Green Synthesis and Characterization of Silver Nanoparticles by Leaf Extract of *Andrographis paniculata* (Burm.f.) Nees

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Abstract

In this research, green synthesis of silver nanoparticles (Ag-NPs) from the leaves extract of *Andrographis paniculata* (Burm.f.) Nees was investigated. The process of Ag-NPs observed a rapid reduction of silver ions leading to the formation of stable crystalline Ag-NPs in the solution. Plant extracts were used for the synthesis of Ag-NPs, in various concentrations of silver nitrate solutions (5mM; 10mM, 15mM). The ratio of silver nitrate and leaves extract is 1:4. The resulting Ag-NPs were characterized by scanning electron microscopy (SEM) and Fourier Transform Infrared spectroscopy (FT-IR). The resulting silver nanoparticles, the characteristic absorption peak at 515.99 cm^{-1} was observed in the FT-IR spectrum. The results of X-ray diffraction (XRD) were calculated by using Debye-Scherrer's equation and the sizes of Ag-NPs were 12 nm to 20 nm. The antimicrobial activity of Ag-NPs was also determined by Agar-well diffusion method on six selected organisms, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescnes*, *Bacillus pumilus*, *Saccharomyces cerevisiae* and *Escherichia coli.* Ag-NPs from the leaves sample responded medium activity on all organisms with the exception of *Pseudomonas fluorescnes*.

Keywords; green synthesis, Ag-NPs, FT-IR, X-ray diffraction, antibacterial activity

Introduction

Green-nanotechnology is the application of green engineering and chemistry to this field. Green synthesis of nanoparticles makes use of environmental friendly, non-toxic and safe reagents (Mohanpuria, *et al.*, 2008).

Nanotechnology is a modern field of science which plays a significant role in today life aspects. Nanotechnology is the study and application of small object which can be used across all fields such as physics, chemistry, biology, material science and engineering. Nanotechnology deals with production, manipulation and use of material ranging in nanometers (Kavitha,*et al*.,2013).

Nanoparticles can be made of materials of diverse chemical nature, the most common being metals, metal oxides, silicates, non-oxide ceramics, polymers, organics, carbon and biomolecules. Nanoparticles exist in several different morphologies such as spheres, cylinders, platelets, tubes etc. (Geiser, 2010).

Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. With their antioxidant or reducing properties, they are usually responsible for the reduction of metal compounds into their respective nanoparticles(Begum, 2009 and Gurunathan, 2011)

Generally ,silver nanoparticles can be prepared through a top-down or bottom-up method. The top-down approach involves chopping down the bulk metals by mechanical means and the resulting particles are stabilized by colloidal protecting agent(Ahmed, *et al.*, 2016).

The bottom-up approach produces nanoparticles through processes such as reduction of metals salt by chemical means, electrochemically, or through use of chemical and biological method to control the decomposition of metastable organometallic compound in solution(Elghanan , *et al*., 1997).

Scientific Classification

Botanical name - *Andrographis paniculata (*Burm.f.) Nees

Family - Acanthaceae

Common name - Say khar gyi

Part used - Leaves

Medical us - anticancer, diabetes, malaria high blood pressure,ulcer, leprosy, bronchitis and skin diseases,

Figure 1 Leaves of Say khar gyi

(Kumar, 2004)

Materials and Methods

Sample Collection

For the research work, the leaves of *Andrographis paniculata* (Burm.f.) Nees were collected from Meza village, Indaw Township, Sagaing Region, Myanmar. The collected sample was with distilled water and dried in air, then crushed into small pieces. Those pieces were stored in well stoppered bottle to prevent moisture changes and contamination.

Preliminary Phytochemical Tests for the leaves of Sample

The phytochemical tests were carried out to detect the presence or absence of organic constituents in the leaves of the sample (Harborne,J.B. 1973) .

Preparation of leaves Extract

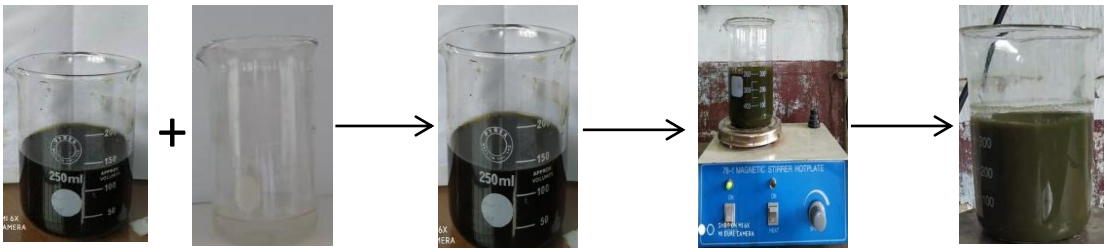
20 g of dry powder samples were boiled with 400ml of deionized water for 30 minutes. The extracted solution was filtered through Whatman No.1 filter paper and cooled. The solution was stored in refrigerator for further use (Mason, 2012).

Preparation of Silver Nitrate Solution (5mM, 10mM, 15mM)

Silver nitrate solutions were prepared by dissolving in 0.339g, 0.679g and 1.019g of $AgNO_3$ in each 400mL of deionized water to obtain concentrations of 5mM, 10mM and 15mM of silver nitrate solutions.

Synthesis of silver Nanoparticles

Silver nanoparticles were synthesized by dry leaves of *Andrographis paniculata* (Burm.f.) Nees as follows. The 30ml of silver nitrate solution was mixed with 120ml of prepared dry leaves extract. The ratio of silver nitrate and leaves extract is 1:4. This mixture was stirred on a magnetic stirrer at 300 rpm for 5 hr.The greenish brown precipitates were obtained. The observed change in color from brown to green with time indicates the formation of Ag-NPs. The resultant precipitates were washed three time using distilled water and acetone. The pH value of the green synthesis of silver nanoparticles has been maintained at 8.25. (Sahu, 2012), (Amudha, 2014).



Leaves extract of Say khar gyi | Silver nitrate | After mixing | Stirred 300rpm | After 5hr stirring

Figure 2 Color changes for process of synthesis of silver nanoparticles

Centrifuge method

Centrifuged technique was used to separate the silver nanoparticles from the solution. The solution was centrifuged at 6000rpm for 20 min. The centrifuged supernatant liquid was collected and then centrifuged twice at 10000rpm for 20 min.The suspended precipitate was purified using acetone (Mitra, 2012).

Calcination method

The purified precipitates were calcined at 300°C for 2 hr in Muffle furnace. After the evaporation of solvent and water molecules, the nanoparticles have been collected in powder form. The dry powder was obtained which showed the crystals. This powder was used for further characterization.

Scanning electron microscopy method

After preparating the nanoparticles, the shape and size of silver nanoparticles synthesized by the green method were determined by scanning electron microscopy. For SEM analysis, the purified silver nanoparticles were sent to Department of Chemistry ,West Yangon University. SEM observation was carried out on a ZEISS EVO 40 EP Electron microscope (Yamini 2011).

Functional Groups Determination of Synthesized Silver Nanoparticles

The functional groups contained in synthesized silver nanoparticles were measured by FT-IR spectroscopic method at Monywa University. The infrared spectrum informs the functional groups of organic and inorganic compounds contained in green synthesized of silver nanoparticles. (Silverstein, 1998)

Characterization of Silver Nanoparticles

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

$$D(nm) = K\lambda/\beta\cos\theta \quad (1)$$

D=average crystallite size, K=constant (shape factor),

β= FWHM (full width at the half maximum) peak

λ= wave length of x ray (constant), θ= the angle of diffraction

Determination of Antimicrobial activity on AgNPs (5mM)

For the measurement of antimicrobial activity of silver nanoparticles from the leaves extract of *Andrographis paniculata* (Burm.f.) Nees was determined by using Agar- well diffusion method on six selected organisms. It was sent to Botany Department, Magwe University. To determine antimicrobial activity of the silver nanoparticles, 70% ethanol was used as control. (Chio,2008 and Garima Singhal, 2011).

Results and Discussion

Phytochemical Tests for Leaves of Say khar gyi

From the experiment, alkaloid, flavonoid, glycoside, phenolic, polyphenol, reducing sugar, steroid, saponin and tannin were present in Say khar gyi leaves.

Amount of Silver Nanoparticles at Different Concentrations

From to experiment, when concentration increase the amount of silver nanoparticles also increase. The ratio is 1:4 v/v of silver nitrate and leaves extract. The amount of silver nanoparticles is 0.05g for 5mM, 0.06g for 10mM and 0.09g for 15mM.

SEM Analysis of Silver Nanoparticles (5mM of $AgNO_3$ solution)

Scanning electron microscopy was used to determine the size distribution. The surface morphology of sample, Say khar gyi was observed by Scenning electron microscopy image as shown in figure (4).

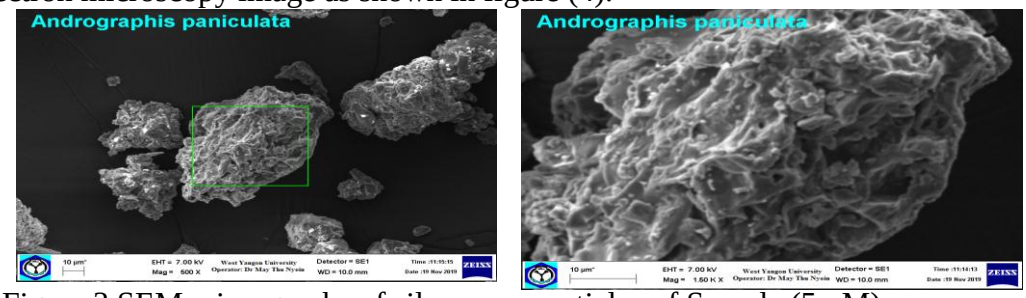


Figure 3 SEM micrographs of silver nanoparticles of Sample (5mM)

According to SEM micrograph, it was found that Ag-NPs were nanocluster.. Scanning electron microscopy provided further insight into the morphology and size details of the Ag-NPs. The size of the silver nanoparticles synthesized at 5mM (silver nitrate solution and leaves extract) was recorded to be 10 µm.

FT-IR Spectrum of Silver Nanoparticles (5mM of $AgNO_3$ solution)

The results of the infrared spectrum of silver nanoparticles were illustrated in Table (1).

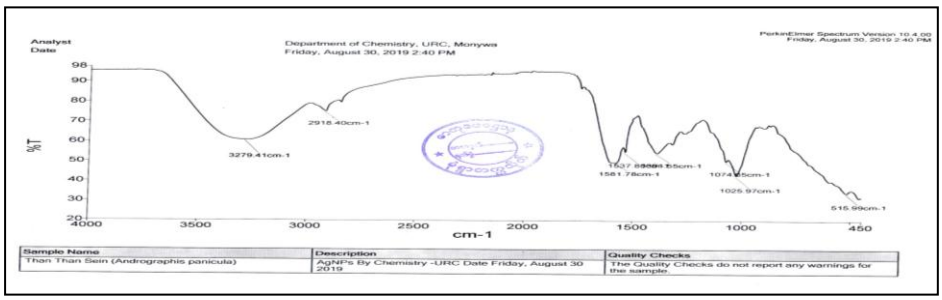


Figure 4 FT-IR spectrum of silver nanoparticles

From the FT-IR spectrum for 5mM silver nitrate solution, it was found that the silver nanoparticles from leaves extract of Say khar gyi contain O-H stretching viberation at 3279.41 cm^{-1} ,C-H stretching of sp^3 hydrocarbon at 2918.40 cm^{-1} , C=C stretching of alkene at 1581.78 cm^{-1} , C-O stretching vibration of alcohol group at 1079.65 cm^{-1},1025.97 cm^{-1} and Ag-O stretching vibration at 515.99 cm^{-1} (Harish, *et al*, 2018), (Silverstein, R.M and Webster, F.X, 1998).

XRD Analysis of Silver Nanoparticles

The crystalline sizes and Interplanar spacings of silver nanoparticles were determined by XRD method. XRD spectrums of silver nanoparticles of different concentrations of $AgNO_3$ (5mM, 10mM, 15mM) were shown in figure (5 to 7) and the results were shown in Table (1 to 3).

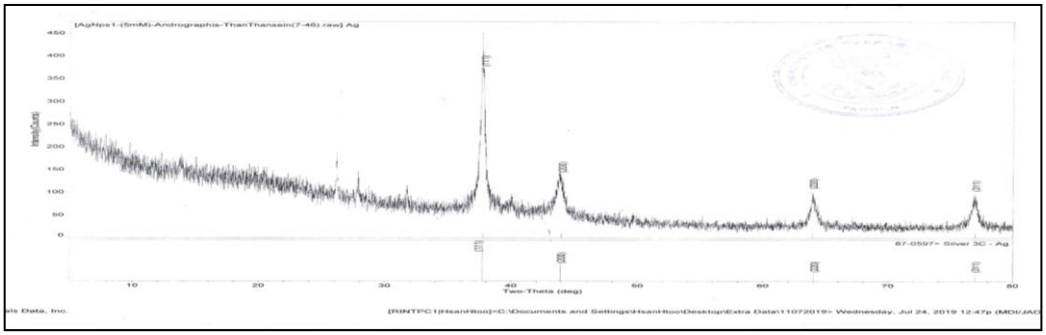


Figure 5 XRD spectrum of silver nanoparticles (5mM of $AgNO_3$ Solution)

Table (1) XRD Results of Silver Nanoparticles (5mM of $AgNO_3$ Solution)

Sr. No	Braggangle 2θ	Miller Indices (hkl)	FWHM peak β	d-spacing (nm)	Crystallite size (D)nm
1	37.710	(111)	7.8×10^{-3}	0.238	17.70
2	43.930	(200)	10.1×10^{-3}	0.205	1.38
3	63.933	(220)	8.6×10^{-3}	0.145	16.02
4	76.964	(311)	8.9×10^{-3}	0.123	15.49

According to XRD results, the crystallite sizes of silver nanoparticles were found within the range of 1.38 nm to 17.70 nm. The average crystallite size of AgNPs was 12.65nm. Interplanar spacing between silver nanoparticles were found within the range of 0.205 nm to 0.238nm.

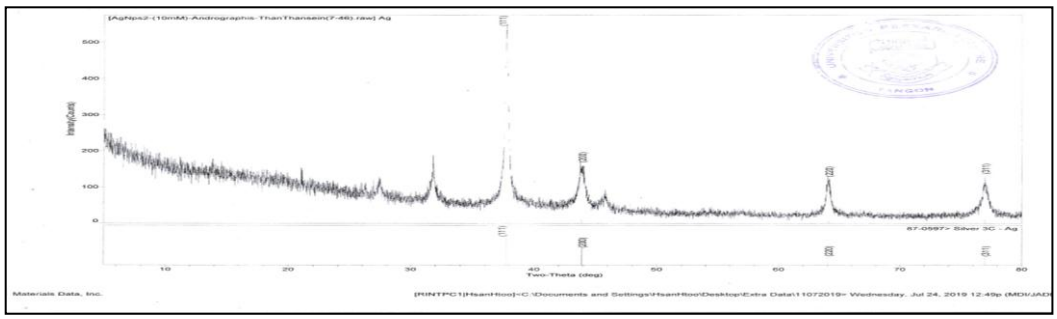


Figure 6 XRD spectrum of silver nanoparticles (10mM of $AgNO_3$ Solution)

Table (2) XRD results of Silver Nanoparticles (10mM of $AgNO_3$ Solution)

Sr. No	Bragg angle 2θ	Miller Indices (hkl)	FWHM of peak (β)	d-spacing (nm)	Crystallite size (D) nm
1	37.699	(111)	6.1×10^{-3}	0.238	24.41
2	43.852	(220)	9.3×10^{-3}	0.206	14.80
3	64.060	(220)	7.8×10^{-3}	0.145	20.74
4	76.986	(311)	7.3×10^{-3}	0.123	23.95

According to XRD results, the crystallite sizes of silver nanoparticles were found within the range of 14.80 nm to 24.41 nm. The average crystallite size of AgNPs was 20.98nm. Interplanar spacing between silver nanoparticles were found within the range of 0.206 nm to 0.238 nm.

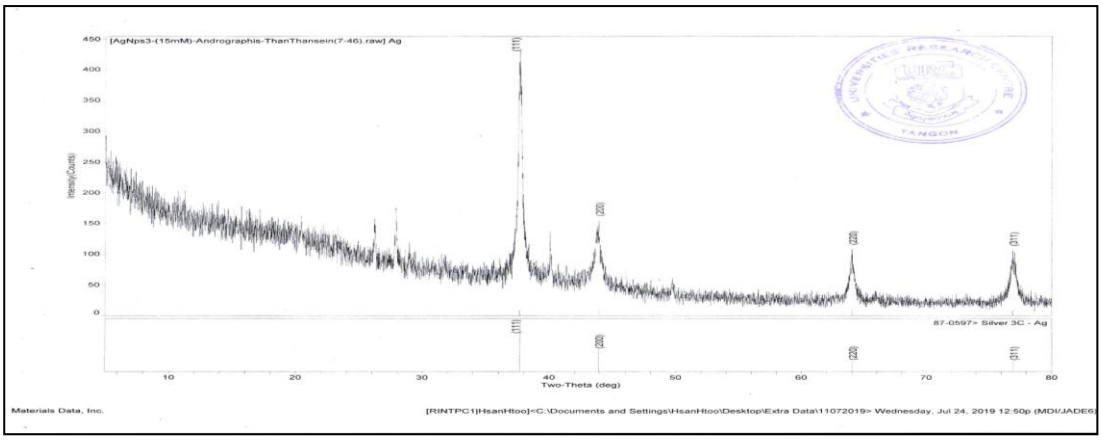


Figure 7 XRD spectrum of silver nanoparticles (15mM of $AgNO_3$ Solution)

Table (3) XRD results of Silver Nanoparticles (15mM of $AgNO_3$ Solution)

Sr. No	Bragg angle 2θ	Miller Indices (hkl)	FWHM of peak (β)	d-spacing (nm)	Crystallite size (D) nm
1	37.649	(111)	6.3×10^{-3}	0.238	23.67
2	43.892	(200)	8.0×10^{-3}	0.206	18.66
3	64.012	(220)	6.9×10^{-3}	0.145	23.43
4	76.920	(311)	8.5×10^{-3}	0.123	16.25

According to XRD results, the crystallite sizes of silver nanoparticles were found within the range of 16.25 nm to 23.67nm. The average crystallite size of AgNPs was 20.50nm. Interplanar spacing between silver nanoparticles were found within the range of 0.123nm to 0.238nm.

Antimicrobial activity Silver Nanoparticles from the Leaves Extract of Sample (5mM of $AgNO_3$ solution)

For the study of antimicrobial activity of silver nanoparticles from the leaves extract of Say khar gyi, the result was showed in Table (5).

Table (4) Antimicrobial activity of Silver Nanoparticles

Test Organism	Inhibition Zone (mm)	
	EtOH Control	Ag-NPs
	C	AgNPs I
Bacillus subtilis	-	15(++)
Staphylococcus aureus	-	15(++)
Pseudomonas fluorescnes	-	12(+)
Bacillus pumilus	-	16(++)
Saccharomyces cerevisiae	-	15(++)
Escherichia coli	-	15(++)

Agar well ~ 10mm
10mm ~ 14mm = low activity (+)
15mm ~ 19mm = medium activity (++)
20mm ~ above = high activity (+++)

Ag-NPsI= Silver nanoparticles from the leaves extract of Say khar gyi

According to this table, silver nanoparticles from the leaves sample responded medium activity on all organisms except *Pseudomonas fluorescnes.*

Conclusion

The different concentrations (5mM, 10mM, and 15mM) of silver nitrate solutions were used in this investigation. When the concentrations increase, the amount of silver nanoparticles also increase. According to the report of XRD peak of silver nanoparticles from the leaves extract of Say khar gyi, the average crystallite sizes of silver nanoparticles were found 12.65nm for 5mM, 20.98nm for 10mM and 20.50nm for15mM. Among them, the XRD spectrum of silver nanoparticles by using 5mM of $AgNO_3$ shows clear and sharp peaks. It was selected to characterize by SEM, FT-IR and antimicrobial activity.

The resulting nanoparticles were characterized by FT-IR and the characterized peak of silver nanoparticles for the selected plant was observed at 515.99cm^{-1} as Ag-O stretching vibration.

Furthermore, the sample of leaves extract of silver nanoparticles solution can inhibit the six types of microorganisms. The leaves extract of silver

nanoparticles solution have medium effectiveness on activity to the microorganisms.

Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis, and in this method, there is no need to use high pressure, energy, temperature and toxic chemicals.

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