Study on Antibacterial Activity of Synthesized Silver Nanoparticles Using Guava Leaf Extract

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Abstract

The green synthesis of the nanoparticles using plant extracts is non-toxic, very rapid and low-cost. In this research, watery extract of leaf of *Psidium guajava* L. was chosen to synthesize the silver nanoparticles as the reducing agent and the silver nitrate solution was used as the starting material. The phytochemical compounds present in *Psidium guajava* L. leaves were examined by phytochemical test. The leaves contain valuable phytochemical compounds. The elemental composition of *Psidium guajava* L. leaves was measured by Energy Dispersive X-ray Fluorescence spectroscopy. The leaves have no toxic element. Silver nanoparticles were synthesized using silver nitrate solution and watery extract of *Psidium guajava* leaves. The synthesized silver nanoparticles were characterized by SEM, FT-IR and XRD techniques. From the XRD results, the size of silver nanoparticles was found to be in the range of nano scale. Antibacterial activity of *Psidium guajava* leaves extract and silver nanoparticles of leaves extract on four selected microorganism was measured by agar well diffusion method. The silver nanoparticles of leaves extract give high activities on *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*. The results show that green synthesized silver nanoparticles, using *Psidium guajava* L. (Guava) leaf extract, have a potential to inhibit the growth of bacteria.

Keyword: phytochemical compounds, elemental composition, nanoparticles, silver nitrate, Antibacterial activity

Introduction

Nanomaterials and nanostructures, usually ranging from 1 to 100 nm, based on the specific characteristic such as size, morphology and distribution exhibit their remarkable potential in the field of biology and medicine (Wang *et al*., 2005).

Nano-crystalline silver particles have been found tremendous applications in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics (Jiang H, *et al*., 2004).

Because of their wide applications beneficial to humans, there is a need to develop rapid and reliable experimental protocols for the synthesis of silver nanoparticles. The synthesis of silver nanoparticles using biological entities is gaining momentum as biological methods are providing nontoxic and environmentally acceptable " green chemistry" procedures (Song and Kim, 2009).

In this research, silver nanoparticles were synthesized by bioreduction process using aqueous silver nitrate solution and watery leaves extract of *Psidium guajava* L. The antimicrobial activities of watery extract of *Psidium guajava* L and synthesized silver nanopartiles were examined.

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Botanical Description


Figure (1) Plant and Leaf of *Psidium guajava* L.

Family name - Myrtaceae
Scientific name - *Psidium guajava* L.
Myanmar name - Malaka
English name - Guava

Materials and Method

Sample collection

The leaves of *Psidium guajava* L. (English name- Guava) were collected from Mandalay Technological University Campus. They were washed and then cut into small pieces and used for the experiment.

Preliminary phytochemical test of leaves of *Psidium guajava* L.

Phytochemical tests were done on the various extracts of leaves of *Psidium guajava* L. using standard methods (Harborne J. B, 1973).

Determination of elemental composition of leaves of *Psidium guajava* L.

The elemental analysis of leaves of *Psidium guajava* L. was carried out by using EDXRF (Energy Dispersive X-ray Fluorescence) Spectroscopy at Department of Chemistry, Monywa University.

Preparation of watery extract of leaves of *Psidium guajava* L.

The fresh leaves of *Psidium guajava* L. **(**Guava) were washed with distilled water and cut into small pieces. The 16 g of leaves pieces was refluxed with 200mL of distilled water for one hour and the mixture was cooled. The leaves extract was filtered. The watery extract was obtained.

Preparation of 10 mM of silver nitrate solution

10 mM of silver nitrate solution (100mL) was prepared using silver nitrate and distilled water in the laboratory.

Synthesis of silver nanoparticles using 10 mM $AgNO_3$ solutions and *Psidium guajava* L. leaves extract

The 25 mL of 10mM silver nitrate solution were mixed with 100 mL of freshly prepared *Psidium guajava* L. leaves extract. The mixture was stirred on a magnetic stirrer with 500 rpm at room temperature for 6 hours. Then, the mixture was centrifuged with 6000 rpm for 10 minutes. The precipitates of silver nanoparticles were obtained. They were washed with acetone and then placed to a petridish.

Characterization of the synthesized silver nanoparticles

The Fourier Transform-Infrared (FT IR) technique is used to identify the organic and inorganic functional group present in the synthesized silver nanoparticles. FTIR transmittance spectra were reported in the 4000 - 400 cm^{-1} region by using KBr pellet technique. (Silverstein,1998).

Morphology and size distribution of the synthesized silver nanoparticles were determined by SEM and the crystallite sizes of nanoparticles were measured by XRD method. The particle sizes were estimated by using Debye-Scherrer's equation.

Determination of antibacterial activities of leaves of *Psidium guajava* L.

The antibacterial activities of watery extract of leaves of *Psidium guajava* L. and the synthesized silver nanoparticles were studied by agar well diffusion method on four tested microorganisms such as *Esherichia coli, Pseudomonas aeruginosa, Bacillus cereus and Staphylococcus aureus* at Department of Biotechnology, Mandalay Technological University.

Results and Discussion

Phytochemical constituents of leaves of *Psidium guajava* L.

According to results of phytochemical tests, alkaloids, flavonoids, glycosides, phenolic compounds, polyphenols, reducing sugars, saponins, steroids, tannins, terpenes and lipophenols were presented in Leaves of *Psidium guajava* L.

Yield percent of silver nanoparticles using aqueous extract of leaves of *Psidium guajava* L.

The yield percent of silver nanoparticles synthesized by using aqueous extract of leaves of *Psidium guajava* L. was found to be 0.50%**.**

Characterization of the synthesized silver nanoparticles

The FTIR spectrum of the synthesized silver nanoparticles is shown in Figure 2. From the FT IR spectrum, it was found that the synthesized silver nanoparticles contained alcohol, amine groups (3259, 1318.46cm^{-1}) alkenic group (2920, 1604 cm^{-1}) and ether group (1039.92cm^{-1}). These groups would be in watery extract of leaves of *Psidium guajava* L. They were covered on the synthesized silver nanoparticles.

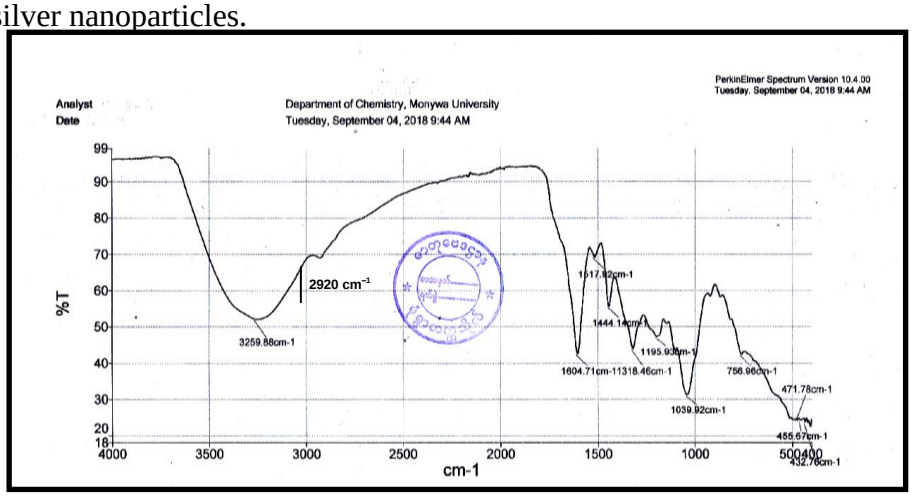


Figure (2) FT IR spectrum of Silver Nanoparticles using Leaves of *Psidium guajava* L.

The surface morphology of the synthesized silver nanoparticles observed by Scanning Electron Microscopy is shown in Figure (3). From the SEM results, the crystallite structures of the synthesized silver nanoparticles were found to be cubic.

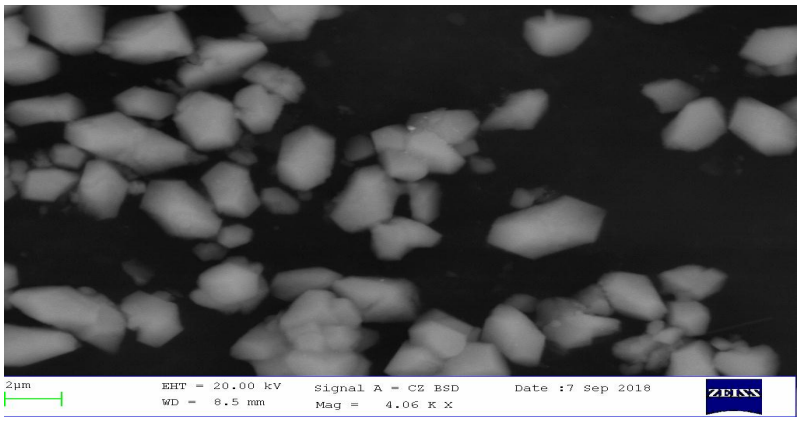


Figure (3) SEM Image of the Synthesized Silver Nanoparticles

The crystallite sizes and inter planar spacing of the synthesized silver nanoparticles were determined by XRD analysis. The speaks obtained by XRD studies of synthesized silver nanoparticles at 2 Θ angles 32.117, 38.093, 44.313, 64.310 confirmed that the particles were face centered cubic structure with (200), (111), (200) and (220) planes respectively. But the synthesized metallic silver peak shows other impurity crystallite phase. The results are in accordance with Joint Committee on Powder Diffraction Standards (JCPDS) data file No.04-0783 and other reports (Parida, U.K. and Bindhani,B.K, 2013) (Ghassan, M.S., *et al* 2013). The X-ray diffractogram of synthesized silver nanoparticles and JCPDS file No.04-0783 are shown in Figure (4). The crystallite size of silver nanoparticles formed in the bioreduction process was determined using Debye-Scheer's formula and was found to be range of 15.93-38.51 nm. The average crystallite size of silver nanoparticles was 25.21 nm .

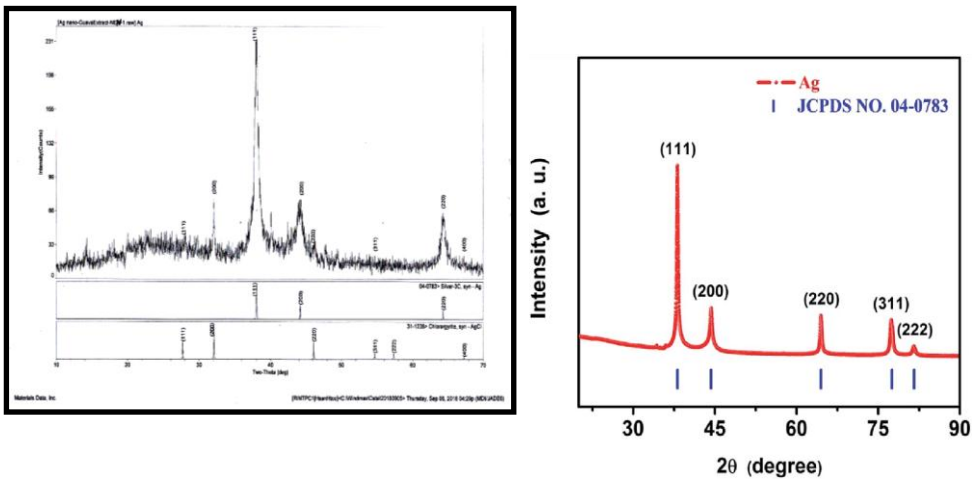


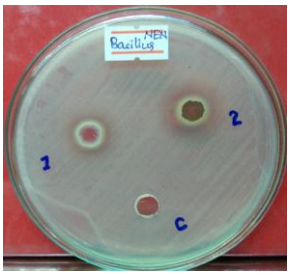
Figure (4) X-ray Diffractogram of Synthesized Silver Nanoparticles and X-ray Diffractogram of Silver (JCPDS 04-0783)

Antimicrobial activities on leaves of *Psidium guajava* L.

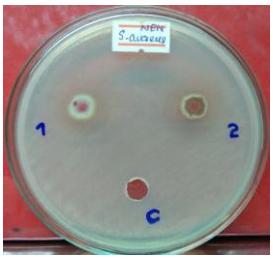
Table (1) Antibacterial Activity on Leaves Extract of *Psidium guajava* L. and Silver Nanoparticles

Test microorganisms (m)	Inhibition Zone Diameter (mm)	
	Guava Leaves Extract	Silver Nanoparticle of Leaves Extract
Bacillus cereus	14	15
Staphylococcus aureus	16	20
Escherichia coli	12	13
Pseudomonas aeruginosa	-	16

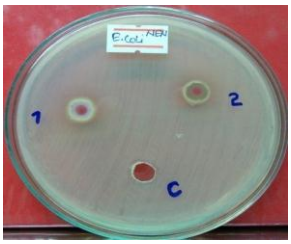
The antibacterial activity of leaves extract of *Psidium guajava* L. shows high activity on *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli*, no activity on *Pseudomonous aeruginosa*. The antibacterial activity of silver nanoparticles of leaves extract gives high activities on *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonous aeruginosa*. The synthesized silver nanoparticles binding with secondary metabolites of the watery extract of the leaves of *Psidium guajava* L. should be used as antibacterials. The results were tabulated in Table (1) and Figure (5).



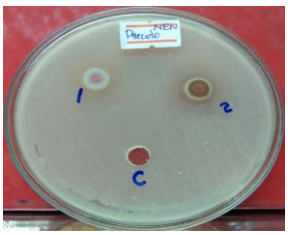
Bacillus cereus



Staphylococcus aureus



Escherichia coli



Pseudomonas aeruginosa

Control well = 8 mm

Figure (5) Antibacterial Activity on Leaves Extract of *Psidium guajava* L. and Silver Nanoparticles of Leaves Extract

Conclusion

In this research work, the leaves of *Psidium guajava* L. were selected for the synthesis of silver nanoparticle. The phytochemical tests of leaves of *Psidium guajava* L give positive tests for flavonoids, glycoside, reducing sugar, saponin and tannin, respectively. The phytochemical analysis indicated that the leaves sample contained the valuable phytochemical constituents. From the FT IR spectrum, it was seen that the synthesized silver nanoparticles contained the biomolecules of watery extract of leaves of *Psidium guajava* L. The results also confirmed that secondary

metabolites are binding to the silver nanoparticles in clusters.From the SEM study, the morphology of silver nanoparticles synthesized by using watery extract could be determined. The results showed that the structures of crystals of synthesized silver nanoparticles were cubic.According to XRD results, the crystallite sizes of synthesized silver nanoparticles were found to be within the range of 15.93-38.51 nm. The peaks in XRD pattern of the synthesized silver nanoparticles also showed that the metallic silver peak and other impurity crystallite phase were detected.

From the examination of antibacterial activities, the leaves extract of *Psidium guajava* L., have high activity on *Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*, no activity on *Pseudomonous aeruginosa*. The antibacterial activity of silver nanoparticles of leaves extract show high activities on *Bacillus cereus, Staphylococcus aureus*, *Escherichia coli* and *Pseudomonous aeruginosa*. Novelty of this present study is that the plant extract is very cost effective, eco-friendly, economic and effective alternative for the large scale synthesis of silver nanoparticles for antibacterial and other applications.

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