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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING LEAVES OF THREE PLANTS FROM FABACEAE FAMILY

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Abstract

In the present study, the leaves of *Sesbania grandflora* (L.) Poir, *Senna siamea* (Lam.) Irwin & Barney and *Tamarindus indica* L. were collected from Yadanabon University Campus, Amarapura Township, Mandalay Region, Myanmar. Firstly, preliminary detection of phytochemical compounds present in three kinds of leaves were carried out. This phytochemical dectection gave rise to positive tests for alkaloid, phenolic, polyphenol, steroid and saponin. The mineral content of three kinds of leaves sample were analyzed by using EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. The green synthesis was done by using the aqueous solution of *Sesbania grandflora* (L.), *Senna siamea* (Lam.) and *Tamarindus indica* L. and $AgNO_3$. Silver was particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the colour change was observed which proved the formation of nanoparticles. The size distribution of nanoparticles, surface and morphological features of synthesized silver nanoparticles were studied by Scanning Electron Microscope (SEM). Moreover, the crystal size and crystalline nature of synthesized silver nanoparticles were determined by X-ray diffraction (XRD). Finally, the prepared silver nanoparticles were found to possess antibacterial activities on six selected microorganisms by agar-well diffusion method.

Keywords: silver nanoparticles, *Sesbania grandflora* (L.), *Senna siamea* (Lam.), *Tamarindus indica* L. green synthesis, agar-well diffusion method

Introduction

Nanotechnology is one of the most active research areas in the modern material science. Nanobiotechnology is rapidly growing as an interdisciplinary eco-friendly research area and used in broad research section such as biology, chemistry, physics, biomedicine and material engineering

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(Daniel and Astruc, 2004). Nanotechnology deals with the production and usage of material with nanoscale dimension. Nanoscale dimension provides nanoparticles, a large surface area to volume ratio and thus very specific properties.

Nanoparticles have started being considered as nano antibiotics because of their antimicrobial activities. The novel properties of nanoparticles (NPs) have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices (De, Ghosh and Rotello, 2008). It deals with various shapes and size of particles in the range of 1 to 100 nm.

Plant extract is a more attractive option as they are nontoxic and therefore environmentally safe especially for their application in food, pharmacy or in medicine (Devi *et al.,* 2015). Nanoparticles show completely new or unproved properties, such as size, distribution and morphology of the particles etc (Kaviya, 2011). Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeuties, catalysis and micro-electronics (Jiang *et al*., 2004).

Silver is well known for possessing an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes (RO, B., 1999). In medicines, silver and silver nanoparticles have a sample application including skin ointments and creams containing silver to prevent infection of burns and medical devices. In textile industry, silver-embedded fabrics are now used in sporting equipment (Klaus *et al*., 1999).

In the recent days, silver nanoparticles have been synthesized from the naturally occuring sources and their products like green leaf, *Carica papaya* leaf, *Aloe vera* plant extract and lemon grass leaf, etc. (Vijayaraghavan *et al*., 2012). These leaves contain vitamin A, C and E which are antioxidants. Plant leaf extracts act as both reducing agents and stabilizing agents in the synthesis of nanoparticles (Yadav *et al*., 2010). Biologically active compounds present in the plant extracts, such as proteins, polysaccharides and vitamins play a major role in the reduction of silver nitrate into silver nanoparticles (Daniel and Astruc, 2004). Silver nanoparticles have potential in treating variety of diseases, including retinal neovascularization, immunodeficiency syndrome, infection and cancer (Mankin *et al*., 2009).

The aim of the present study is to determine the synthesis and characterization of silver nanoparticles by using three kinds of leaves shown in figure1. All of these plants are belonging to Fabaceae family.

Sesbania grandiflora (L.) Poir
Paukpanbyu

Senn asiamea (Lam.) Irwin & Barneby
Mezali

Tamarindus indica L.
Magyi

Figure 1: Three different leaves used for synthesis of silver nanoparticles

Materials and Methods

Silver nitrate, deionized water and three different leaves were used. Preliminary photochemical analysis was performed by using test tube method. The apparatus used were EDXRF (Energy dispersive X-ray fluorescence spectrometer), SEM (Scanning electron microscope) and XRD (Bruker X-Ray diffractometers). Antibacterial activities of the prepared silver nanoparticles were tested by agar-well diffusion method.

Sample Collection

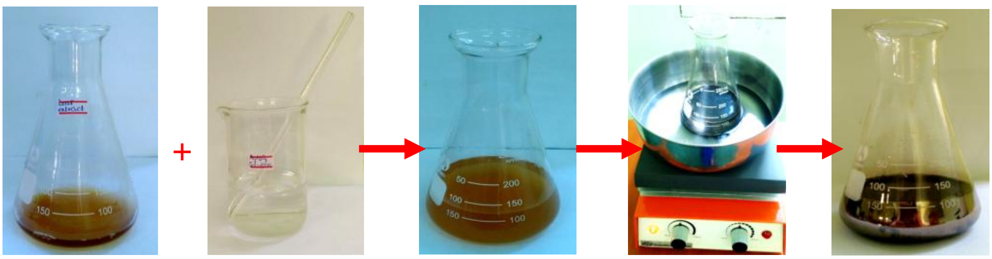
Three different leaves (*Sesbania grandflora* (L.), *Senna siamea* (Lam.) and *Tamarindus indica* L.) were collected from Yadanabon University Campus.

Preparations of Leaves Extract

The leaves were washed thoroughly in running tap water and distilled water. Then, the leaves were kept for drying at room temperature followed by weighing and then crushing it using a mortar. Each sample 75g was boiled in 600mL of deionized water for 30 min at room temperature. The solution was filtered by using Whatman filter paper and the obtained clear solution was used as leaves extract. The solution was stored in refrigerator at 16°C.

Synthesis of Silver Nanoparticles

One millimolarity of silver nitrate solution was prepared by dissolving 0.0169 g of $AgNO_3$ in 100mL of deionized water. The 100 mL of this solution were mixed with the 25 mL of the freshly prepared leaves extract. This mixture was stirred on a magnetic stirrer with 300 rpm. This reaction mixture was maintained at 50°C for reaction times intervals of 1, 3, 5, 7 h for the reduction of silver. Each mixture was centrifuged with 6000 rpm for 30 min and the supernatant poured out. The dark paste obtained was redispersed in deionized water to remove excess biological molecules. The process of centrifugation and redispersion in deionized water was repeated three times to completely purify the nanoparticles. The dark paste collected was then dried in petridish. The weight of dark paste silver nanoparticles was shown in Table 3.



Leaves extract | Sliver nitrate solution | After mixing | Stirred 300 rpm, 50°C | Prepared AgNPs

Figure 2: Procedure for synthesis of silver nanoparticles

Results and Discussion

Phytochemical Test for Three Kinds of Leaves Sample

Preliminary phytochemical analysis was performed in order to know different types of chemical constituents present in the leaves sample.

Table 1: The Results of Phytochemical Test for Leaves Sample

No.	Tests	Reagents	Observation	Results		
				I	**II**	**III**
1	Alkaloid	Wagner's solution	Reddish brown ppt	+	+	+
		Dragendroff's solution	Pale orange ppt	+	+	+
2	Phenolic	10 % $FeCl_3$	Brown colour solution	+	+	+
3	Saponin	Distilled water	Forth	+	+	+
4	Steroid	Acetic anhydride, Conc: H_2SO_4	Blue green colour solution	+	+	+
5	Polyphenol	1 % $FeCl_3$ + 1 % $K_3[Fe(CN)_6$	Greenish blue colour solution	+	+	+

(+) = presence of constituents

I = Paukpanbyu, II = Mezali, III = Magyi

Elemental Analysis of Three Kinds of Leaves Powder

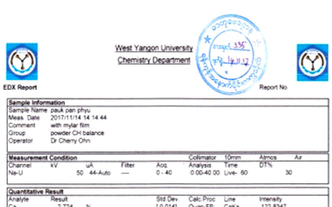
The elements that contain in three kinds of leaves sample were analyzed by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy at Department of Chemistry, West Yangon University.

Table 2: Elemental Analysis for Leaves of Three Kinds of Samples Powder

No.	Element	Symbol	Result (%)		
			Paukpanbyu	**Mezali**	**Magyi**
1	Calcium	Ca	2.724	2.020	1.575
2	Potassium	K	2.342	1.069	0.504
3	Sulfur	S	0.424	0.259	0.178
4	Iron	Fe	0.023	0.010	0.012
5	Strontium	Sr	0.010	0.008	0.008
6	Manganese	Mn	0.005	0.003	0.002
7	Zinc	Zn	0.002	0.002	0.002
8	Bromine	Br	0.002	0.002	-

From the experimental results, the higher amount of calcium and potassium were found in the leaves of three samples. Moreover, sulphur, iron, strontium, manganese, zinc and bromine were also found in that order. These mineral contents indicate that the leaves of three samples powder were rich source of mineral.

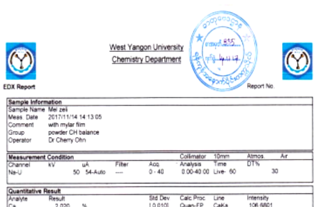
Elemental Analysis of Leaves Samples


West Yangon University
Chemistry Department
EDX Report
Report No.

Sample Information
Sample Name paukpan phyu
Meas. Date 2017/11/14 14:14:44
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air
Channel kV uA Filter Acq. Analysis Time DT%
Na-U 50 44-Auto ---- 0 - 40 0.00-40.00 Live- 60 30

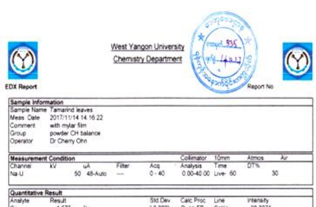
Quantitative Result
Analyte Result Std Dev. Calc Proc Line Intensity


West Yangon University
Chemistry Department
EDX Report
Report No.

Sample Information
Sample Name Mei zeli
Meas. Date 2017/11/14 14:13:05
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air
Channel kV uA Filter Acq. Analysis Time DT%
Na-U 50 54-Auto ---- 0 - 40 0.00-40.00 Live- 60 30

Quantitative Result
Analyte Result Std Dev. Calc Proc Line Intensity


West Yangon University
Chemistry Department
EDX Report
Report No.

Sample Information
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Meas. Date 2017/11/14 14:16:22
Comment with mylar film
Group powder CH balance
Operator Dr Cherry Ohn

Measurement Condition Collimator 10mm Atmos. Air
Channel kV uA Filter Acq. Analysis Time DT%
Na-U 50 48-Auto ---- 0 - 40 0.00-40.00 Live- 60 30

Quantitative Result
Analyte Result Std Dev. Calc Proc Line Intensity

Figure 3: Elemental analysis of *Sesbania grandflora* (L.), *Senna siamea* (Lam.) and *Tamarindus indica* L. of leaves sample by EDXRF method

Table 3: The Results of the Amount of Silver Nanoparticles from Three Kinds of Leaves Extract

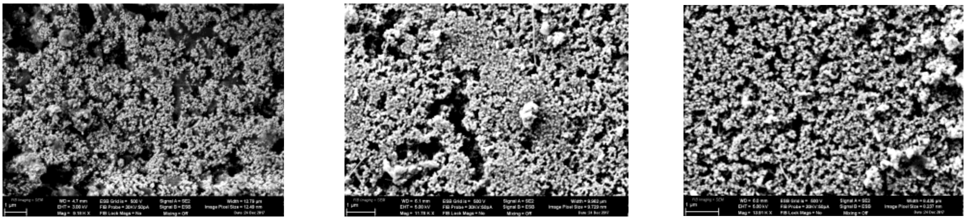
Sample : $AgNO_3$	Time (hr)	Weight of AgNPS (g)		
		Paukpanbyu	Mezali	Magyi
1 : 4	1	0.0102	0.0101	0.0093
1 : 4	3	0.0115	0.0112	0.0101
1 : 4	5	0.0130	0.0128	0.0119
1 : 4	7	0.0155	0.0143	0.0135

volume ratio → Leaves extract : $AgNO_3$

1 : 4

According to the Table 3, silver nanoparticles were determined by using different time intervals. It was found that the amount of silver nanoparticles depends on the contact time. When the stirring time increased, the amount of silver nanoparticles also increased.

SEM Micrographs of Silver Nanoparticles



Paukpanbyu **Mezali** **Magyi**

Figure 4:SEM micrographs of silver nanoparticles using three different leaves

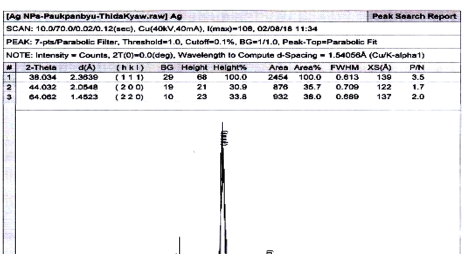
According to all SEM micrograph, it was found that AgNPs was spherical shapes. SEM studies provided further insight into the morphology and size details of the silver nanoparticle. The results of the SEM studies on silver nanoparticles synthesized using Paukpanphyu leaves, Mezali leaves and Magyi leaves extract revealed the formation of stable silver spherical shaped nanoparticles. The size of the silver nanoparticles synthesized using three different leaves were recorded to be 1 μm.

XRD Analysis of Silver Nanoparticles

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

Table 4: XRD Results of Average Crystallite Size of Silver Nanoparticle by using Paukpanbyu Leaves Extract

Sample name	2θ (°)	FWHM (radian)	β (radian)	Crystallite size (D) nm	d-spacing (nm)	d(Å)
Paukpanbyu	38.034	0.613	10.70×10^{-3}	13.71	0.2129	2.3639
	44.032	0.709	12.38×10^{-3}	12.08	0.1849	2.0548
	64.062	0.689	12.03×10^{-3}	13.59	0.1307	1.4523
			Average	13.12		



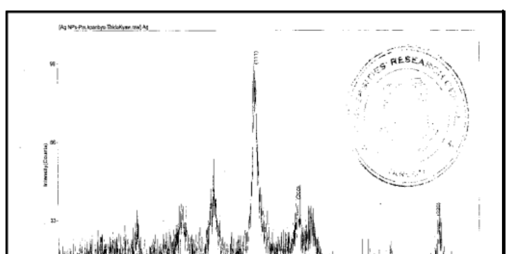


Figure 5: XRD diffratogram of silver nanoparticles using Paukpanbyu leaves

Table 5: XRD Results of Average Crystallite Size of Silver Nanoparticles by using Mezali Leaves Extract

Sample name	2θ (°)	FWHM (radian)	β (radian)	Crystallite size (D) nm	d-spacing (nm)	d (Å)
Mezali	37.958	0.459	8.032×10^{-3}	18.25	0.2132	2.3685
	44.112	0.593	10.377×10^{-3}	14.41	0.1846	2.0513
	64.233	0.532	9.31×10^{-3}	17.58	0.1304	1.4489
			Average	16.74		

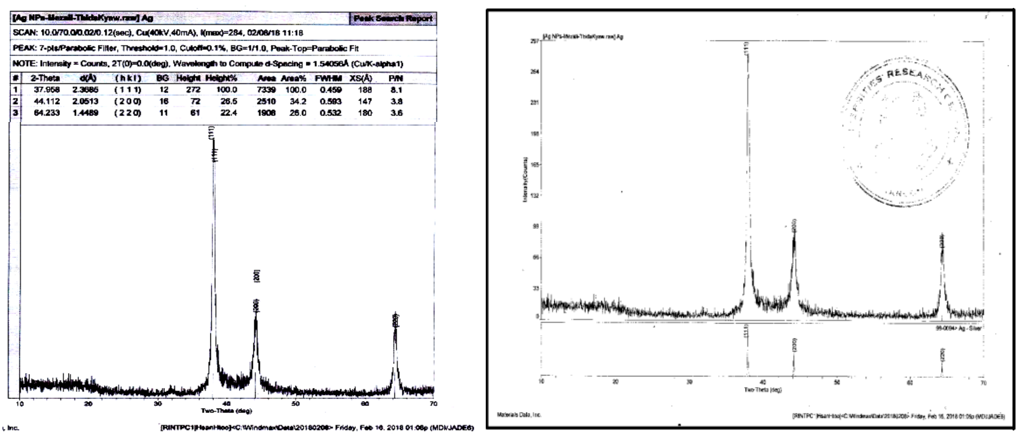


Figure 6: XRD diffratogram of silver nanoparticles using Mezali leaves

Table 6: XRD Results of Average Crystallite Size of Silver Nanoparticles by using Magyi Leaves Extract

Sample name	2θ (°)	FWHM (radian)	β (radian)	Crystallite size (D) nm	d-spacing (nm)	d(Å)
Magyi	37.940	0.482	8.435×10^{-3}	17.57	0.2132	2.3696
	44.133	0.566	9.905×10^{-3}	15.10	0.1845	2.0504
	64.217	0.365	6.388×10^{-3}	25.62	0.1304	1.4492
			Average	19.43		

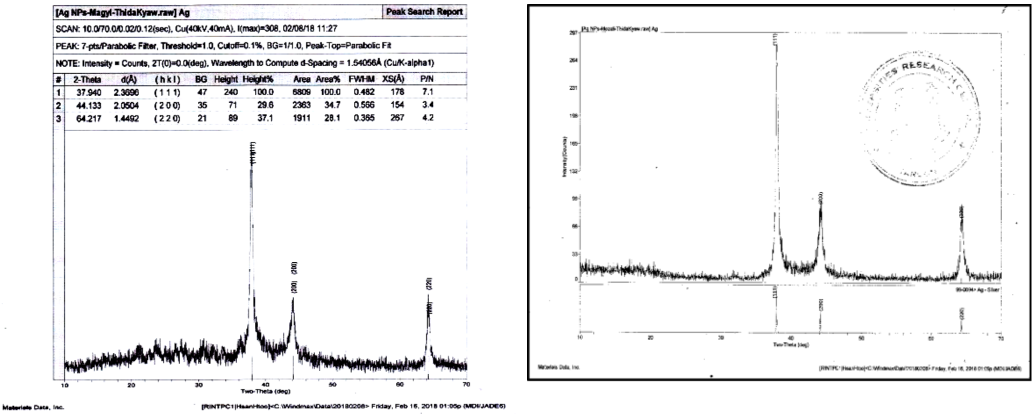


Figure 7: XRD diffratogram of silver nanoparticles using Magyi leaves

According to XRD results, the crystallite size of silver nanoparticles was found within the range of 12.05-13.67nm prepared by using Paukpanbyu leaves extract. Interplanar spacing between silver nanoparticles was found within the range of 0.1304-0.2127 nm. The crystallite size of silver nanoparticles was found within the range of 14.41-18.25 nm prepared by using Mezali leaves extract. Interplanar spacing between silver nanoparticles was found within the range of 0.1304-0.2132 nm. The crystallite size of silver nanoparticles prepared from Magyi leaves extract was found within the range of 15.10-25.62 nm. Interplanar spacing between silver nanoparticles was found within the range of 0.1304-0.2132 nm.

Scherer's Equation for Estimation of Crystalline Size

$$D = \frac{K\lambda}{\beta \cos\theta} \text{Å}, \quad d = \frac{K\lambda}{2\sin\theta}$$

Where, D = the average crystalline size in A°
K = the shape factor
λ = the wavelength of X-ray (1.5406 A°)
β = full-width at half maximum (FWHM) of different peaks (radian)
θ = the Bragg angle
d = interplanar spacing

Antibacterial Activity of the Prepared Silver Nanoparticles

Silver nanoparticles have been using in many industries such as the health, pharmaceuticals, water treatment, paint, food storage because of its antibacterial properties (Venu *et al*., 2011). In the present study, the antibacterial activity of prepared AgNPs was tested against six different human pathogens. It is apparent that the AgNPs showed inhibition zone against six tested organisms (Table 7 and Figure 8).

Table 7: Results for Antibacterial Activities of the Prepared Silver Nanoparticles (AgNPs)

Source of leaves extract	Inhibition zone diameters of the prepared silver nanoparticles against various organisms (mm)					
	I	II	III	IV	V	VI
Paukpanbyu	-	15	12	13	19	15
Mezali	-	10	13	12	15	14
Magyi	10	13	18	10	17	14
Control(water)	11	13	-	-	11	-

Agar-well (7-8 mm)

Organisms

I = *Escherichia coli*
II = *Staphylococcus aureus*
III = *Shigella boydii*
IV = *Salmonella typhii*
V = *Pseudomonas aeruginosa*
VI = *Bacillus cereus*

According to the Table 7, the AgNPs prepared by using Paukpanbyu and Mezali leaves extract did not show antibacterial activity on *E. coli*. AgNPs prepared by using Paukpanbyu showed the highest activity on *Staphylococcus aureus, Salmonella typhii, Pseudomonas aeruginosa* and *Bacillus cereus*.

Escherichia Coli | *Staphylococus aureus* | *Shigella boydii* | *Salmonella typhi* | *Pseudomonas aeruginosa* | *Bacillus cereus*

A = AgNPs (Mezali), B = AgNPs (Magyi), C = Control, D =AgNPs (Paukpanbyu)

Figure 8: Antibacterial activity of AgNPs prepared by using three leaves extracts

Conclusion

In this investigation, the phytochemical compounds present in three different leaves were examined by phytochemical tests which informs the presence of alkaloid, phenolic, polyphenol, steroid and saponin respectively. Therefore, these samples contain valuable phytochemical constituents for human's health. Furthermore, the determinations of elemental composition of three different leaves samples were carried out by using EDXRF (Energy Dispersive X-ray Fluorescence) spectroscopy. According to EDXRF spectroscopy, it was found that the amounts of potassium and calcium are higher than that of others.

Silver nanoparticles were synthesized by reduction of silver nitrate with leaves extract at different contact times 1 h, 3 h, 5 h and 7 h. The results of this investigation reveal that synthesis and characterization of silver nanoparticles using leaves extract.

The particle size distribution of nanoparticles surface morphology were characterized with Scanning Electron Microscopy (SEM) that informs nano aggregate structure of silver nanoparticles. SEM studies revealed the formation of spherical shaped nanoparticles and their sizes were 1 µm.

The crystallite size of nanoparticles were determined by using x-ray diffractometer. According to XRD results, the crystallite size of silver nanoparticles were found within the range of 12 to 25 nm. Interplanar spacing between silver nanoparticles were found within the range of 0.1304 to 0.2132 nm. The peaks in XRD pattern of AgNPs prepared by using leaves of Mezali and Magyi extracts contained only metallic silver peak and that prepared by using leaves of Paukpanbyu extracts contained metallic silver as well as peaks of other impurity crystallite peak. The XRD patterns of silver nanoparticles are in high quality accord with the values of standard card (JCPDS card no: 04-0783). According to XRD peak search report of AgNPs, the crystallite size of the AgNPs was found to be 13.09 nm (Paukpanbyu), 16.74 nm (Mezali) and 19.43 nm (Magyi).

Moreover, the antibacterial screening of silver nanoparticles were examined by using agar-well diffusion method against on six selected microorganism namely *Escherichia coli, Staphylococcus aureus, Shigella boydii, Salmonella typhii, Pseudomonas aeruginosa, Bacillus cereus.* We could found that the prepared AgNPs of Paukpanbyu, which has the highest activity on *Staphylococcus aureus, Salmonella typhii, Pseudomonas aeruginosa* and *Bacillus cereus*

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