHESIZED SILVER NANOPARTICLESUSING *ALOE VERA*FOR THEIR INVESTIGATION OF ANTIBACTERIAL ACTIVITY

Shanta Prasain, Hookman Jimi Rai and Anup Basnet
Department of Microbiology, St. Xavier's College, Tribhuvan University, Kathmandu, Nepal.

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Abstract

Silver nanoparticle synthesis is an interesting area in nanotechnology due to their remarkable biomedicalproperties. To prepare silver nanoparticles, reduction of Ag^+ ions to Ag° nanoparticles was done in a medium of *Aloe vera*. The AgNPs were synthesized by mixing extract and silver nitrate then heated at 80°C for 30 minutes maintaining alkaline pH10. The obtained colloidal solution was dried and taken to confirm the formation of silver nanoparticles through X-ray diffractometer. The average sizes of AgNPs were found to be in a range of 5 to 8 nm in controlled environment. The formation of smaller size silver nanoparticles was seen at higher concentration of extract (25gm/100ml), silver nitrate(4mM) and higher temperature (70°C to 80°C).The resulting silver nanoparticles were found to be effective against *Staphylococcus aureus*ATCC 25923and *Escherichia coli*ATCC 25922, *Klebsiella pneumoniae*ATCC 700603. However synthesized silver nanoparticles were observed less effective against *Pseudomonas aeruginosa*ATCC 27853. The resulting silver nanoparticles showed a synergism of *Aloe vera* extract and silver nanoparticles on bactericidal effect.

Introduction:-

Nanoparticles represent a particle with a nanometer size of 1–100nm [1]. Different studies have been done in silver and its nanoparticles and it was concluded that silver has strong antimicrobial property. Silver containing materials has been used in different medical procedures, for example, to reduce infection in burn treatment and arthroplasty as well as to prevent bacteria colonization on prostheses, catheters, vascular grafts, dental materials. Silver containing materials are also used in various industries like water treatment and for elimination of microorganisms on textile fabrics [9].

Silver nanoparticles can be synthesized by biological route which provides non-toxic, eco-friendly and reliable synthesis of nanoparticles [7]. Synthesis by biological method is relatively easy and cost effective compared to the physical and chemical method [1].

The green synthesis of silver nanoparticles from Aloe vera proceeds when the plant extracts come in contact with silver nitrate solution with phytochemicals present in Aloe vera (AloinA and AloinB, Flavanoids, Lupeol, Mannose, Resveratol, Emodin) [15] reduce silver nitrate into Ag^+ to Ag° nanoparticles or the extract is used to reduce the

Corresponding Author:- Shanta Prasain
Address:- Department of Microbiology, St. Xavier's College, Tribhuvan University, Kathmandu, Nepal.

silver ions to form silver nanoparticles in the pH and temperature-controlled environment also called as one-step hydrothermal method[12].

To relate the scope of nanoparticles in bioscience and involvement in improving the welfare through food fortification and medicinal purpose, food packaging system, there has not been enough researches and application of the best possible benefit/scope of nanoparticles come in practice yet. Nepal's involvement and concern in this field is far fledged. However few researchers have researched regarding the metal nanoparticles and discussed its scopes in very individual level [8].

In recent years, increasing antibiotic resistance by microbes is imposing serious threat to the health sector. Silvernanoparticles have proved to be a likely candidate for antimicrobialagent since their large surface to volume ratio ensures a broadrange of attack on bacterial surface. AgNPs exhibited significant antibacterial activityagainst*Escherichia coli*,*Staphylococcusaureus*and antifungalactivity against*Trichophyton*,*Trichosporonbeigelii*and*Candidaalbicans*[13].

Materials and Methods:-

Collection of plant material

Healthy leaves of Aloe vera were collected from Kalanki, Kathmandu.

Preparations of leaf extract (reducing agent)

Fine pieces of Aloe verawere boiled for 20 minutes and filtered [10].

Synthesis and optimization of silver nanoparticles

Synthesis and optimization process was done according to [6] with slight changes.

Effect of variation of Aloe veraConcentration

20ml of 1mM $AgNO_3$ was added to each 25 mL of 10gm/100ml Aloe vera extract adjusting pH at10 using 0.1M NaOH solution and placed in a shaking water-bath at temperature of 80°C for 20 minutes. The above procedure was repeated for plant extract concentrations of 15gm/100ml, 20g/100ml, and 25g/100ml.

Effect of Variation of $AgNO_3$Concentration

20ml of 1mM $AgNO_3$ was added to 25 ml of 10g/100ml Aloe veraextractadjusting pH to10 using 0.1M NaOH solution and placed in a shaking water-bath at temperature of 80°C for 20 minutes. The above procedure was repeated for $AgNO_3$ concentration values of 2mM, 3mM and 4mM.

Effect of Variation of temperature

20 ml of 1mM $AgNO_3$ was added to 25 mL of 10g/100ml *Aloe vera*extractadjustingpHat 10 using 0.1M NaOH solution. Then placed in a shaking water-bath at temperature of 40°C for 20 minutes. The above procedure was repeated for temperature values of 50°C, 60°C and 70°C.

Recovery of nanoparticles

Thesolutions were centrifuged at 5000rpm for 30 minutes to recovered and washed. Then the nanoparticles were dried at 60°C according to Poudel et al [8].

UV-spectrophotometer

UV-Visspectrophotometer in the wavelength range of 250– 700 nm to obtain the UV-Visible spectra of the sample. The distilled water used as a blank reference[6].

XRD (X-ray diffraction)

The dried powdered of silver nanoparticles were used to determine crystalline structure of silver and the XRDwas done at NAST, Khumaltar, Nepal using D2 phazer machine of Bruker Company with Cu-Kα (λ=1.54056 Å) radiation. The diffraction pattern was recorded from diffraction angle range of 10º to 90º at a 2θ pattern. The average crystalline size was calculated from the width of the XRD peaks using the Scherrer formula:$D = K\lambda / \beta\cos\theta$ Where,

λ is the X-ray wavelength in nanometer (nm)β is the peak width of the diffraction peak profile at half maximum height resulting from small crystallite size in radians θ is the angle of incidence of X-ray and K is a constant related to crystallite shape and taken as 0.9[8].

Assessment of Antimicrobial activity silver nanoparticle

Antimicrobial activity of synthesized silver nanoparticle was tested by agar well diffusion method.Bacteria including *Klebsiella pneumoniae*ATCC 700603, *Escherichia coli*ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 were used as hosts to test the antimicrobial activity. Antibiotics such as Streptomycin 10mg/disc, Ciprofloxacin 30mcg/disc and Gentamycin 10mcg/disc were used as positive control[12].

Results:-

UV-Spectrophotometric analysis

A distinct color change from pale yellow to brown was observed after addition of aqueous Aloe vera extract to silver nitrate solution.

***Aloe vera* extract concentration variation**

At 10gm/100ml of *Aloe vera* extract peak absorbance was 0.185 at 440nm wavelength. Followed by 15gm/100ml of *Aloe vera* extract concentration, the peak absorbance was 0.288 at 423nm wavelength. Then at 20gm/100ml extract peak absorbance was 0.367 at 420nm. Then at 25gm/100ml extract peak absorbance was 0.671 at 441nm wavelength as shown in figure 1.

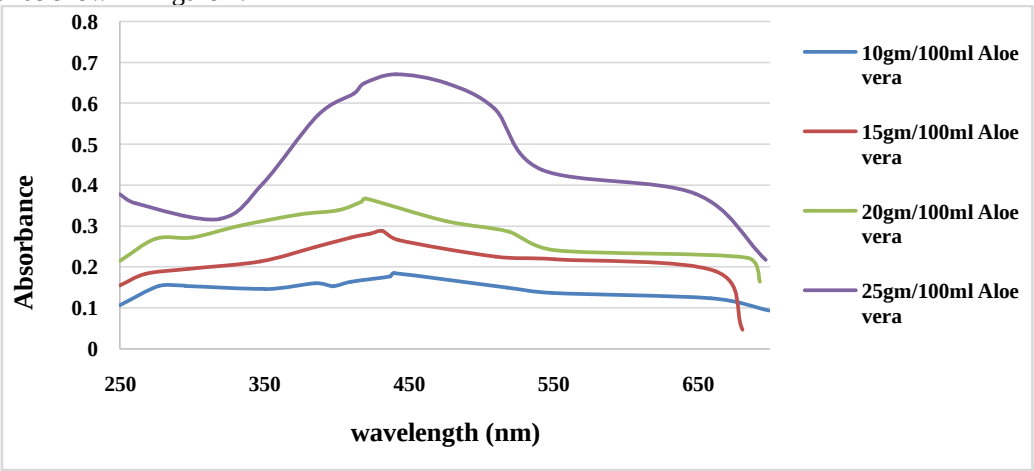


Fig. 1:- UV–vis absorbance spectra of AgNPs synthesized using *Aloe vera* under different *Aloe vera* concentrations.

$AgNO_3$ concentration variation

At 1mM of $AgNO_3$ peak absorbance was 0.577 at 422nm wavelength. Followed by 2mM of $AgNO_3$ concentration, the peak absorbance was 0.729 at 429nm wavelength. Then at 3mM of $AgNO_3$ peak absorbance was 0.770 at 411nm. Then at 4mM of $AgNO_3$ extract peak absorbance was 1.684 at 416nm wavelength as shown in figure 2.

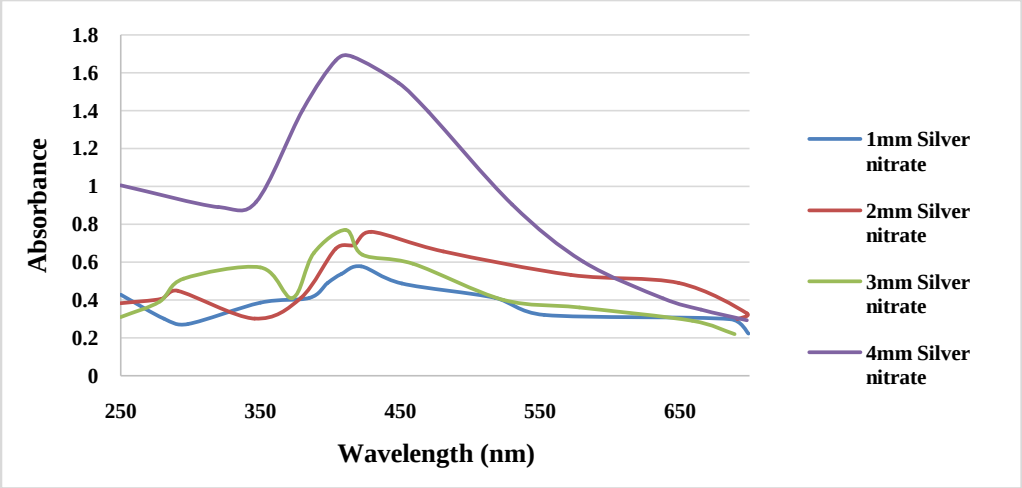


Fig.2:- UV–vis absorbance spectra of silver nanoparticles synthesized under different $AgNO_3$ concentrations.

Temperature variation

At 40°C peak absorbance was 0.399 at 422nm wavelength. Followed by 50°C the peak absorbance was 0.423 at 417nm wavelength. Then at 60°C peak absorbance was 0.577 at 411nm. Then at 70°C of $AgNO_3$ extract peak absorbance was 1.243 at 410nm wavelength as shown in figure 3.

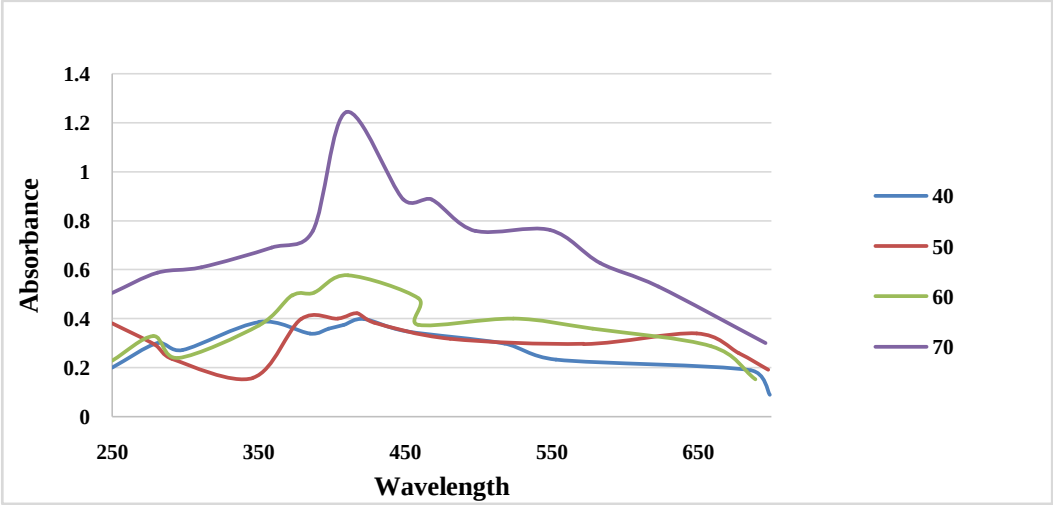


Fig. 3:- UV–vis absorbance spectra of silver nanoparticles synthesized under different temperature.

X-ray diffraction of AgNPs at Aloe vera concentration variation

The average size of the silver nanoparticle synthesized from Aloe vera extract at the concentration of 10gm/100ml of extract was 8.92, Likewise at 15gm/100ml was 7.75, at 20gm/100ml was 7.54 and at 25gm/100ml was 6.52 as shown in table 1.

Table 1:- XRD particle size evaluation of Ag-NPs at *Aloe vera* concentration variation using Scherrer equation.

S.N	FWHM	β	2θ	θ	radian	crystallite size (D)	average size/nm
	0.89	0.01553	32.4	16.21	0.2830	9.29595	
	0.94	0.01640	38.1	19.05	0.3325	8.9413	
	0.86	0.01501	54.7	27.36	0.4776	10.4015	
	1.15	0.02007	64.4	32.23	0.5625	8.16641	
10gm/100ml	1.3	0.02268	77.3	38.65	0.6746	7.82536	8.9261
	1.21	0.02111	32.2	16.13	0.2816	6.83475	
	1.12	0.01954	38.1	19.07	0.3329	7.50521	
15gm/100ml	1.19	0.02076	44.3	22.17	0.3869	7.20883	7.7570

	0.9	0.01570	64.6	32.33	0.5643	10.4469	
	1.5	0.02618	77.4	38.73	0.6760	6.78957	
	1.05	0.01832	32.0	16.02	0.2796	7.87168	
	1.14	0.01989	37.9	18.97	0.3311	7.36911	
	1.18	0.02059	44.1	22.09	0.3856	7.26606	
	1.39	0.02426	64.2	32.14	0.5609	6.7497	
20gm/100ml	1.2	0.02094	77.3	38.65	0.6745	8.47688	7.5466
	1.41	0.02460	32.0	16.04	0.2799	5.86248	
	1.14	0.01989	37.9	18.97	0.3311	7.36911	
	2.06	0.03595	44.1	22.09	0.3856	4.16211	
	1.26	0.02199	64.4	32.24	0.5626	7.45429	
25gm/100ml	1.31	0.02286	77.3	38.65	0.6746	7.76563	6.5227

The total average size of crystallite is 7.68814.

X-ray diffraction of AgNPs at $AgNO_3$ concentration variation

The average size of the silver nanoparticle synthesized from Aloe vera extract at the concentration of 1mM of $AgNO_3$ was 10.39, Likewise at 2mM was 7.31, at 3mM was 9.68 and at 4mM was 4.26 as shown in table 2.

Table 2:- XRD particle size evaluation of AgNPs at $AgNO_3$ concentration variation using Scherrer equation.

S.N	FWHM	β	2θ	θ	radian	Crystallite size (D)	Average size/nm
1mM	0.89	0.01553	32.0	16.0	0.28003	9.287982	10.391
	0.62	0.01082	37.9	18.9	0.33135	13.55046	
	0.77	0.01343	44.1	22.1	0.38563	11.135	
	0.88	0.01535	64.3	32.1	0.56121	10.66322	
	1.39	0.02426	77.3	38.6	0.67465	7.318684	
2mM	1.08	0.01885	32.1	16.0	0.28056	7.655139	7.3153
	0.87	0.01518	38.1	19.0	0.33274	9.661302	
	1.36	0.02373	44.2	22.1	0.38624	6.305938	
	1.25	0.02181	64.4	32.2	0.56278	7.514334	
	1.87	0.03263	77.3	38.6	0.67457	5.439712	
3mM	0.74	0.01291	38.1	19.0	0.33274	11.35856	9.6887
	1.01	0.01762	44.2	22.1	0.38571	8.489357	
	0.99	0.01727	64.4	32.2	0.56269	9.487273	
	1.08	0.01885	77.3	38.6	0.67465	9.419418	
4mM	1.31	0.02286	32.4	16.2	0.28274	5.82355	4.2682
	1.47	0.02565	38.2	19.1	0.33335	5.106767	
	1.99	0.03473	44.4	22.2	0.38755	3.696041	
	1.87	0.03263	64.4	32.2	0.56278	3.593089	
	1.99	0.03473	77.1	38.5	0.67308	3.121433	

The total average size of crystallite is 7.9158.

X-ray diffraction of AgNPs at temperature variation

The average size of the silver nanoparticle synthesized from Aloe vera extract at temperature of 40°C was 6.15, Likewise at 50°C was 5.73, at 60°C was 5.58 and at 70°C was 5.27 as shown in table 3.

Table 3:- XRD particle size evaluation of Ag-NPs at temperature variation using Scherrer equation.

S.N	FWHM	β	2θ	θ	radian	Crystallite size (D)	average	total average
40	1.07	0.01868	32.08	16.04	0.27995	7.72532	6.1523	5.6857
	1.84	0.03211	37.96	18.98	0.33126	4.56578		

	1.45	0.02531	54.61	27.31	0.47656	6.16583	
50	1.02	0.0178	32.26	16.13	0.28152	8.10769	5.737
	1.32	0.02304	38.15	19.08	0.33292	6.36806	
	2.54	0.04433	44.37	22.19	0.3872	3.37773	
	1.8	0.03142	64.48	32.24	0.56269	5.218	
	1.81	0.03159	77.13	38.57	0.67309	5.61338	
60	1.02	0.0178	32.25	16.13	0.28143	8.10748	5.5806
	1.37	0.02391	38.14	19.07	0.33283	6.13546	
	1.75	0.03054	44.36	22.18	0.38711	4.90236	
	2.01	0.03508	64.48	32.24	0.56269	4.67284	
	2.49	0.04346	77.28	38.64	0.6744	4.08468	
70	1.14	0.0199	32.44	16.22	0.28309	7.25755	5.2728
	1.82	0.03176	38.31	19.16	0.33432	4.62083	
	3	0.05236	44.37	22.19	0.3872	2.85981	
	1.48	0.02583	64.67	32.34	0.56435	6.35287	

The total average size of crystallite was 5.6857.

Antibacterial activity of AgNPs against bacterial pathogens at Aloe vera concentration variation

The antibacterial activity of the colloidal solution was compared with various antibiotics. The AgNPs at 25gm/100ml concentration of Aloe vera extract showed highest antimicrobial activity against S. aureus, E. coli and K. pneumoniae with zone of inhibition 16mm, 8mm and 15mm respectively. whereas no inhibitory activity was seen against P. aeruginosa. Synthesized AgNPs of 10gm/100ml concentration of Aloe vera did not show inhibitory activity either as shown in figure 4.

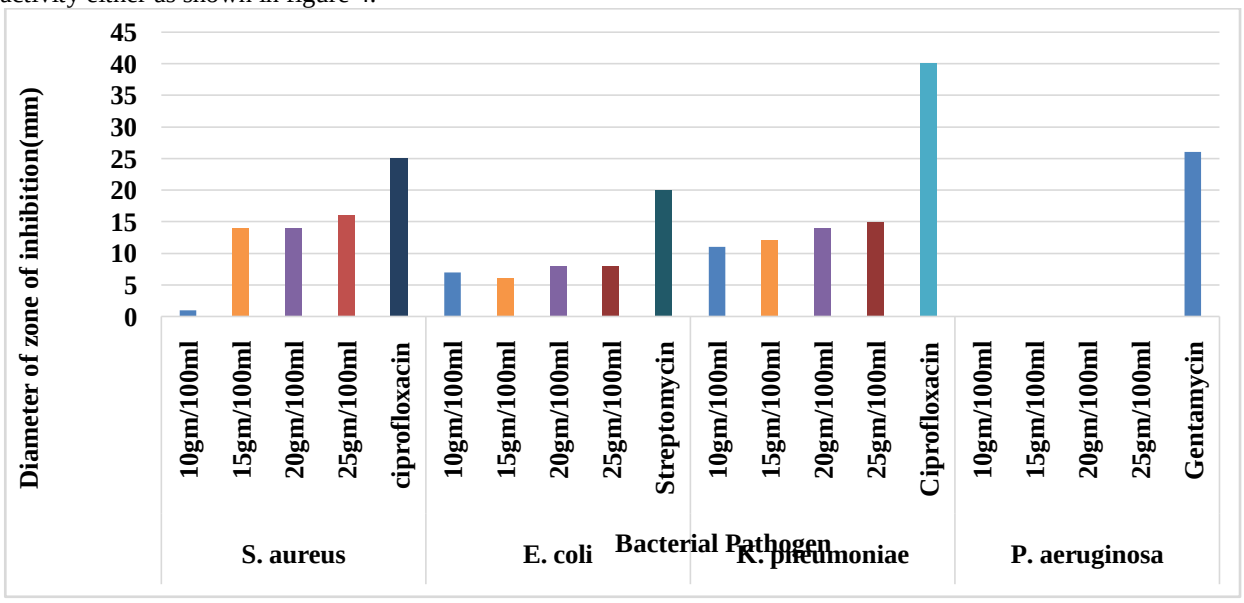


Fig. 4:-Zone of inhibition shown by AgNPs against pathogenic bacteria at different *Aloe vera*extract concentration.

Antibacterial activity of AgNPs against bacterial pathogens at $AgNO_3$ concentration variation

The AgNPs at 4mM concentration of $AgNO_3$ extract showed highest antibacterial activity against S. aureus, E. coli and K. pneumoniae and P. aeruginosa with 19mm, 16mm, 21mm and 18mm respectively as shown in figure 5.

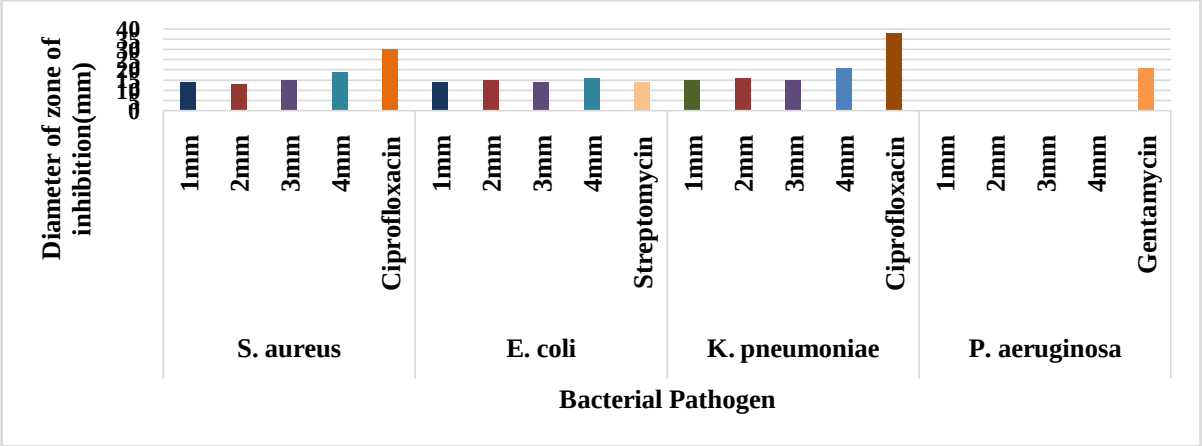


Fig. 5:-Zone of inhibition shown by AgNPs against pathogenic bacteria at different $AgNO_3$concentration.

Antibacterial activity of AgNPs against bacterial pathogens at temperature variation

The higher temperature i.e., 70°C showed the better inhibition against S. aureus, E. coli and K. pneumoniae with zone of inhibition 20mm, 12mm, 18mm respectively. However, no inhibitory activity was observed against P. aeruginosa. Also, at 40°C synthesized AgNPs could not inhibit S. aureus as shown in figure 6.

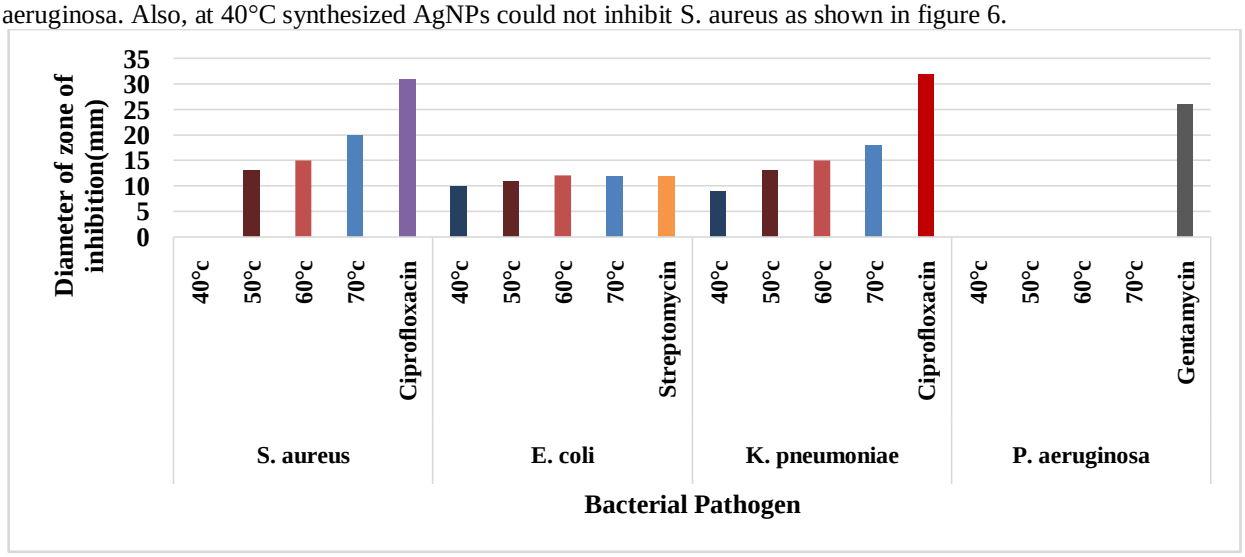
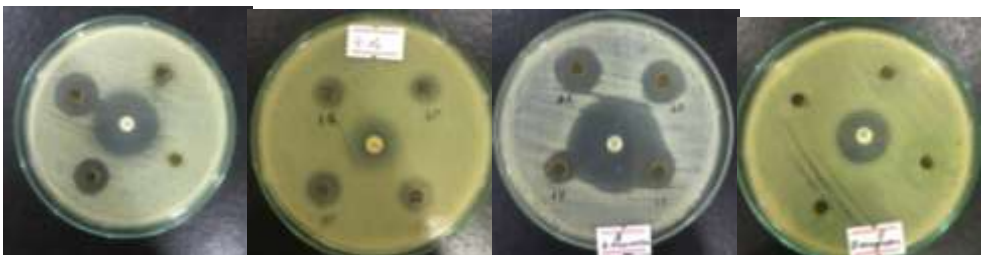


Fig. 6:-Zone of inhibition shown by AgNPs against pathogenic bacteria at different temperature.

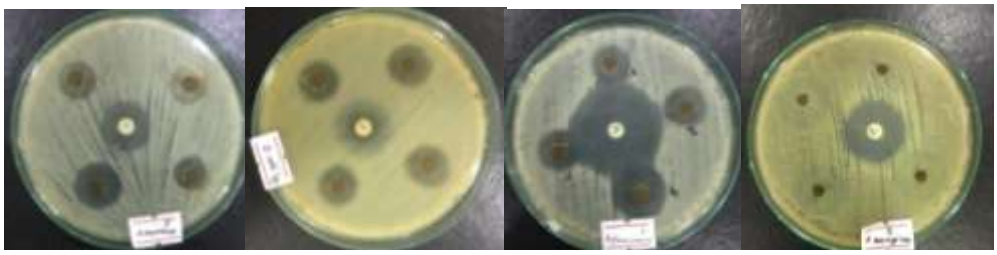
Preparing Aloe vera extract

Synthesized AgNPs from *Aloe vera* extract

Synthesized AgNPs

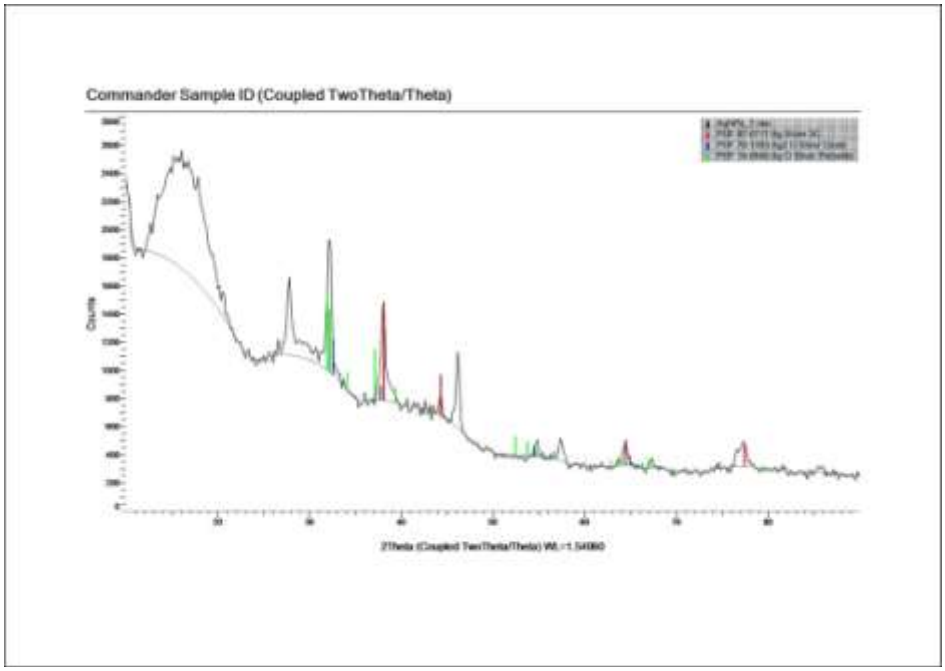


Photograph 4:- Antibacterial activity of *Aloe vera*-mediated AgNPs at *Aloe vera* extract concentration variation against bacterial pathogens: (a) *S. aureus* (b) *E. coli* (c) *K. pneumoniae* (d) *P. aeruginosa*



Photograph 5:- Antibacterial activity of *Aloe vera*-mediated AgNPs at $AgNO_3$ concentration variation against bacterial pathogens: (a) *S. aureus* (b) *E. coli* (c) *K. pneumoniae* (d) *P. aeruginosa*

Photograph 6:- Antibacterial activity of *Aloe vera*-mediated AgNPs at temperature variation against bacterial pathogens: (a) *S. aureus* (b) *E. coli* (c) *K. pneumoniae* (d) *P. aeruginosa.*



Photograph 7:- XRD pattern of synthesized AgNPs from *Aloe vera* at 4mM $AgNO_3$ concentration.

Discussion:-

Bioactive compounds such as aloin, emodin, flavonoids, tannins[14] present in Aloe vera act as excellent natural reducing, capping and stabilizing agent. The alkaline environment is very important as it provides hydroxide ions which results the increment of reduction capacity of silver ions [10]. Also, at greater pH than 7 agglomeration is discouraged due to all groups are deprotonated favoring the repulsion between the nanoparticles [1]. The color of solution was changed because of surface plasmon resonance (SPR) and this band is ascribed to excitation of valence electrons [10, 8].

At higher concentration of extract, $AgNO_3$ and temperature, high absorbance and smaller size nanoparticles were observedin UV spectrophotometry followed by XRD.The reason may be related to abundant availability of reducing agent present in these plants same result was observed in [6]. Also, at higher concentration of $AgNO_3$there is abundance of silver ions and at higher temperature, the absorbance was maximum. At high temperature may be due to increase in reaction rate, leading in the formation of nuclei from the silver ions and preventing the secondary reduction process on the surface of the pre-formed nuclei. Which implies that the extract concentration, silver nitrate concentration and temperature directly proportion to the formation of smaller size silver nanoparticles [13]. The broadenedof absorbance peak was due tothe shift in the plasmon resonance peak indicates a change in the size of AgNPs and hence any shift of the peak towards the higher wavelength is accompanied by a decrease in the size of the prepared AgNPs as suggested by [1].

The positive antibacterial activity shown by silver nanoparticles is because AgNPs can attach to the negatively charged cell surface altering the physiochemical components of cell membrane [2,5] then easily invade the inner cell components and cause severe damage to the cells by interacting with sulfur and phosphorus-containing compounds, such as proteins and genetic materials, which leads to complete cell. Also, the formation of free radicals, the inactivation of proteins in the cell by silver ions and the production of reactive oxygen species (ROS) may be another reason[4].

The inhibitory activity of synthesized silver nanoparticles was quite satisfying against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa.500mg/ml concentration of AgNPs were added against pathogens since according to [3], 500µg/ml showed larger inhibition zone than 400µg/ml, 300µg/ml and 200µg/ml. In this study antibacterial activity of silver nanoparticles compared with antibiotics(ciprofloxacin, Streptomycin and Gentamycin) because these are strong antibiotics and shows good antibacterial activity against tested bacteria.

The antibacterial mechanism of silver nanoparticles is species dependent and also on the size of the nanoparticles [11]. The zone of inhibition was larger at high concentration of extract, $AgNO_3$ and temperature synthesizing smaller size of AgNPsagainst almost four pathogens.

However, atAloe vera extract variation there was no zone of inhibition seen against P. aeruginosa. At highest condition such as at 25gm/100ml concentration of Aloe vera, 4mM concentration of $AgNO_3$ and at 70°C the zone of inhibition was largeri.e., 16mm, 15mm and 20mm respectively against S. aureus. The strongest reason about the susceptibility of Staphylococcus aureus against nanoparticles may be due to their cell wall plasmolysis or separation of cytoplasm from their cell wallas according to [11].

Conclusion:-

This study showed the ability of synthesizing silver nanoparticles using Aloe vera. In addition, synergistic effect of synthesis of silver nanoparticles with different parameters was also studied which means with the increasing concentration of plant materials, silver nitrate and the temperature the size of silver nanoparticle got smaller. Through X-ray diffraction method the average size of silver nanoparticles was detected and confirmed which was 5-8nm. The synthesized silver nanoparticles exhibited antibacterial activity against Staphylococcus aureus, *Escherichia coli, Klebsiella pneumoniae*and*Pseudomonas aeruginosa*.It was proved that Pseudomonas aeruginosa was less susceptible to silver nanoparticles. It supported the evidence that silver nanoparticles seem to be alternative antibacterial agents to antibiotics and have the ability to overcome the bacterial resistance against antibiotics.

Abbreviations

AgNPs: Silver nanoparticles

$AgNO_3$: Silver nitrate
ATCC: The American Type Culture Collection
DNA: deoxyribonucleic acid
NaOH: Sodium hydroxide
NAST: Nepal Academy of Science and Technology
nm: Nanometer
NPs: Nanoparticles
ROS: Reactive oxygen species
SPR: Surface plasmon resonance
UV-vis: Ultra violet- visible
XRD: X-ray diffraction

Authors Contribution
SP has contributed to the plan of the research work, sample collection, sample processing, data analysis, intellectual content design and result interpretation. HJR helped in collection of samples, interpreting result. AB supervised the research project. All the authors drafted the manuscript and agreed for its publication.

Competing interests
No competing interests

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Ethical approval and consent
Not applicable.

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