Characterization of Silver Nanoparticles by Using Biosynthesis,and Evaluation of their Antimicrobial Activities

Nwe Thin Ni[1], Aung Kyaw Moe[2]

Abstract— From this study, fabrication of silver nanoparticles from watery extracts of *Cymbopogon citratus*, Stapf leaves was conducted by using biosynthesis. Silver nitrate was used as a metal precursor and *Cymbopogon citratus* leaves extracts was applied as biomimetic routes of stabilizing agent as well as reducing agent for synthesis and characterization of silver nanoparticles (AgNPs). The surface morphology of fabricated silver nanoparticles (AgNPs) was conducted with FESM (Field Emission Scanning Electron Microscopy). From the result of XRD (X-ray diffraction), the average particle size of AgNPs (30 nm-35nm) was observed. The size distribution of prepared (AgNPs) was analysed on advanced techniques Zetapotential-DLS (Dynamic Light Scattering). The localized surface plasmon resonance band for formation of AgNPs (435 nm) was found under UV-visible spectrometer. Moreover, the reducing agent or capping agent of bio-based extracted matter such as proteins, phenols and etc., was determined by using the FTIR (Fourier Transform-Infrared Spectroscopy). The effect of selected stabilizing agent of *Cymbopogon citratus* leaves extract and the effect of stirring time on synthesis of AgNPs were studied. In addition, the reflected beam was occurred in the formation of AgNPs by the Tyndall effect under the provision of laser beam. Furthermore, determination of antimicrobial activity of reducing agent of *Cymbopogon citratus* leaves and fabricated AgNPs were studied against six species of microorganisms by using agar well diffusion method.

Index Terms— bio-based synthesis, *Cymbopogon citratus*, AgNPs, morphology, average particle size, size distribution

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1 Introduction

THE properties of nanoparticles change as their size (1 to100 nm) and approach the nanoscale as the percentage of the surface [4]. Nanoparticles can be divided into two groups: (i) organic nanoparticles and (ii) inorganic nanoparticles. Organic nanoparticles contain carbon nanoparticles. Inorganic nanoparticles involve magnetic nanoparticles, noble nanoparticles (like gold and silver), semiconductor nanoparticles (like titanium dioxide and semiconductor nanoparticles) possess optical properties [6]. The synthesis of NPs are broadly divided into two main classes: (1) bottom-up approach and (2) top-down approach. The breakdown (top-down) method by which an external force is applied to a solid that leads to its break-up into smaller particles [2]. The build-up (bottom-up) method that produces nanoparticles starting from atoms of gas or liquid based on atomic transformation or molecular condensation [4] [5]. Silver nanoparticles are used in various fields, especially in biomedical industry or diagnosis, drug delivery, cell imaging, and implantation. Biosynthesis of silver nanoparticles could be advantages than photochemical reduction and chemical reduction methods [3]. In biosynthesis including plant, bacteria, fungi and yeast have been used to prepare nanoparticles [9]. Therefore, in this research proposal, biosynthesis of silver nanoparticles in the environmental friendly was conducted. Then, characterization of fabricated silver nanoparticles was studied by applying advanced modern techniques. After that, the determination of inhibitory effect of reducing agent and prepared silver nanoparticles were studied against on six kinds of microorganisms. The *Cymbopogon* genus (lemon grass) belongs to the family of *Gramineae*, and is a herb of worldwide for its high essential oil content. It is widely distributed in the tropical and subtropical regions of Africa, Asia and America. In Myanmar, it is widely distributed throughout the country. Traditional applications of *Cymbopogon citratus* in different countries show its diversity as a common tea, medicinal supplement, insect repellant, insecticide, and as an anti-inflammatory, analgesic, cures for stomach upset, malaria therapy and as an antioxidant [1].

2 Procedure for Paper Submission

2.1 Sample Collection

The leaves of *Cymbopogon citratus* Stapf. were collected from Nwe-kway-ywa, Htauk-kyant Township, Yangon Region, Myanmar in the middle of December. After cleaning, the leaves were air-dried at room temperature for three weeks and the dry sample was ground into powder by grinder. The dried powdered sample was stored separately in air-tight containers to prevent moisture changes and other contamination. These plants were identified at the Department of Botany, University of Yangon [11] ; [12].

[1] Department of Chemistry, Taungoo University, Taungoo, Myanmar, drntni@gmail.com
[2] Chulalongkorn University, Petroleum & Petrochemical College, The Centre of Excellent in Petrochemical and Material Science, Bankok, Thailand, s11005@alumini.wmu.se

2.2 Preparation of Reducing Agent from *C. citratus*

The collected sample (30 g) of *C. citratus* was boiled in 100 mL distilled water under 20 min to obtain the extract. This obtained cooled extract was filtered through Whatman filter paper No.1. The filtrate was cooled down at 4 °C. The extracted matter can be applied the capping agent as well as the reducing agent for the reduction of silver ions.

2.3 Preparation of $AgNO_3$ solution

The molar mass of $AgNO_3$ is 169.87 $gmol^{-1}$. For 1M solution of $AgNO_3$, 169.87 g of $AgNO_3$ can be used in 1 liter of distilled water. In the preparation of 0.001 M of $AgNO_3$ solution, 0.034 g of $AgNO_3$ in 200 ml of distilled water was used in this biosynthesis of silver nanoparticles.

2.4 Preparation of Silvernanoparticles (AgNPs)

Two to three milliliter of prepared extracted matter from *C. citratus* leaves was added to the 0.001 M of silver nitrate solution in 100 mL conical flask and incubated at 40 °C for (60-90) min. Then, this reaction process was carried out in dark to avoid unnecessary photochemical reactions [10]. The colour change of silver nitrate solution was occurred and photoreduced sample constituents was observed under UV-vis spectroscopy. This obtained AgNPs was purified through centrifugation at 6000-8000 rpm for 20 minutes. The dispersion of AgNPs was washed with deionized water and dried in an oven at 100°C for 24h.

2.5 Phytochemical Screening of *C. citratus* and Prepared AgNPs

The main chemical constituents of carbohydrate, and proteins etc., present in reducing agent of *C. citratus* and fabricated AgNPs were mentioned by using the FTIR spectroscopic method.

2.6 Characterization of Prepared AgNPs

The FESEM (Field Emission Scanning Electron Microscopy) techniques was provided to analyzes the surface morphology of fabricated AgNPs. Then, the measurement of average particle size of AgNPs was detected under XRD (X-ray Diffractometer) method. In addition, determination of particle size distribution of AgNPs was measured by using Zeta potential-DLS instruments at 25°C with percent intensity. The localized surface plasmon resonance band of AgNPs were observed at 435 nm by using Shimadzu UV-1800 spectrometer.

2.7 Determination of Antimicrobial Activity of Fabricated AgNPs

By measuring the inhibition zone diameters, the antimicrobial activity of extracted reducing agent and fabricated AgNPs were evaluated by using agar well diffusion method against test strains of six microorganisms.

3.RESULTS AND DISCUSSION

3.1 Sample Collection and Preparation of Reducing Agent

The leaves of locally grown *C. citratus* were collected from Nwe-kway-ywa, Htauk-kyant Township, Yangon Region, Myanmar. The watery extract of *C. citratus* leaves was used as reducing agent as well as capping agent in the preparation of AgNPs.

3.2 Preparation of Silver Nanoparticles (AgNPs)

The silver nitrate (0.001 M) was used as metal precursor. The fabricated silver nanoparticles (AgNPs) was mentioned under visual condition. AgNPs was formed with a colour change from yellow to brownish-black colour during the reaction period within 15 min. The colour change of brownish-black was observed in the formation of AgNPs and it was the effect of reducing agent of *C. citratus* leaves extracts.

3.3 Effect Of Concentration of Silver Nitrate Solution And Stirring Time

The capping agent or reducing agent (20 mL) of *C. citratus* leaves extract was added to (60, 80,100) mL of silver nitrate solution (reducing agent: $AgNO_3$) (1:3,1:4,1:5) solution in a conical flask. The flask was heated with magnetic stirrer at 50°C. After stirring time for (1,2,3) h and it was kept in the dark place. The colour intensity was raised with the increased of stirring time (Figure 1,2,3).

(a) (b) (c) (d) (e)

Figure 1 Colour Changes of AgNPs (reducing agent: $AgNO_3$), (1:3)

(a) $AgNO_3$ Solution (0.001M)
(b) Fabrication of silver (after 10min)
(c) Fabrication of silver (after 1h)
(d) Fabrication of silver (after 2h)
(e) Fabrication of silver (after 3h)

(a) (b) (c) (d) (e)

Figure 2 Colour Changes of AgNPs (reducing agent: $AgNO_3$), (1:5)

(a) $AgNO_3$ Solution (0.001M)
(b) Fabrication of silver (after 10min)
(c) Fabrication of silver (after 1h)
(d) Fabrication of silver (after 2h)
(e) Fabrication of silver (after 3h)

Figure 3 Appearance of fabricated AgNPs
(a) (1:3) (reducing agent:$AgNO_3$), (after stirring 1,2,3) hr
(b) (1:4) (reducing agent:$AgNO_3$), (after stirring 1,2,3) hr
(c) (1:5) (reducing agent:$AgNO_3$), (after stirring 1,2,3) hr

3.4 Characterization of Fabricated AgNPs

3.4.1 FESEM Analysis

The field emission scanning electron microscopy (FESEM) images of silver nanoparticles in the ratio of (1:5) after one hour stirring time was shown in Figure 4. The surface morphology of silver nanoparticles were observed in spherical nature. From this result, *C. citratus* leave extract was used as reducing or capping agent in the production of silver nanoparticles [7],[8].

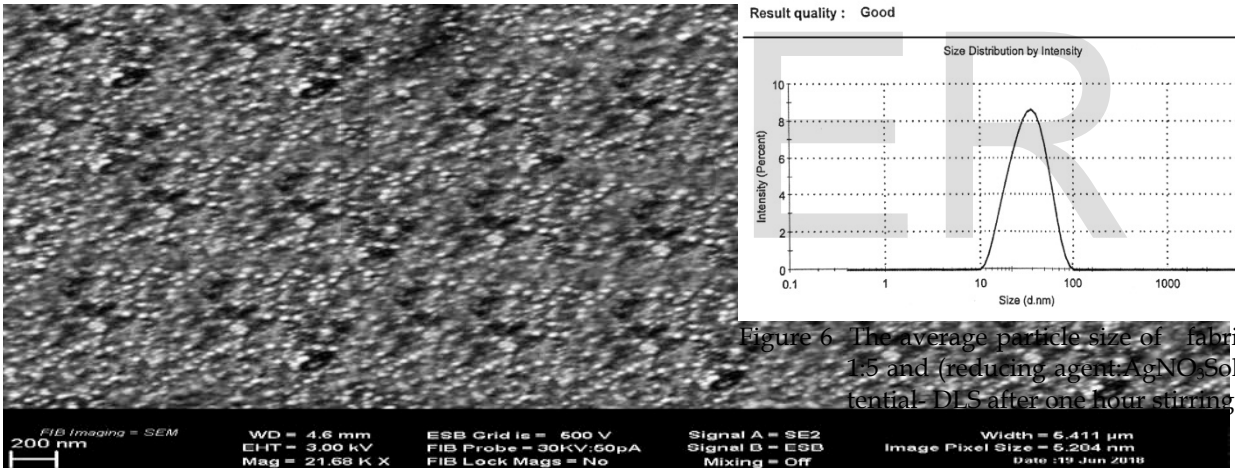


Figure 4 FESEM spectrum of fabricated AgNPs in the ratio of (1:5) reducing agent: $AgNO_3$ solution after one hour stirring time

3.4.2 XRD Analysis

From the XRD result, the average crystalline size of silver nanoparticle was determined by using the Debye-Scherrer equation, it was as shown in Figure 5. The data was collected in the 2θ. $D=\frac{K\lambda}{\beta\cos\theta}$

Where, D= average crystalline domain size perpendicular to the reflection plane

λ = X -ray wavelength

β = full width at half maximum intensity (FWHM)

θ = diffraction angle (Bragg's Angle)

K = a dimensionless shape factor with the value closes to unity

The modified formula is valid only when the average crystalline size is smaller than 100 nm. The four distinct diffraction peaks at 2θ values of (30.128, 36.401, 29.879 and 44.566) can be indexed to (200, 220, 111 and 311) reflection planes of face centered cubic structure of silver. From the width of half maximum of Debye-Scherrer equation, the estimated average size of particle was observed (25-35) nm nm respectively. This may be due to the bio-organism compounds occurring on the surface of silver nanoparticles.

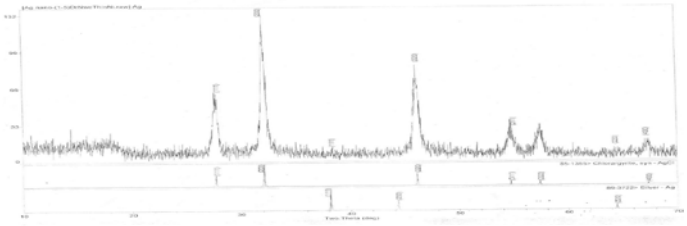


Figure 5 XRD spectrum of AgNPs in the ratio of 1:5 (reducing agent:$AgNO_3$) after one hour stirring time

3.4.3 Zeta Potential - DLS Analysis

The size distribution of Fabricated AgNPs was reported with intensity using zeta potential-DLS, it was shown in Figure 6. From this result, the size distribution of AgNPs was observed between (1-100) nm range.

Figure 6 The average particle size of fabricated AgNPS into 1:5 and (reducing agent:$AgNO_3$Solution) on zeta potential- DLS after one hour stirring time

3.4.4 UV-Visible spectral Analysis

The formation and stability of metal nanoparticle in aqueous solution was determined by using the UV-visible spectroscopy and it is an important technique to exhibit UV-visible absorption maximum in the range of 300-360 nm due to the excitation of surface plasmon vibration. The localized surface plasmon resonance band of 1:3 1:4 and 1:5 ratio of reducing agent and $AgNO_3$ were observed at 435 nm by using Shimadzu UV-1800 spectrometer. The increase in the concentration of the silver nitrate will be increased the absorbance intensity but the wavelength was not changed it was shown in Figure 7.

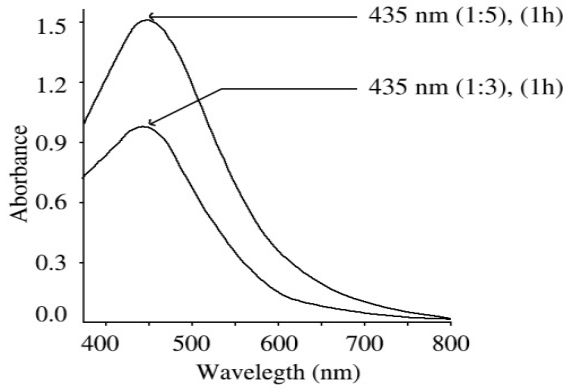


Figure 7 Absorption spectra of AgNPs in different ratio of (reducing agent: $AgNO_3$) after one hour stirring time

3.4.5 FTIR Analysis

According to FTIR spectral data, the absorption band of the reducing agent powdered and prepared silver nanoparticles were described in Figure 8 and 9. In the spectrum of capping agent of *C. citratus* leaves, the peaks were observed at 3728 cm^{-1} which may be due to the overlapping of O-H and N-H stretching bands, 2928 cm^{-1} represents aliphatic C-H stretching , 1725,1624 cm^{-1}indicates N-H bending, 1386 cm^{-1}also indicates C-H bending, 1078 cm^{-1}indicates also C-O stretching, respectively. It was also confirmed the reducing agent of *C. citratus* is effected in formation of AgNPs. After reaction with silver, the new moderate intensity peak was observed at 540cm^{-1}. It may be confirmed that, the amino group (N-H group) present in capping agent which can be provided reduction of Ag^+ to Ag^0.

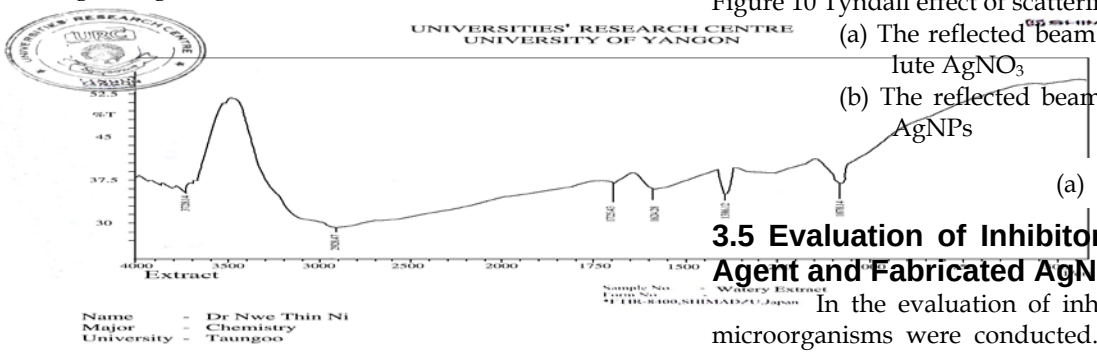


Figure 8 FTIR spectrum of reducing agent, *C. citratus*

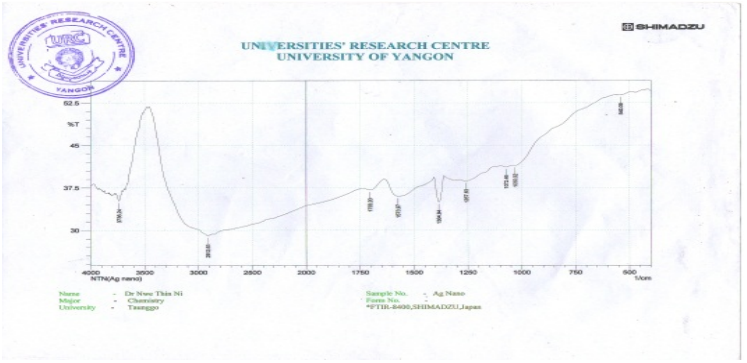


Figure 9 FTIR spectrum of fabricated AgNPs in the ratio of (1:5) reducing agent: $AgNO_3$ solution

3.4.6 Tyndall effect

The Tyndall effect, also known as Willis-Tyndall scattering is light scattering by particles in a colloid or in a very fine suspension. The particle even large enough that they can be scattered light, the Tyndall effect was occurred. Since the presence of a colloidal suspension can be monitored by the reflection of a laser beam from the particles because a laser pointer emitted that the polarized light, and the pointer can also be oriented that the beam appear to disappear. If the colloidal particles are present, the laser beam passed and if the particles are absent, the beam did not pass through as shown in Figure 10.

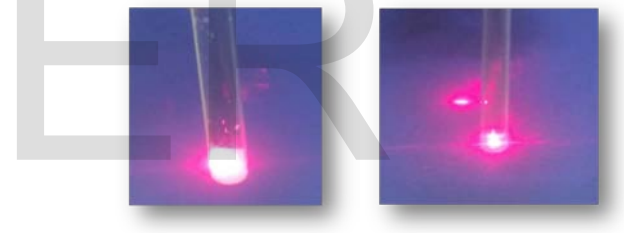


Figure 10 Tyndall effect of scattering light
(a) The reflected beam did not pass through absolute $AgNO_3$
(b) The reflected beam passed through prepared AgNPs

(a)

(b)

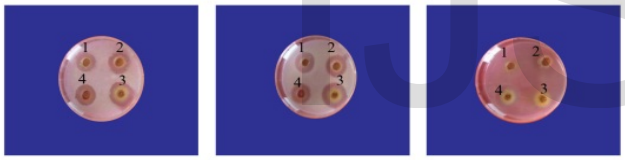
3.5 Evaluation of Inhibitory Effect of Reducing Agent and Fabricated AgNPs

In the evaluation of inhibitory effect, six strains of microorganisms were conducted. Among them, each tested materials watery extract of capping agent and different ratio of performed AgNPs were possessed inhibitory effect by using the agar well diffusion method. Moreover, it was observed that the most potent activity of AgNPS in the ratio of 1:5 (reducing agent: AgNPS) than remaining tested materials against on *Escherichia coli* and *Bacillus pumilus*. It was shown in Table 1 and Figure 11.

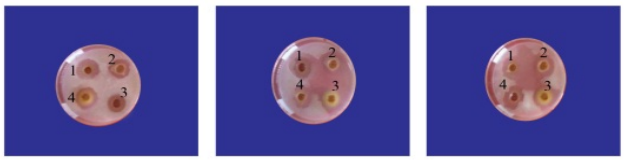
Table 1 Inhibition Z ımeter of Reducing agent and Prepared AgNPs Against Six Microorganisms by Agar Well Diffusion Method

No.	Micro-organ-isms	Inhibition Zone Diameter (mm)			
		wa-tery	(1:3) AgNP	(1:4) AgNP	(1:5) AgNP
1	*Bacillus Subtilis*	18 (++)	13 (++)	17 (++)	19 (++)
2	*Staphy-lococcus aureus*	17 (++)	12 (+)	18 (++)	18 (++)
3	*Pseudo-monas aeru-ginosa*	15 (++)	11 (+)	18 (++)	19 (++)
4	*Bacillus pumilus*	18 (++)	14 (+)	17 (++)	20 (+++)
5	*Candida albicans*	16 (++)	12 (+)	17 (++)	19 (++)
6	*Esche-richia coli*	18 (++)	13 (+)	17 (++)	20 (+++)

Agar well -10 mm
10mm ~ 14 mm(+), 15mm ~19 mm (++), 20 mm above (+++)



Bacillus subtilis *S.aureus* *P.aeruginosa*



Bacillus pumilus *Candida albicans* *E.coli*

1. capping agent
2. AgNPs, (1:3), (reducing agent: $AgNO_3$)
3. AgNPs, (1:4), (reducing agent: $AgNO_3$)
4. AgNP, (1:5), (reducing agent: $AgNO_3$)

Figure 11 Inhibition zone diameter of reducing agent and AgNPS against six microorganisms

4. CONCLUSION

From the overall assessment of this research work, in the bio- based synthesis of silver nanoparticles was conducted. This method is environmental friendly and non-toxic effect in environment. From this research, *C. citratus* was used as reducing agent as well as capping agent. The surface morphology of AgNPs was obtained spherical shape by using FESEM. In addition, the particle size distribution of AgNPs was showed with intensity in the range of (10-100) nm range under the zeta potential-DLS. The average particle size of AgNPs (25nm -35nm) range were observed by the aids of XRD result. The intensity colour of fabricated AgNP (435 nm) in the ratio of 1:5 were conducted under the UV- visible spectrometer. According to FTIR analysis, the constituents of amino group present in the stabilizing agent of *C. citratus* was denoted that reduction of Ag^+ to metallic silver nanoparticles. The presence of a colloidal suspension can be monitored by the reflection of a laser beam from the particles because a laser pointer emitted that the polarized light, and the pointer can also be oriented that the beam appear to disappear. If the colloidal particles are present, the laser beam was passed and if the particles were absent, the beam did not pass through it. Furthermore, the evaluation of inhibitory effect, by comparing the zone diameters, the fabricated AgNPs in the ratio of 1:5 was observed the more potent activity than other tested materials by using agar well diffusion method. Therefore, nanoparticles were generally more active than reducing agent (plant extract) against selected microorganism. It is reasonable to state that the binding of the particles to the bacteria depends on the surface area available for interaction. This is because, the silver nanoparticles may attach to the surface the cell membrane and disturb its power function such as permeability and respiration.

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