LEAF-EXTRACT- MEDIATED GREEN SYNTHESIS OF SILVER NANOPARTICLES (AgNPs) COLLOIDS USING *Amherstianobilis*LEAF EXTRACT AND THEIR ANTIBACTERIAL ACTIVITIES

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Abstract

Plant- extract-mediated green synthesis of nanomaterials has been increasingly gaining popularity due to its eco-friendly nature and cost-effectiveness. In this study green synthesis of (AgNPs) colloids were synthesized using 1mM silver nitrate and the aqueous extract of *Amherstia nobilis* Leaf as the reductant and the capping agent at room temperature. The formation of AgNPs were confirmed by Ultraviolet-Visible (UV-Vis) spectroscopy exhibiting a λ_{max} at 430nm. The electrochemical result shows that the biosynthesized AgNPs exhibit good electrochemical activity which were analyzed by cyclic voltammetry (CV) measurement. FTIR study performed that the *Amherstia nobilis* Leaf extract identifies the functional groups of carbonyl, hydroxyl, amine and protein molecule which were not only functioned as a bioreductant but also stabilized the surface of the AgNPs by acting as a capping agent. The synthesized AgNPs colloids and *Amherstia nobilis* Leaf extracts were separately tested to examine their antibacterial activities. The activities were tested against various bacteria land fungal microorganisms including Candida albicans, Micrococcus luteus, Pesudomonas fluorencens, Staphylococcus aureus. These results evidently show that the inclusion of *Amherstia nobilis* Leaf extracts improves the solubility of AgNPs, which led to a significant enhancement in the toxicity of the NPs against the assessed microorganisms.

Keywords: Green Synthesis, AgNPs colloids, antibacterial activities

Introduction

Nowadays, nanoscience is a rapidly developing field contributed to produce a wide range of various synthesized metal nanoparticles (MNPs). Owing to the unique physicochemical properties of MNPs and their shapes, a promising scientific area of research appeared for biotechnical applications in biomedicine, environmental bioremediation, optical and electronic fields as well as usage in drug delivery and bioimaging [7]. The metal nanomaterials have received particular attention for their positive impact on improving many economy sectors including consumer products, energy, transportation, cosmetics, pharmaceuticals, antimicrobial agents and agriculture [1]. Nanoparticles are particles that have a size of 1 to 100 nm in at least one dimension and possess unique physical and chemical properties due to their large surface area to volume ratio and smaller size [2]. There are two basic approaches used in nanoparticle synthesis: the top-down (communication and dispersion) approach and the bottom-up (nucleation and growth) approach. The decision on which method to adopt depends on the approach that can deliver the specified properties and on cost [6]. Among the metal nanoparticles, Silver nanoparticles have unique optical, electrical, and thermal properties that play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering [8]. Recently, Silver nanoparticles have been widely employed due to their physicochemical properties and are currently used as anti-bacterial agents in food storage, textile and health industries, for biolabeling and as biosensors. In addition, silver nanoparticles (AgNPs) can also be used in clothing, sunscreen, cosmetics, and food industry due to its antimicrobial properties. It is also used for waste water treatment. It has been found that when silver nanoparticles are exposed into waste

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water, the number of nitrifying bacteria present in sludge is reduced. Silver nanoparticles, like their bulk counterpart, show effective antimicrobial activity against gram positive and gram negative bacteria, including highly multiresistant strains such as methicillin resistant Staphylococcus aureus[5]. The three main methods of nanoparticle synthesis are physical, chemical and biological. Each method has advantages and disadvantages with common problems being costs, stability, particle sizes and size distribution and so on [4-9]. Biosynthesis of nanoparticles from green synthesis is advantageous over chemical and physical methods as it is a cost effective and environmental friendly method and it is not necessary to use high pressure, energy, temperature and toxic chemicals. Plants provide a better platform for nanoparticle synthesis as they are free from toxic chemicals as well as provide natural capping agents. Moreover, the use of plant extracts also reduces the cost of microorganism isolation and culture media enhancing the cost competitive feasibility over nanoparticle synthesis by microorganisms[8]. In this work, we report the biosynthesis of silver nanoparticles using a plant extract of *Amherstia nobilis*. The synthesized silver nanoparticles were characterized by UV-Vis spectroscopy, Cyclic voltammetry analysis and FT-IR spectroscopy. UV-Vis spectroscopy is used to confirm AgNPs formation by showing the Plasmon resonance [3]. Cyclic Voltammetry Characterization is the electrochemical activity of the synthesized nanoparticles. Extracts from bio-organisms may act both as reducing and capping agents in silver nanoparticles synthesis. The reduction of Ag+ ions by combinations of biomolecules found in these extracts such as enzymes/proteins, amino acids, polysaccharides, and vitamins [10]. The antibacterial activity of the synthesized copper nanoparticles for the selected bacterial strains was also investigated using Agar disc diffusion method.

Materials and Methods

Silver nanoparticles were synthesized from *Amherstia nobilis* leaves and were then tested for their antimicrobial activity against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli) bacteria. Fresh and healthy leaves of *Amherstia nobilis* leaves were collected from Yangon University, Myanmar. The fresh leaves 25 grams of the plant were collected and washed thoroughly with distilled water to remove the dust particles and were air dried at room temperature. Leaves were chopped into small pieces and mixed into 100 ml distilled water and boiled for 20 min. It was allowed to cool and filtered with Whatman filter paper no. 1. The filterate of the samples were stored at 4°C for further experiments. The concentration 1mM silver nitrate ($AgNO_3$) was weighed using an analytical weighing balance and then transferred into a 250 mL volumetric flask that contained 100 mL of distilled water. This was followed by stirring for 20 min to ensure that all the solid $AgNO_3$ dissolved. The prepared extract 5ml was added to 45ml of aqueous AgNO3 (1mM solution) at room temperature. The mixture was stirred continuously for 20 minutes. The synthesis of NPs using leaves extract can be visually observed because of the change in coloration from pink to reddish-brown. After 24 hours with the appearance of AgNPs colloids changed to brownish-black colour which confirms the formation of silver nanoparticles.

Colloidal AgNPs Characterization

UV-Visible Spectroscopy Characterization : Ultraviolet–visible (UV-Vis) spectrophotometry is the most important and simple technique to confirm the formation of nanoparticles. The absorbance spectrum of synthesized AgNPs was recorded after the time duration of 24 hrs, using a Perkin Elmer UV/Vis Spectrometer (Lamda 35), the wavelength range from 190 to 800 nm.

Cyclic Voltammetry Characterization: The electrochemical activity of the synthesized nanoparticles was studied using a BASi EC Epsilon potentiostat complete with cell stand and data processor. The three-electrode system is composed of Glassy Carbon Electrode (GCE) as the working electrode. The experimental parameters used were initial potential-1000 mV, switching potential +1100 mV, and final potential -1000 mV. The scan rates used ranged from 20 mV/s to100 mV/s. The cyclic voltammogram obtained exhibited a distinct anodic peak at 290 mV at a scan rate of 100 mV/s. The electrochemical experiments were carried out at room temperature (25°C).

Fourier transform infrared (FTIR) spectroscopy Characterization: The plant extracts were freeze-dried and then analyzed at 400–4000 cm^{-1} using a Fourier transform infrared (FTIR) spectrometer (Shimadzu, Koyoto, Japan). FTIR analysis was performed to classify the biomolecules in the plant extracts surrounding the nanoparticles.

The antibacterial activity analysis: For antimicrobial studies, the bacterial strains used in this study were Candida albicans, Micrococcus luteus, Pesudomonas fluorencens, Staphylococcus aureus. These samples were collected from Department Botany Yadanabon University, Myanmar. Bacterial cultures were maintained on Nutrient Agar plates and Slants. They were subcultured and subsequently stored at 4°C.

Results and Discussion

The present study, colloidal AgNPs was synthesized using *Amherstia nobilis* Leaf extracts. In this work, reduction of silver nitrate ions (Ag^{+}) to Ag^{0} was achieved rapidly within 30 min of the incubation period. Silver nanoparticles colloids showed, colour changes from pink toward reddish-brown in aqueous solution due to the excitation of surface plasmon in silver nanoparticles. This change in color is a morphological indicator for the formation of colloidal solution of AgNPs. The synthesized silver nanoparticles showed the following absorption spectrum at the wavelength range of 400nm – 800nm. The results of the UV-vis spectra recorded, SPR peak at around 430nm confirmed the formation of silver nanoparticles as shown in Fig-1.

Electrochemical behavior of Ag+/0 was studied by carrying out cyclic voltammetry in the potential range +0.5 V to -0.5V vs Ag/ AgCl in a repetitive scanning mode at a scan rate of $50mVs^{-1}$, consuming almost 200s for one cycle of forward cathodic and reverse anodic scan. Fig- 2 shows the record of cyclic voltammograms of 1mM silver salt solution in aqueous 0.1M

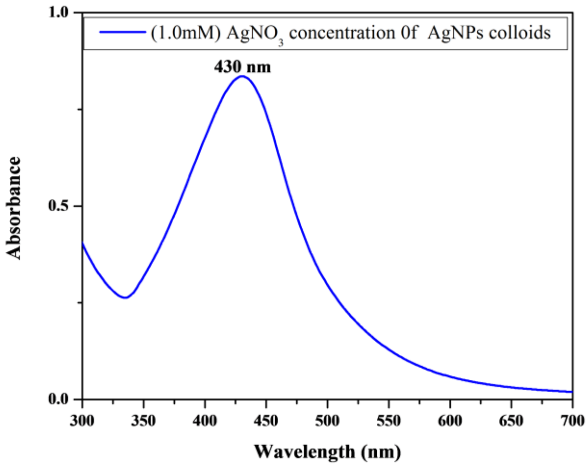

Fig-1. UV–visible absorption spectrum of synthesized colloid solution of AgNPs

KNO_3 medium. The synthesized AgNPs cyclic voltammograms a well-defined redox signal with cathodic peak at +0.55V (E_{pc}) vs Ag/AgCl electrode correspond to the electrochemical re-oxidation Ag^+ to Ag^0 during first anodic scan. The anodic current (I_{pa}) is 37µA produced by chemical reduction of Ag^+ giving rise to cathodic current along with electrochemical diffusion current (EC mechanism). Further, shift of anodic peak shifted towards more positive potential and marked decrease in peak current observed in subsequent cycles is also an indication of stabilized Ag^0 undergoing oxidation.

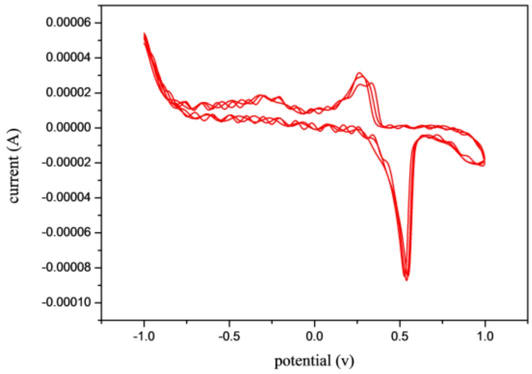

Fig-2 Cyclic voltammograms of synthesized AgNPs colloids.

FTIR spectroscopy is used to analyze the chemical structure and determine the function group of the colloidal silver nanoparticles. FTIR analysis of the AgNPs synthesized using *Amherstia nobilis* Leaf extracts are shown in Fig-3. The sharp peak FTIR spectrum 3273.262 cm-1represents O–H or N-H stretching Hbonded alcohols and phenols or amines and amides. The peak 1725.645 cm-1 assigned C=O carbonyl group, which corresponds aldehydes and carboxylic acid. The peak found 1601.763 cm-1 shows the bond for (N–H) bending, which corresponds to primary amines. Plant extracts play a dual nature role as a reducing agent and stabilizing agent. These results show that aldehydes, glycoside, phenolic, and carboxylic acids of the *Amherstia nobilis* Leaf extracts are mainly involved in the construction of AgNPs. The reduction of silver nanoparticles was accomplished due to the phenolics, terpenoids, polysaccharides and flavones compounds present in the extract. The amino acid residues and proteins compounds present in the extract were claimed to be responsible for the stabilization of nanoparticles. Possible chemical constituents of the plant extracts are responsible for the bioreduction of metal ions.

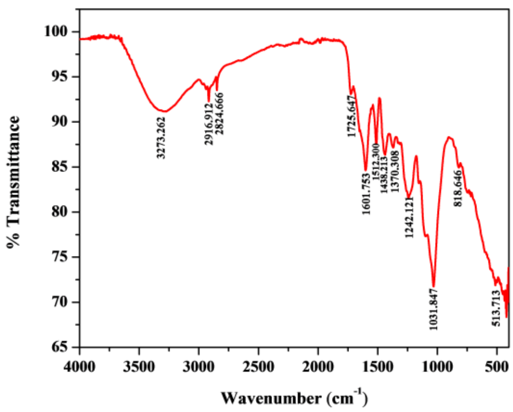

Fig-3 FTIR spectrum of AgNPs using *Amherstia nobilis* leaf extract

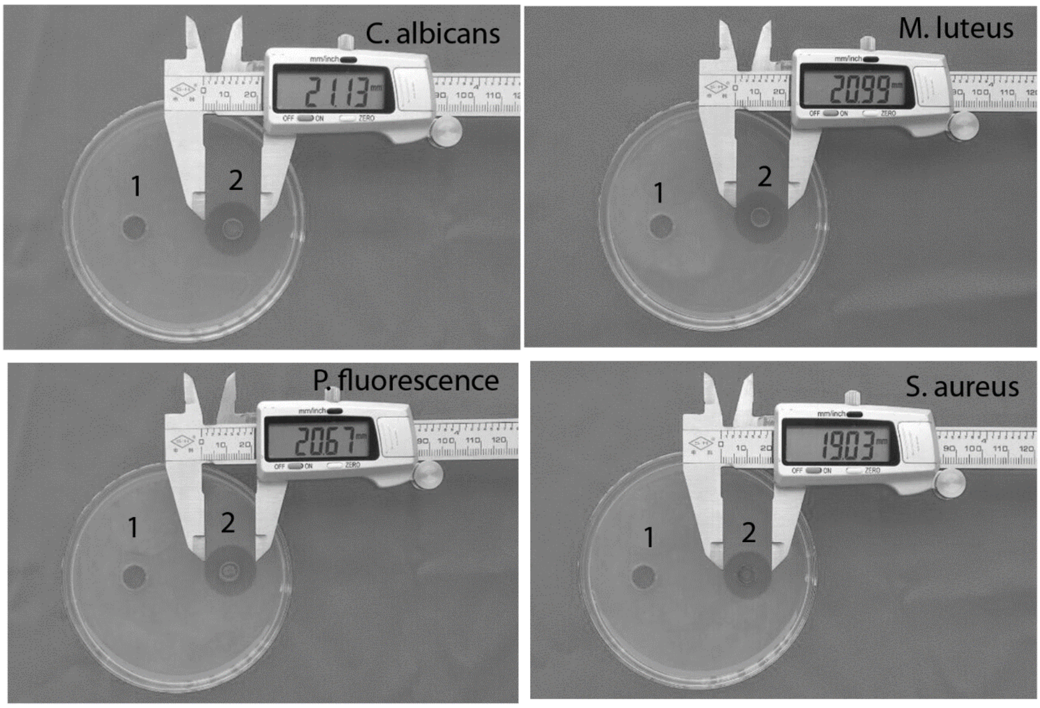

Fig-4. The antibacterial activity of Ag NPs against microorganisms of AgNPs colloids.

The agar well diffusion method was used to determine the antibacterial activity of the aqueous *Amherstia nobilis* leaf extracts and the synthesized AgNPs colloids via the bioreductive ability of the extract. In Fig- 4, the disc diffusion results of AgNPs colloids are shown, with the corresponding zone of inhibition presented in Table 1. against gram-positive (C. albicans, M. luteus, and S. aureus) and gram-negative (P. fluorescence) microbial. Regarding these results, it can be concluded that the bio-synthesized AgNPs colloids have higher antimicrobial activity than *Amherstia nobilis* leaf extract, as measured against all the cited bacteria. This may be because of the bacterial cell death due to accumulation of metallic NPs inside the cell membrane and the release of cellular compounds. Since smaller sized NPs had a larger surface area for interaction with bacteria, their antimicrobial activity was higher than that of larger sized NPs, due to large surface to volume ratio of NPs.

Table 1. The zone inhibition (mm) of AgNPs Colloids using *Amherstia nobilis* leaf extract against tested microbial.

Compound	zone inhibition (mm)			
	C. albicans	M. luteus	P. fluorescence	S. aureus
Amherstia nobilis leaf extract	16.99	16.57	16.79	15.78
AgNPs Colloids	21.13	20.99	20.67	19.03

Conclusion

Silver nanoparticles (AgNPs) colloids were successfully synthesized using the aqueous *Amherstia nobilis* leaf extract within 30 min of reaction time at room temperature. The UV-Vis spectroscopy analysis confirmed the formation of silver nanoparticles (AgNPs) colloids. The electrochemical characterization performed by using cyclic voltammetry shows significant response for change in reduction potential of Ag^+ ion from higher oxidation state to Ag^0 oxidation state. Corresponding FT-IR to the functional groups of carbonyl, amino and hydroxyl showed that aldehydes, carboxylic acids, amino acid, and phenolics-components of the leaf extract play an important role in AgNPs formation. The zone inhibition results showed that in comparison with aqueous leaf extract, the AgNPs colloids have a larger potential in terms of antimicrobial activity. The green synthesis of AgNPs colloids were eco-friendly nature and cost-effectiveness synthesizing method. The antimicrobial activity of the bio-synthesized AgNPs may suggest the use of plant extracts for the preparation of AgNPs.

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