SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING LEAVES EXTRACT OF *Aloe vera* (L.) Burm.f. *

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ABSTRACT

In this research work, the leaves of *Aloe vera* were collected from Amara Pura Township, Mandalay Region Myanmar. The preliminary detection of phytochemical constituents present in leaves was carried out by standard method. The elemental compositions of these leaves were also determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy. Silver nanoparticles were synthesized by using *Aloe vera* leaves extract. (Sample: Silver nitrate) (1:4 ratio) of leaf extract and different concentrations of (10mM, 5mM, 1mM) silver nitrate solution were used. After preparing silver nanoparticles, the characterization of these particles was determined by using X-ray Diffraction (XRD), Fourier Transform Infrared spectroscopy (FTIR) techniques. The antibacterial activity of leaf extract of silver nanoparticles solution was tested by Agar well diffusion method on four selected organisms such as *Bacillus cereus*, *Staphylococcus aureus*, *Escherchia coli* and *Pseudomonas aeruginosa*.

Keywords : *Aloe vera*, elemental compositions, Silver nanoparticles, XRD, antibacterial activity

1. INTRODUCTION

In the modern material science, nanotechnology plays a remarkable role with its eminent salient features such as manipulating nanoscale structures, engineering of atoms and designing of materials with improved properties (Chen, H., et al, 2008). The application of nanoscale materials and structure is an emerging area of nanoscience and nanotechnology. Nanoparticles possess unique electrical, optical as well as biological properties and are applied in catalysis, biosensing, imaging, drug delivery, nanodevice fabrication and in medicine (Hett .A & Zurich, 2004).

Among various metals, silver nanoparticles (AgNPs) are particular and localized surface plasmon resonance properties which render them unique properties such as broad-spectrum antimicrobial. (Franci,G., *et al*. 2015).Silver nanoparticles (AgNPs) are increasingly used in various fields including medical, food, health care consumer and industrial purposes, due to their unique physical and chemical properties. (Gurunathan, S.,*et al*., 2010). Recently, AgNPs have been frequently used in many textiles, keyboard, wound dressings and biomedical devices (Li, C.Y.; 2014, Sondi, *et al*. 2003). Biologically active compounds present in the plant extracts, change from silver nitrate into silver nanoparticle (Wilkinson, J.B.; *et al.*, 1990). Silver nanoparticles have been synthesized using various plant leaf extracts such as *Aloe vera*. Silver nanoparticles have potential in treating a variety of diseases, including retinal neovascularization, immunodeficiency syndrome, infection and cancer. Recently, AgNPs have been shown much interest because of their therapeutic applications in cancer as anticancer agents, in diagnostics and in probing. Taken literature into consideration, in this research we focused on recent developments in synthesis, characterization, properties and bio-applications mainly on the antibacterial properties of AgNPs in a single platform.

1.1 Botanical Description

Family name - Asphodelaceae
Botanical name - *Aloe vera (*L) Burm.f
English name - Barbados Aloe
Myanmar name - Shar-zaung-let-pat
Part used - Leaves

(Perkins, Cyndi.2016).

Fig: 1 The whole Plant of *Aloe vera*

2. MATERIALS AND METHODS

2.1 Sample Collection

The leaves of *Aloe vera* were collected from Amara Pura Township, Mandalay region, Myanmar. Then, they are cut into small pieces and used for the experiment.

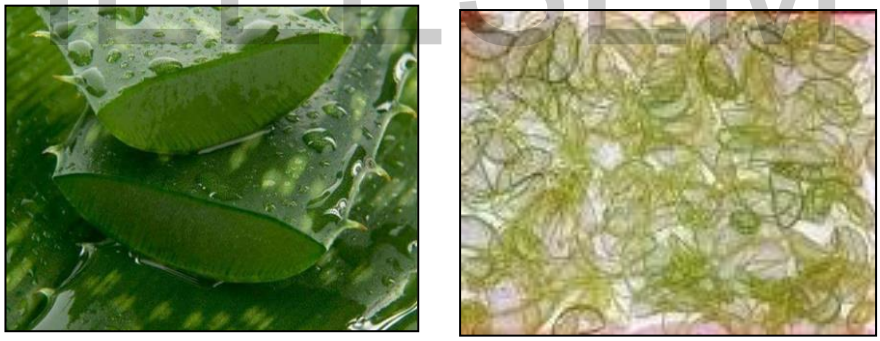


Fig: 2 Small Pieces of Plant Sample

2.2 Preliminary Phytochemical Test of Leaves of Sample

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the sample. Phytochemical tests were done on the various extracts of sample at Department of Chemistry, University of Mandalay. (Harbone J. B., 1973)

2.3 Determination of Relative Composition of Some Elements by EDXRF Spectrometry

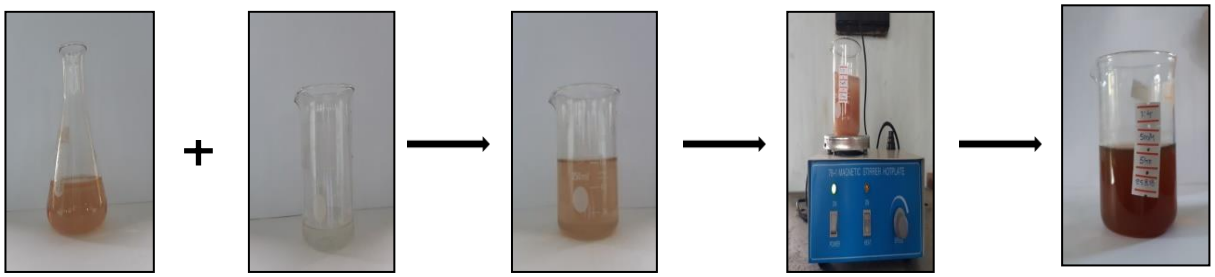
The sample was placed in the sample chamber of EDXRF spectrometer that can measure the 12 samples at a time. The chamber was pumped up to vacuum. Rhodium target was used in EDXRF spectrometer. Each sample was run for a counting time of about 100 seconds and the spectrum obtained was stored and analyzed in PC based multichannel analyzer using EDX-8000 software. The elemental analysis of leaves of *Aloe-vera* was determined by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy at Department of Chemistry, Monywa University. (SPECTRO XEPOS EDXRF Spectrometer, Germany)

2.4 Preparation of Plant Leaf Extract

The collected samples were washed distilled water and cut into small pieces. The 20g of this samples were boiled in 200ml of distilled water until complete extraction was achieved. The extract was then filtered.

2.5 Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized from *Aloe vera* leaves as following produceure. Ten millimolarity silver nitrate solution was prepared by dissolving by 0.08g of $AgNO_3$ in 50ml of deionized water. The 30ml of this solution were mixed with 120ml of freshly prepared plant extract. This mixture was stirred on magnetic stirrer with 300 rpm. The mixture was centrifuged with 6000 rpm for 10mins. The precipitate of silver nanoparticles were obtained. The resultant silver nanoparticles were washed with deionized water and acetone and then dried in petridish.



Plant extract silver nitrate after mixing stirred 300rpm after five hour

Fig: 3 Color Changes for the Process of Synthesis of Silver Nanoparticles

2.6 Characterization of Silver Nanoparticles

The size of nano crystallites were measured by X-ray diffraction (XRD) method. Estimation of particle size is carried out by using Debye-Scherre's equation;

$$L = K\lambda/\beta \cos\theta,\ d = K\lambda/2 \sin\theta \qquad (1)$$

L = average crystallite size
K = constant (shape factor)
λ = wave length of x ray
β = the peak width of the diffraction peak profile at half maximum height (FWHM)
θ = the angle of diffraction
d = spacing

2.7 Determination of Antibacterial Activities

For the measurement of antibacterial activities, the leaves extract of silver nanoparticles solution were determined by Ager well diffusion method. They were sent to Biotechnology Department, Mandalay Technology University. The four bacteria strains were used to screen the antibacterial activity.These bacteria strains were *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*. To determine antibacterial activity the leaves extract of silver nanoparticles solution, 70% ethanol was used as control. The bacteria strains were included on nutrient, both and incubated at 37ºC for 6 hrors. After that, the plates were spread with each strain. The wells were made on the medium. Then, the mixture of the extracts of silver nanoparticles solution and ethanol (as control) were filled into the wells. All plates were incubated at 37ºC for 24 hours to observe the activities. (Magaldi, 2004) (Valgas, 2007)

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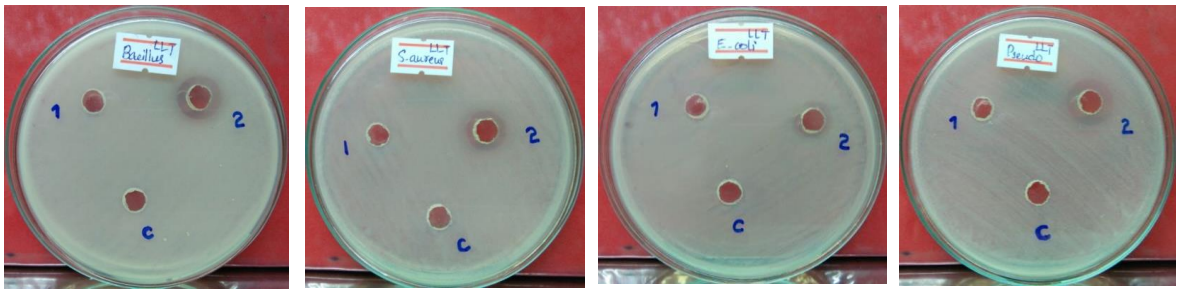

Bacillus cereus ***Staphylococcus aureus*** ***Escherichia coli*** ***Pseudomonas***

Fig: 4 Antibacterial Activity of (1) Leaves Extract, (2) Silver Nanoparticles Solution and (c) Control

3. RESULT AND DISCUSSION

3.1 Phytochemical Constituents of Leaves of *Aloe vera*

Leaves of *Aloe vera* were tested by phytochemical screening and this result was shown in Table (1).

Table 1 The Result of Phytochemical Tests for Leaves of *Aloe vera*

No	Test	Reagent	Observation	Results
1	Alkaloid	(i)Wagner's reagent (ii)Dragendorff's reagent	Reddish brown ppt Orange ppt	+
2	Carbohydrate	α – napthol, sulphuric acid	Yellow solution	–
3	Flavonoid	10% lead acetate	Yellow solution	–
4	Glycoside	10% lead acetate	White ppt	+
5	Phenolic	10% $FeCl_3$	Brown solution	+
6	Polyphenol	$FeCl_3$, 1% $K_3(Fe^+(CN_6))$	Greenish blue solution	+
7	Reducing sugar	Benedict's solution	Orange solution	+
8	Saponin	Distilled water	Forth	+
9	Steroid	$CHCl_3$, acetic anhydride, conc: H_2SO_4	Yellow solution	–
10	Tannin	2%NaOH, 10% ferrichloride	Yellowish brown ppt	+

.(+) =presence of constituents, (-)= absence of constituents

According to this table, alkaloid, glycoside, phenolic, polyphenol, reducing sugar, saponin, and tannin were present in the sample.

3.2 Determination of Mineral Content of Leaves of *Aloe vera*

The mineral content for leaves of *Aloe vera* were determined by using EDXRF method at Chemistry Department, University of Monywa. This results shown in Table (2).

Table 2 The Results of Mineral Content for leaves of *Aloe vera*

No	Element	Symbol	Relative abundance %
1	Potassium	K	1.341
2	Calcium	Ca	1.206
3	Silicon	Si	0.191
4	Phosphorus	P	0.169
5	Sulfur	S	0.115
6	Iron	Fe	0.005
7	Strontium	Sr	0.004
8	Copper	Cu	0.002
9	Maganese	Mn	0.001
10	Zinc	Zn	0.001
11	Rubidium	Rb	0.001

According to this table, the higher amountof potassium and calcium were found in leaves of *Aloe vera*. Moreover, silicon, phosphorus, sulfur, iron, strontium, copper, maganese, zinc and rubidium were also found in that order. Potassium is a very important mineral for the function of all cells, tissues, and organs in the humans body. Calcium is a very important mineral in human metabolism, present mainly in the bones and teeth. *Aloe vera* leaves is a rich source of minerals.

3.3 The Amounts of Silver Nanoparticles of Sample of Different Concentrations

The weights of silver nanoparticles of the sample (Sample: Silver nitrate), (1:4 ratio) at different concentrations (10mM,5mM,1mM)were found to be 0.6%, 0.3% and 0.1%. The yield percent of silver nanoparticles were determined by using different concentrations intervals. It was found that, the amount of silver nanoparticles depend on the contact concentration. When concentration increase, the amounts of yield of silver nanoparticles also increse.

3.4 XRD Analysis of Silver Nanoparticles of Sample

The crystallite size and inter planar spacing of silver nanoparticles were determined by XRD analysis.

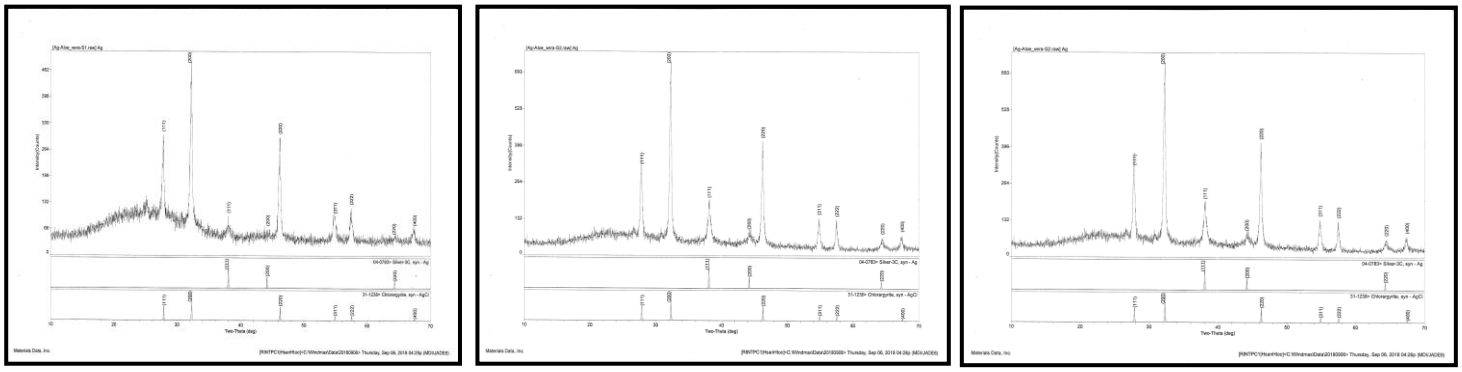

Fig: 4 XRD Spectrums of Silver Nanoparticles using Leaves Extract of *Aloe vera* (1:4 ratio), 10 mM, 5mM and 1mM of $AgNO_3$ Solution

Table 3 XRD Results of Crystallite Size of Silver Nanoparticles of Sample using (10 mM) of $AgNO_3$ Solution

Sr. No	Bragg angle 2θ	Miller Indices (hkl)	FWHM of peak (β)	Crystallite size (L) nm	d-spacing (nm)
1	13.889	(111)	5.1×10^{-3}	26.44	0.166
2	16.106	(200)	5.9×10^{-3}	22.57	0.192
3	19.015	(111)	7.5×10^{-3}	18.20	0.225
4	22.138	(200)	2.6×10^{-3}	49.39	0.261
5	23.104	(220)	6.3×10^{-3}	20.24	0.272
6	27.441	(311)	9.2×10^{-3}	12.68	0.319
7	28.717	(222)	6.5×10^{-3}	31.10	0.333
8	32.103	(220)	15.2×10^{-3}	07.72	0.368
9	33.755	(400)	9.4×10^{-3}	12.26	0.385
			Average	22.29	

According to XRD results, the crystallite size of silver nanoparticles were found within the range of 07.72nm-49.39 nm and average crystallite size is 22.29nm. Interplanar spacing between silver nanoparticles were found within the range of 0.368nm to 0.261nm.

Table 4 XRD Results of Crystallite Size of Silver Nanoparticles of Sample using (5 mM) of $AgNO_3$ Solution

Sr. No	Bragg angle 2θ	Miller Indices (hkl)	FWHM of peak (β)	Crystallite size (L) nm	d-spacing (nm)
1	13.915	(111)	6.1×10^{-3}	25.99	0.166
2	16.121	(200)	5.3×10^{-3}	25.13	0.192
3	19.017	(111)	7.3×10^{-3}	17.95	0.225
4	22.072	(200)	11.1×10^{-3}	11.57	0.254
5	23.130	(220)	5.8×10^{-3}	21.98	0.272
6	27.386	(311)	7.5×10^{-3}	16.41	0.318
7	28.744	(222)	5.5×10^{-3}	22.10	0.333
8	32.233	(220)	8.1×10^{-3}	14.47	0.369
9	33.720	(400)	6.9×10^{-3}	16.71	0.384
			Average	19.14	

According to XRD results, the crystallite size of silver nanoparticles were found within the range of 11.57nm-25.99nm and average crystallite size is 19.14nm. Interplanar spacing between silver nanoparticles were found within the range of 0.254nm to 0.166nm.

Table 5 XRD Results of Crystallite Size of Silver Nanoparticles of Sample using (1 mM) of $AgNO_3$ Solution

Sr. No	Bragg angle 2θ	Miller Indices (hkl)	FWHM of peak (β)	Crystallite size (L) nm	d-spacing (nm)
1	13.929	(111)	7.1×10^{-3}	18.95	0.166
2	16.149	(200)	8.6×10^{-3}	15.48	0.192
3	19.046	(111)	3.7×10^{-3}	35.42	0.226
4	22.075	(200)	4.2×10^{-3}	30.59	0.260
5	23.147	(220)	7.4×10^{-3}	17.22	0.272
6	27.415	(311)	7.9×10^{-3}	15.57	0.319
7	28.892	(222)	5.7×10^{-3}	21.29	0.334
8	32.049	(220)	6.7×10^{-3}	17.54	0.367
9	33.740	(400)	5.9×10^{-3}	19.54	0.385
			Average	21.29	

According to XRD results, the crystallite size of silver nanoparticles were found within the range of 15.48nm-35.42 nm and average crystallite size is 21.29 nm. Interplanar spacing between silver nanoparticles were found within the range of 0.192nm to 0.226nm.

3.5 FT IR Assignments of Silver Nanoparticles of Sample

The infrared spectrums of silver nanoparticles from *Aloe vera* was carried out by FT-IR instrument at Department of Chemistry, University of Monywa. The results obtained were illustrated in followings.

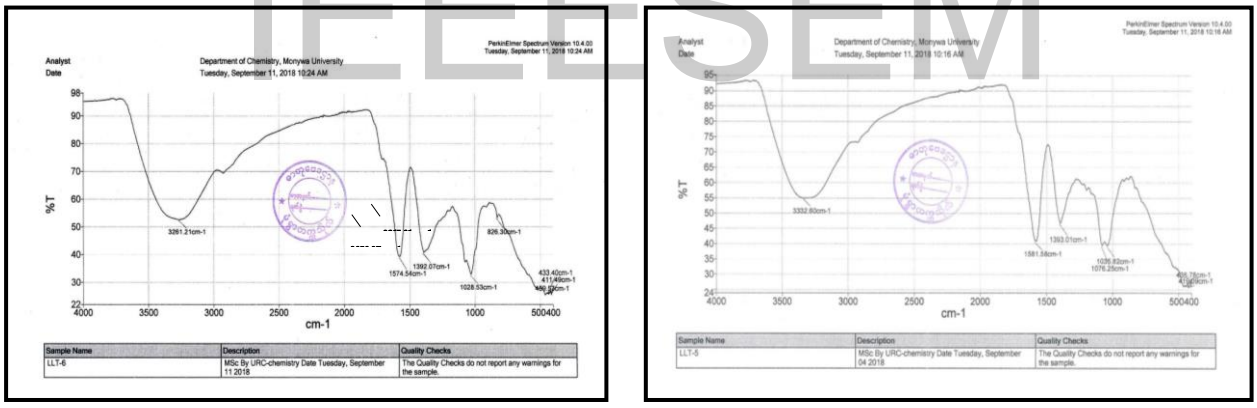

Fig: 5 FT IR Spectrums of Silver Nanoparticles (1:4 ratio) ,(10 mM) and (5mM) of $AgNO_3$ Solution

Table 6 FT IR Assigments of Silver Nanoparticles of Sample (10 mM)

Sr. No	Wavelength (cm^{-1})	Assignments (functional groups)
1	3261.21	O-H stretching vibration of alcohol
2	3050.27	C-H stretching vibration of sp^2 hydrocarbon
3	2915.01	C-H stretching vibration of sp^3 hydrocarbon
4	1574.54	C=C streching vibration of alkene group
5	1392.07, 1028.53	C-O stretching vibration of ether group

From the FT-IR spectrum for 10mM of silver nitrate solution, it was found that the silver nanoparticles of sample contain O-H stretching vibration, C-H stretching vibration of sp^2 and sp^3 hydrocarbons, C=C stretching vibration and C-O stretching vibration of ether group.

Table 7 FT IR Assingments of silver nanoparticles of Sample (5 mM)

Sr. No	Wavelength (cm^{-1})	Assignments (functional groups)
1	3332.60	O-H stretching vibration of alcohol
2	3070.78	C-H stretching vibration of sp^2 hydrocarbon
3	2901.05	C-H stretching vibration of sp^3 hydrocarbon
4	1581.58	C=C streching vibration of alkene group
5	1393.01	C-O stretching vibration of ether group

From the FT IR spectrum for 5 mM of silver nitrate solution, it was found that the silver nanoparticles of sample contain O-H stretching vibration, C-H stretching vibration of sp^2 and sp^3 hydrocarbons, C=C stretching vibration and C-O stretching vibration of ether group.

3.6 Antibacterial Activities for the Leaf of *Aloe vera*

The study of antibacterial activities for the leaf extract of silver nanoparticles was performed by Agar-well diffusion method on four microorganisms. There results are tabulated in Table (8).

Table 8 Antibacterial Activities for Leaf Extract of Silver Nanoparticle Solution

Test microorganisms (mm) Sample	Inhibition Zone	
	Control (Ethanol) (mm)	Leaf extract of silver nanoparticles solution (mm)
Bacillus cereus	8	15
Staphylococcus aureus	8	13
Escherichia coli	8	13
Pseudomonas	8	14

8mm-11mm(low), 12mm-14 (medium), 15mm above (high)

According to the experimental data,leaf extract of silver nanoparticles solution can inhibit that *Bacillus cereus* is high activity, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* are medium activities on the bacteria.

4. CONCLUSION

In this research work, the synthesis of silver nanoparticles (AgNPs) silver nitrate have been used as the metal precursor and *Aloe vera* leaves, water extract as reducing agent. According to the preliminary phytochemical screening results, *Aloe vera* leaves contained alkaloid, glycoside, phenol, polyphenol, reducing sugar, saponin and tannin. From the results of EDXRF data, the *Aloe vera* leaves were observed that the amount of potassium, calcium and silicon higher than the others. The different concentrations (10 mM, 5 mM, 1 mM) of silver nitrate solutions were used in this investigation.The observe the crystallite size of nanoparticles were determined by X-ray diffraction (XRD) method. By using Scherrer's equation, for (10mM) ,it was found that the crystallite site of silver nanoparticles was the range from 49.39nm to 7.72nm and average crystallite size is 22.29nm. From the table 4, 5mM of silver nitrate solution ,the crystallite size of silver nanoparticles were found within the range of 11.57nm-25.99nm and average crystallite size is 19.14nm. From table 5, 1mM of silver nitrate solution, the crystallite size of silver nanoparticles were found within the range of 15.48nm-35.42 nm and average crystallite size is 21.29 nm.

Moreover, FT IR assignments of silver nanoparticles (10 mM), in table (6) ,O-H stretching vibration of alcohol at 3261.21 cm^{-1}, C-H stretching vibration of sp^2hydrocarbon at 3050.27 cm^{-1}, C-H stretching vibration of sp^3hydrocarbonat 2915.01 cm^{-1}, C=C stretching vibration of alkene group at 1574.54 cm^{-1}, C-O stretching vibration of ether group at 1392.07 cm^{-1} and 1028.53 cm^{-1} were observed. From another FTIR spectrum of silver nanoparticles (5 mM), O-H stretching vibration of alcohol at 3332.60 cm^{-1}, C-H stretching vibration of sp^2 hydrocarbon at 3070.78 cm^{-1}, C-H stretching vibration of sp^3 hydrocarbon at 2901.05 cm^{-1}, C=C stretching vibration of alkene group at 1581.58 cm^{-1} and C-O stretching vibration of ether group at 1393.01 cm^{-1} were observed. The plant extract compounds including OH and CO groups have a vital role in reducing and stabilization of silver nanoparticles.Then the antibacterial activity of the AgNPs from leaves extracts was measured by Agar-well diffusion method. The leaf extract of silver nanoparticles solution can inhibit the four types of microorganisms. The leaf extract of silver nanoparticles solution have more effective antibacterial activity to the bacteria.

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