SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *Azolla pinnata* PLANT EXTRACTS

By

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DECLARATION

I declare that this thesis entitled "Synthesis and Characterization of Silver Nanoparticles using *Azolla Pinnata* Plant Extracts" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : ________________________________

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Synthesis and Characterization of Silver Nanoparticles using *Azolla pinnata* Plant Extracts

ABSTRACT

Plant extracts have recently drawn attention in the field of nanotechnology for the synthesis of silver nanoparticles. Current nanotechnology research uses a lot of chemicals, which are potential threat to both environmental and public health. Therefore, the need to find more environmental friendly procedures for the production of nanoparticles arises. This research is done in with the purpose of synthesizing silver nanoparticles (AgNP) from *Azolla pinnata* plant extracts while studying their characterization properties. *Azolla pinnata* plants has been used because of its rich source of polyphenolic compounds used for the reduction and capping of silver nanoparticles. The present work focuses on extraction of compounds from *Azolla pinnata* plants using Soxhlet extraction method. The plant extract was mixed with 1 mM silver nitrate solution and it can be observed that the colour changed into dark brown indicated the reduction process of Ag^+ to Ag^0. The synthesized silver nanoparticles were then being characterized using UV-Vis spectrophotometer, Fourier Transform Infrared (FTIR) and SEM. UV-Vis spectrophotometer showed peak at 425 nm due to the excited plasmon surface resonance vibrations. FTIR spectra analysis reveals the functional groups presence in the silver nanoparticles. SEM study showed the morphological structure of the synthesized silver nanoparticles which were irregular spherical shape with average size of less than 80 nm. As a result of the research, it is determined that local plants of *Azolla pinnata* is an alternative to a safer, more eco-friendly alternative of synthesizing silver nanoparticles.

Sintesis dan Pencirian Nanozarah Argentum menggunakan Ekstrak Tumbuhan *Azolla pinnata*

ABSTRAK

Kebelakangan ini, ekstrak tumbuhan telah menarik perhatian ramai pihak dalam bidang nanoteknologi untuk menghasilkan nanozarah argentum. Kajian nanoteknologi terkini menggunakan terlalu banyak bahan kimia yang menjadi ancaman terhadap alam sekitar dan kesihatan awam. Oleh itu, timbulnya keperluan untuk mencari alternatif bagi presedur menghasilkan nanozarah. Kajian ini dijalankan bertujuan untuk mengsintesis nanozarah argentum (Ag) dari tumbuhan Azolla pinnata serta mengkaji pencirian nanozarah yang terhasil. Azolla pinnata telah digunakan berdasarkan sifatnya yang kaya dengan kandungan polifenol yang boleh menjadi agen pengurangan lantas menjadikan proses ini mesra alam. dari Ag^{+} ke Ag^{0} . Oleh itu, kajian ini bertujuan untuk mengsintesis nanozarah argentum dengan menggunakan ekstrak *Azolla pinnata* dan mencirikan nanozarah yang telah diperolehi. Kajian ini memfokus kepada pengekstrakan sebatian dari *Azolla pinnata* menggunakan kaedah pengekstrakan Soxhlet. Melalui kaedah ini, ekstrak tumbuhan dicampur bersama 1 mM larutan nitrat perak dan hasilnya boleh diperhatikan bahawa warna berubah menjadi coklat gelap yang menunjukkan pengurangan Ag^{+} ke Ag^{0}. Nanozarah argentum yang terhasil kemudiannya dicirikan menggunakan UV-Vis spektrofotometer, FTIR dan SEM. Spektrofotometer UV-Vis menunjukkan puncak pada 425 nm disebabkan resonans permukaan plasmon yang teruja. Spektrum FTIR mengesahkan kehadiran kumpulan berfungsi dalam nanozarah argentum. SEM menunjukkan struktur morfologi nanozarah argentum yang disintesis dalam bentuk sfera yang tidak teratur dengan saiz purata kurang daripada 80 nm. Secara keseluruhannya, telah terbukti bahawa tumbuhan tempatan seperti tumbuhan *Azolla pinnata* boleh menjadi alternatif kepada kaedah mengsintesis nanozarah argentum yang lebih selamat dan mesra alam.

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LIST OF ABBREVIATIONS

AgNP	Silver Nanoparticles
UV-Vis	Ultraviolet Visible Spectrophotometer
FTIR	Fourier Transform Infrared Spectroscopy
SEM	Scanning Electron Microscopy
Ag	Silver
Au	Gold
Pt	Platinum
Pd	Palladium
NP	nanoparticles
kg	kilogram
g	gram
nm	Nanometer
mM	milimolar
mL	millilitre
NM	nano-materials
NH_2	amide
NH	ammonia

LIST OF SYMBOLS

±	Plus or minus
°C	Degree Celcius

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The field of nanotechnology is one of the most rapidly growing research areas as it has potential applications in the field of information and communication technologies, biotechnology and medicine, cosmetics, environmental health catalysis and optics. It deals with the study of extremely minute structures and the prefix "nano" is a Greek word which means "dwarf or miniature".

The nanoparticles (NPs) have a high surface area to volume ratio. Due to that, they are often able to react very quickly and efficiently. This can be utilised in areas where high surface area are critical for success. Nanoparticles exhibit new or improved properties based on specific characteristics such as size, distribution, and morphology. They have received a particular attention for their positive impact in improving many sectors of economy, including consumer products, pharmaceutics, cosmetics, energy and agriculture and are being increasingly produced for a wide range of new applications within the industry.

Nanoparticles are created in the dimension of 1-100 nm (Abou El-Nour *et al.*, 2010) and this is equal to the size of proteins in human body. The uniqueness of the structural characteristics generally brings out new physical and chemical properties compared to their bulk counterparts. Additionally, nanoparticles have also been an important element in the production of anti-reflective optical coatings. Recent agriculture and medicine also have been applying nanoparticles in multiple ways. Having tonnes of unique properties and advantages compared to its bulk material, it is believed that it is just a matter of time until more of these properties will be exploited.

Amongst all types of NPs, metallic nanoparticles are conceived to be most tempting for the researcher. Nowadays, nanoparticles of noble metals such as silver (Ag), gold (Au) and platinum (Pt) have been widely applied in manufacturing of products which focusing on human skin. Thus, there is an increase demands in developing environmentally friendly process of synthesizing nanoparticles. In that case, the growing need of nanoparticles without any interference of toxic substances is very high especially in medical industry (Sundaravadivelan *et al.*, 2013).

Among the other metal nanoparticles, silver nanoparticles (AgNPs) are the most commercialized because of its antibacterial, antifungal, larvicidal and anti-parasitic characteristics (Chanda, 2013). Silver has been not only proven as an effective tool for retarding and preventing the bacterial infections but also they are found to exhibit wound healing activity (Vittal, R. R., & Aswathanarayan, J. B., 2011). Hence, attempts have been made to synthesis nanoparticles by using both chemical and biological methods. Synthesis of nanoparticles gets concern in nanotechnology due to the variable size, shapes, chemical composition and controlled dispersity and their potential use in the medical science for the better treatment of human benefits.

Silver nanoparticles can be synthesized by several physical, chemical and biological methods. Generally, conventional physical and chemical methods been clarify as costly and harmful (Zhang, Liu, Shen, & Gurunathan, 2016). On the other hand, according to Zhang et al., (2016) biologically synthesis of AgNPs method show high yield, solubility and stability. In addition, the process also seemed to be time efficient, simple, non-toxic and can be considered as green approaches as it did not give harm or toxic to the environment (Korbekandi *et al.*, 2014).

The potential of aquatic free-floating fern *Azolla pinnata* for the reduction of metal ions to form silver nanoparticles was examined by Korbekandi et al. (2014). It was proven that *Azolla pinnata* can be one among the good reducing agent for environmental friendly synthesis of silver nanoparticles (Korbekandi *et al.*, 2014). Besides that, there are many other reviews on the use of *Azolla pinnata* such as in the field of agriculture which have been stated that this plant is commonly used as bio-fertiliser (Kannaiyan *et al.,* 2005), livestock feed (Kamalasanana *et al.,* 2002), weed control (Edwards *et al.*, 1975) and as water purifier (Jane *et al.*, 1989).

This research is focussing on the synthesis of silver nanoparticle by using *Azolla pinnata* extracts and to also integrate the silver nanoparticles with *Azolla pinnata* extracts. Previous literatures reveals that the plants extract contains biomolecules like flavonoids, alkaloids, proteins, phenolics, etc that can reduce the silver ions into silver nanoparticles. The *Azolla pinnata* extraction was prepared by Soxhlet extraction to yield the compound in the plants. Then, *Azolla pinnata* extract was mixed with silver nitrate ($AgNO_3$) to produce silver nanoparticles. The synthesized silver nanoparticles was then characterized by using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and ultraviolet visible spectrophotometer (UV-Vis).

1.2 Problem Statement

The quest for environmental remediation has led to the synthetic routes involving eco-friendly protocols. Green synthesis methods are gaining more awareness in the field of chemistry and chemical technologies; these cancel out high production cost, and high energy involved in the usual methods of synthesizing silver nanoparticles via the conventional techniques of using hazardous chemicals. Often, chemical synthesis methods leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in certain applications such as medical applications. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route.

The environment that we been living in today have been disturbed by a lot of contaminants and pollutions happened everywhere. So as to mitigate and not adding worse to the Earth, green synthesis method was applied in this research. This method also involves elimination of complex synthesis route, replacement of toxic reducing agent with renewable and sustainable materials. All these are environmentally friendly and are considered as alternative methods for the synthesis of nanomaterials for various applications. Due to many benefits of *Azolla pinnata* plants that have been recorded, I would like to further analyse on the contribution the *Azolla pinnata* plant extracts in the synthesis of silver nanoparticles and next might be applied in the future research for the mosquitoes larvicidal control program. Recently, people had used *Azolla pinnata* for various applications such as livestock feed, biofertiliser and can be used to control mosquitoes because a thick Azolla mat on surface of water helps to prevent breeding and adult emergence. Hence, in this research study, abundantly found *Azolla pinnata* plants have been used to utilise their biomolecules for the synthesis process of silver nanoparticles.

1.3 Objectives

The purpose of doing this research are:

1. To synthesis silver nanoparticles using *Azolla pinnata* plant extracts.
2. To characterize the synthesized silver nanoparticles using *Azolla pinnata* UV-Vis, FTIR and SEM.

1.4 Scope of Study

To achieve the objectives of this research, there are a few main scopes that the study focuses on. The characteristics of the synthesized silver nanoparticles were determined by using Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and ultraviolet visible spectrophotometer (UV-Vis).

1.5 Significance of Study

The silver nanoparticles were synthesized using a local plant (*Azolla pinnata*) because it has a high content of polyphenol. It is also easier to obtain and more cost effective. Furthermore, the biosynthesized silver nanoparticles serve well to a lot of applications like coatings for solar energy absorption and intercalation material for electrical batteries and as catalysts in chemical reactions. This research study also is in line with other people's works and papers where they use different types of plants to synthesize the silver nanoparticles to go against *Aedes aegypti* mosquitoes larvicidal. And also this research aimed to combat the dengue diseases in Malaysia while other researchers in the other country were using many other plants and also other types of nano-materials. So now, as it has been identified in Malaysia that *Azolla pinnata* is very effective because of its' biomolecules content (Ravi R. *et al.*, 2018), so this study strives to prove whether it can be applied in Malaysia. If it is applicable, the impact will be reduced by minimising the concentration used by which increase the effectiveness in only using a small amount of it. This research also integrate the process and to lesser the concentration of plant extract that commonly being used by previous research. The study also maximise the abundantly occur *Azolla pinnata* plants which contains many benefits for many different field of applications (Ravi R. *et al.*, 2018).

CHAPTER 2

LITERATURE REVIEW

2.1 History of Nanomaterials

In the modern research field, the words of nano-science, nanotechnology and nanoparticles have become a common things. Nanoparticles and nano-materials were formed in the 9^{th} century where it is being used by the Mesopotamian to generate glittering effects to pots (P. Kumar, 2011). According to Kumar (2011), the use of copper and silver in making the pots is the reason to how the ceramic have shiny effect. Following the big bang era, the history of nano-materials have begun to create a new branch of technology. There are many other nanoparticles created since then. In recent years, scientists around the world are generally very interested in utilising nanotechnology in their research.

Back when the science began, the use of nanotechnology could also be traced by the ancient artefacts. One fascinating example was the Lycurgus cup (Figure 2.1) (Sudha, Sangeetha, Vijayalakshmi, & Barhoum, 2018). Romans created the cup using glass which composed of Au-Ag alloy NPs. When light passed by the glass, it will change colour in a way that it will look green when light is reflected. Meanwhile, the cup will turned red when light pass through the cup. Another instance of NM was used by Damascans and Romans to create sword. According to Sudha et al. (2018) the sword was very impressive with exceptionally sharp edge and unbeatable strength.

Though there were many products made in those days using nanomaterials, the artisans have yet to discover the term NP.

Figure 2.1 The Lycurgus cup resides in the British Museum in London, an example for use of nanotechnology materials in ancient times. (From F. Heiligtag, M. Niederberger, The fascinating world of nanoparticle research, Mater. Today 16 (7–8) (2013) 262–271. Copyright (2013), Elsevier.)

2.2 Concept of Nanotechnology

The concept of nanotechnology was first being introduced by physicist Richard Feynman at an American Physical Society meeting at the California Institute of Technology (CalTech) on December 29, 1959. In his lecture entitled "There's Plenty of Room at the Bottom", Richard Feynman highlighted how scientist can control the individual atoms and molecules ("History of Nanotechnology," 2018). The word nanotechnology was long ago being firstly used by Japanese scientists Norio Taniguchi (1912-1999) in a 1974 paper on production technology that creates objects and features on the order of a nanometer.

Nanotechnology is expected to play a vital role in various disciplines and is becoming the most innovative scientific field. Nanotechnology mainly concerns with the synthesis of NPs of variable sizes, shapes, chemical compositions and the potential use for human benefits (Niemeyer, 2002; Whitesides *et al.*, 1991). Industries such as food, agriculture, biological, medical and textile industries have been investing and reorganizing their future in the light of nanotechnological development (Ananda *et al.*, 2015).

2.2.1 Importance of Being Nano

NPs are in great demand by the scientific community due to their fascinating properties and for their many technological applications. This interest halts from the radical property varies with the particles size reduce from macro/micro scale to the nanoscale (Caruso, 2014). An increase in the surface area per unit mass is an effect of reduced dimensions of nanocrystals which alters the physical and chemical properties of a material. Owing to high surface area to volume ratio, they have exhibit lower melting point (Buffat and Borel, 1976; Allen *et al.*, 1986) as well as high mechanical strength due to crystal defects compared to their bulk materials. (Mayers *et al.*, 2006).

2.3 Silver Nanoparticles

Silver is a chemical element with symbol Ag and carries atomic number 47. Silver which have the highest electrical and thermal conductivity of any of the metals also is a relatively soft and shiny metal. This lustrous white, malleable metallic element is prepared in various ways depending upon the nature of its occurrence but usually produced as by-product of refining of lead and copper. It occurs naturally in its native form and can be obtained in ores such as argentite.

AgNPs are unique in nanoscale system due to the ease in its synthesis and chemical modifications. Recently, the development of nanotechnology has resulted in the synthesis of nano-scale silver particles which have particular properties that are superior to the bulk silver metal (Hajra, n.d.). Among other types of metal nanoparticles, such as gold and copper, silver nanoparticles (AgNPs) are more commercialized in various kind of industries. Silver can be used in many applications due to its attractive and unique characteristics. AgNPs can be classified as a good conductor, have chemical stability and popular for its antimicrobial activity.

According to a study by Halawani (2017), silver nanoparticles loaded on band aids exhibit strong antimicrobial affects against multi drug resistant bacteria. In the experiment, silver was recorded that it can enhanced the therapeutic efficacy of the band aids. Clearly, silver nanoparticles have portrayed a special ability that can be used in wound treatment (Halawani, 2017). Figure 2.3 shows chemical structure of silver.

According to Kildeby (2005), silver nanoparticles also have found applications in catalysis, optics, bactericidal, electronics, and other areas due to their unique sized-dependent optical, electrical and magnetic properties (Zhang *et al.*, 2016). Compared to the bulk silver metal, nano scale silver particles have larger surface areas and unique physical, chemical and biological properties.

For instance, the 'Noble Silver Nanoparticles' are also striving towards utilisation in every aspect of science and technology. Hence, the increase in demands for silver nanoparticles have been a driving force for researchers around the globe to find alternative ways on synthesizing of silver nanoparticles which is not only cost effective, but should be environment friendly in parallel. Over the millennial, scientists have been synthesis the silver nanoparticles through different methods which included physical, biological and chemical synthesis (Kildeby, 2005). Unfortunately, most of the methods involved the use of hazardous chemicals or consume high energy requirement (Ahmed *et al.*, 2016).

Although the chemical method of synthesis required a short period of time for synthesis of large quantity of nanoparticles, still it provides negative impacts to the environment. Thus, there is a need to develop an environmental friendly method of synthesizing nanoparticles which are free from the use of toxic and hazardous chemicals. Researchers are now focussing on use of plants and plant parts for green synthesis of nanoparticles considering the low cost of production and simple handling as compared to chemical synthesis method (Pavithra, Ragavendran, Murugan, & Natarajan, 2017).

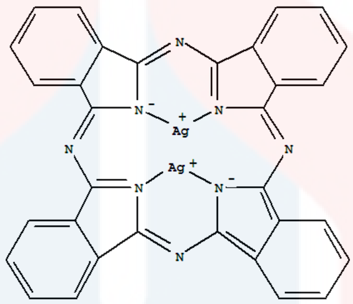

Figure 2.3 Chemical structure of silver

2.4 Synthesis of Silver Nanoparticles from Plant Extract

Over the years, nanoparticles have been synthesized without aggregation using various methods especially chemical methods due to its simplicity and the added advantage of high yield for large scale production (Pacioni *et al.*, 2015). However, chemical substances that involved in the procedures mounts a bias for an eco-friendly and feasible approach for the synthesis of nanoparticles as it may contribute to dangerous effects to the human and environment (Zainal Abidin Ali *et al.*, 2016). The chemical and physical methods are expensive as well as toxic while the biosynthesis avails a feasible alternative. Scaling up physical processes is difficult, the extremely short shelf life of the NPs produced makes addition of a capping agent indispensable and have a very low thermal stability. Chemical processes requires multiple purification, use of explosive solvents, high consumption for process and harmful effects of by-products formed during the process. Hence, plants are used as an alternative trigger for the green synthesis of nanoparticles. Biological means of synthesizing nanoparticles been reported as clean and non-toxic to environment thus it provides an edge over chemical means. Green synthesis offers many advantages as it cost effective, does not involve physical barriers with regard to reducing agents and eliminates the toxic effects of chemicals used for the synthesis (Ilyas *et al.*, 2016). Figure 2.4(a) shows the chemical structure of silver nitrate that can be used to synthesis silver nanoparticles.

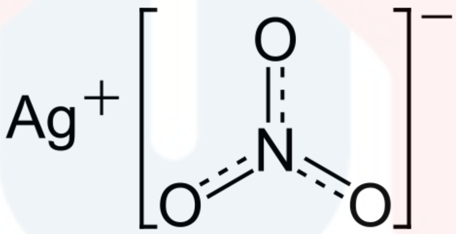

Figure 2.4(a): Chemical structure of silver nitrate

Synthesis of nanoparticles using plants also can potentially eliminate the problem of the presence of some toxic chemical species absorbed on the surface by making the nanoparticles more bio-compatible. Exploration of the plant systems as the potential nano-factories, has increased interest in the biological synthesis of nanoparticles. Previous research studies have reported various kind of plants that have been utilised for the synthesis of silver nanoparticles. Ahmed et al. (2016) reported the potential of plant leaf extracts in the biosynthesis of silver nanoparticles in the paper. The research examined the bioreduction of chloraurate ions and silver ions by extracts of germanium and neem leaf (Ahmed *et al.*, 2016). The silver ion in silver nitrate undergoes reduction to become silver molecule with formula is shown in Equation 2.1.

$$Ag^+ \rightarrow Ag^0$$

Furthermore, the aqueous extract of Bamboo leaf (*P. aurea*) has also been used by researcher to synthesize silver nanoparticles (Yasin *et al.*, 2013). Bamboo leaf contains various phytochemicals like flavonoids, phenolic acids and lactones. The flavonoids play a key role in the reduction process of metal ion for biosynthesis. Accordingly, the high content source of flavonoids and phenolic acids in bamboo extract act as the mechanism to supports the potential bio-reduction of Ag^+ to Ag^0. Figure 2.4(b) shows the chemical structure of flavonoids found in Bamboo which responsible for the reduction of metal ions.

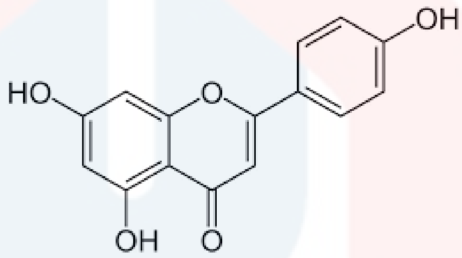

Figure 2.4(b) Chemical structure of flavonoids

In another technique, extract of oven dried leaves of *Pongamia pinnata* (L) Pierre was used for the synthesis of silver nanoparticles. The study produced a stable and crystalline silver nanoparticles by the treatment of aqueous solution of $AgNO_3$ with dried leaf extract of *Pongamia pinnata* (L) Pierre (Raut *et al.*, 2010). In a research done by Pilaquinga et al. (2018) on larvicidal activity of AgNPs that have been successfully synthesized by using extracts of *Ambrosia arborescens*. The plant extracts proved the potential to be developed as an alternative tool for the control of mosquito populations.

Other plants materials that have been studied by previous researcher included *Azima Tetracantha Lam.* leave extracts (Abirami *et al.*, 2016), *Azadirachta indica* leaf exract (Ahmed et al., 2016) and Zizyphus Spina Christi leaf extract (Halawani *et al.*, 2017). This past studies have proved in their research that plant mediated synthesis of silver nanoparticles was able to synthesis silver nanoparticles while contribution no harmful and dangerous effects to the environment. Additionally, the synthesis process occur in a very short time in room temperature (R. Kumar *et al.*, 2017).

2.5 *Azolla pinnata*

2.5.1 Taxonomy of *Azolla pinnata*

Azolla which is from kingdom Plantae can be classified into two sub-genera which are Euazolla and Rhizosperma. *Azolla pinnata* is assigned under the sub-genus of Rhizosperma along with *Azolla nilotica* (Seulthorpe, 1967 ; Lumpkin and Plucknett, 1980 ; Van Hove, 1989 ; Wagner, 1997).

Other than that, *A. pinnata* is also a free-floating water fern with average size of 1.5-2.5 cm long. It can be identified by its tiny scale-like 2-lobed leaves; lobes each 1-2 mm long; leaves green or red, and usually will give giving reddish appearance to the water surface as stated in US Federal Noxious Weed. The main stem with pinnate branches giving the plant a triangular leaves arrangement. The *A. pinnata* appears feathery due to their roots with fine lateral rootlets.

Figure 2.5.1 Physical structure of *Azolla pinnata*

2.5.2 Distribution of *Azolla pinnata*

Azolla plants commonly known as mosquito fern, water fern, water velvet and feathered mosquito fern and most of them are natives to Asia, Africa and the America (Integrated Taxonomic Information System on-line database, 2018). According to Lumpkin and Plucknett (1980), *Azolla pinnata* can be found in most of Asia and the coast of tropical Africa. These plants naturally grow in swamps, marshy ponds, ditches, lakes, paddy fields and rivers. Lumpkin and Plucknett (1980) also stated that *Azolla sp.* has high potential N_2 fixation (biomass rich in nitrogen and protein) and has high rate of multiplication (growth doubling around 2-3 days).

Azolla sp. also can cover the entire areas of water and form a thick mat on the water surface. Ravi et al. (2018) stated that extract from *Azolla pinnata* plants have the ability as bio-fertiliser for the *Pisum sativum* plant. When the aforementioned plant was soaked into the *A. pinnata* extract, the plant did not get wilt or died but somehow it grow healthier.

In another study, *A. pinnata* extracts proved that it cause no harm to the fish. The experiment on Guppy fish, *Poecilia reticulata* toxicity test with *Azolla pinnata* extracts was done by Ravi et al. (2018). Meanwhile, *Azolla pinnata* plant also contribute in aquaculture aspects. *A. pinnata* were widely used as fish feeds, as it promotes growth of fingerlings and adults. On the other hand, another field study report with *A. pinnata* as mosquito breeding control was by Pandey (2015) from Gujerat, India. It was mentioned that *A. pinnata* has an effect on the oviposition of Anopheles culicifacies and An. subpictus mosquitoes in rice fields.

2.6 Characterization of Silver Nanoparticles

The need to characterize nanoparticles in solution before assessing the compound is very important over anything else. Particle size, size distribution, particle morphology, particle composition, surface area, surface chemistry, and particle reactivity in solution are important factors which need to be defined and take into consideration to accurately assess nanoparticle toxicity (Gurunathan *et al.*, 2009).

2.6.1 UV-Visible Spectroscopy

UV-Vis spectroscopy is a very simple yet reliable technique for the primary characterization of synthesized nanoparticles which is also used as a tool to monitor the synthesis and stability of AgNPs. AgNPs have unique optical properties which make them strongly interact with specific wavelengths of light. In addition, UV-Vis spectroscopy is superfast, easy, simple, sensitive, selective for different types of NPs, and very time efficient as it needs only a short period time for measurement. (Zhang *et al.*, 2016). Past research study on the synthesis of silver nanoparticles using (Prunus persica) plant extract by (Kumar et al., 2017) also been using UV-Vis for primary characterization process. It was observed that there was a peak at 440 nm due to the excitation of surface plasmon vibrations.

Ag ions reduction was monitored by observing UV-Vis spectrum between a wavelength ranging of 200 - 700 nm as following to past research study on ‘Green Synthesis of Silver Nanoparticles using Apple Extract’ (Zainal Abidin Ali *et al.,* 2016). Some research study observed un vis spectroscopy in different range of wavelength for example in a study on rapid biosynthesis method using *Zizyphus spina*, Halawani (2017) use 350 to 700 nm on order to analyse the UV-Vis spectra of synthesized AgNPs.

2.6.2 Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is able to provide accuracy, reproducibility and also a favourable signal-to-noise ratio. FTIR spectroscopy is frequently used to find out the functional groups presence in the solution and whether biomolecules was being involved in the synthesis of silver nanoparticles (Zhang *et al.,* 2016). Furthermore, the study of FTIR has also been extend to the nano-scaled materials, such as confirmation of functional molecules covalently grafted onto silver, gold nanoparticles, or interactions occurring between enzyme and substrate during the catalytic process. Recently, further advancement has been made in FTIR method. Therefore, FTIR is suitable, valuable, non-invasive, cost effective, and simple technique to identify the role of biological molecules in the reduction of silver nitrate to silver.

In order to assess the biomolecules that responsible for the reduction of Ag^+ ions in the synthesis of silver nanoparticles, most of the research studies conducted FTIR analysis for determination of functional groups present in the AgNPs. According to a study by (Hajra, n.d), the biomolecules that involved in reduction of Ag ion clearly recorded by FTIR spectra. She observed the wavelength of 400 to 4500 cm^{-1} in the study and detect presence of aromatic group in the sample. In another study on green synthesis of plant mediated silver nanoparticles, FTIR analysis also been chosen as one of characterization tools in determination of functional groups present in synthesized AgNPs. In the study, protein group in the plant extract had been detected as the agent that responsible for reduction of silver ions into silver nanoparticles. (Veerasamy *et al.*, n.d.).

2.6.3 Scanning Electron Microscopy (SEM)

Recently the field of nano-science and nanotechnology has provided a driving force in the development of various high-resolution microscopy techniques in order to learn more about nano-materials using a beam of highly energetic electrons to probe objects on a very fine scale. Among various electron microscopy techniques, SEM is a surface imaging method, fully capable of resolving different particles sizes, size distributions, nano-materials shapes and the surface morphology of the synthesized particles at the micro and nano-scales (Zhang *et al.*, 2016). Other than that, this machine is time consuming and costly. Previous literature also stated that it also provide limited information on the distribution of size of silver nanoparticles (Bhatia, 2016).

SEM analysis is a very helpful tool in determination of nanoparticles morphology as well as examine the size of produced particles. In the field of nanotechnology, SEM have been widely used by researchers to analyse the size and morphological structure of synthesized silver nanoparticles before being assess for various kind of applications. Previous literature have recorded that with the aid of SEM, the surface morphology of synthesized silver nanoparticles using *Prunus persica* extract could be determined. The synthesized AgNPs were roughly spherical in shape and uniformly distributed yet there was agglomeration between particles (Kumar *et al.*, 2017). That research proved the function of SEM in nanoparticles characterization analysis.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Sample Collection

A total of 20 kg fresh and healthy *Azolla Pinnata* aquatic plants was collected from Kuala Krai, Kelantan, Malaysia. It is identified by the morphological structure of phyllotaxis. The fresh *Azolla pinnata* plants were then washed with chlorine free water to remove any impurities from other sources before using it for extraction process.

3.2 Preparation of Plant Extracts

Following Ravi et al. (2018), washed leaves were then leave for sun-dried (30°C ± 4°C room temperature) for 2 days. The dried leaves were then powdered mechanically into fine powder by using electrical blender and were sieved to fine powder. Figure 3.1 shows powdered *Azolla pinnata*.

Figure 3.1 Powdered *Azolla pinnata*

The preparation of plant extract was obtained by using Soxhlet extraction method (Mithraja *et al.*, 2011) with a few changes. According to a study by Ravi et al. (2018), Soxhlet extraction method was very effective compared to other extraction methods because it yield high percentage of biomolecules compound from *Azolla pinnata* plants which give benefit to future use especially for mosquitoes control program. Following recent research study, (Ravi *et al.*, 2018), Soxhlet extraction apparatus (Favorit, Malaysia), 35 g of *Azolla Pinnata* was packed in an extraction thimble. Some cotton wool was placed on the top of thimble to prevent overflowing of sample onto other part of apparatus. Then, the extraction solvent (300 ml of methanol) was added into a round bottom flask. Heat was supplied underneath the round bottom flask via mantle.

The dried and powdered *Azolla Pinnata* (35 g) was extracted with methanol (300 ml) for 8 hours at a temperature not exceeding the boiling point of the solvent. The extraction process was considered finished until the solvent in the siphon arm was observed to become clear. The clear colour of solvent indicate that the plant sample was extracted entirely (Ravi *et al.*, 2018) After that, the extracts were collected by filtration using Whatman filter paper. Once the process has finished, the methanol was evaporated using a rotary evaporator, leaving a small yield of extracted plant material (50 ml) in the glass bottom flask. The obtained extract was weighted and stored in vials at 40^0C for further experimental studies. Figure 3.2 shows the setup of Soxhlet extraction process.

Figure 3.2 Soxhlet extraction setup

3.3 Preparation of Silver Nitrate

Silver nitrate was weigh (Figure 3.3) and transferred slowly into distilled water in a beaker and was stir slowly. After that, the mixture was transferred into volumetric flask and distilled water was added until the mark at volumetric flask.

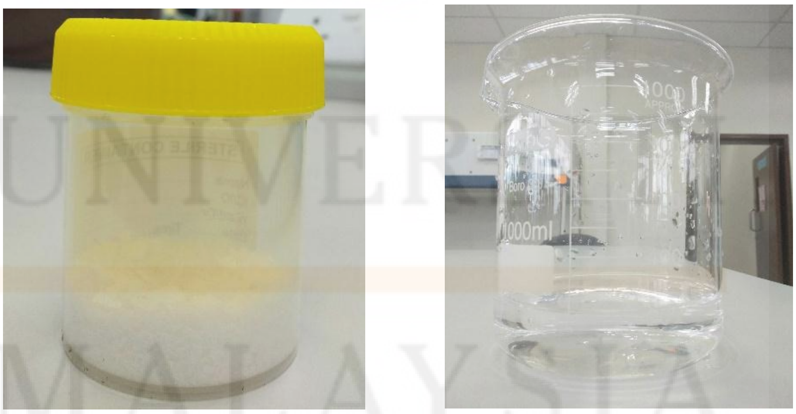

Figure 3.3 shows silver nitrate in powder form (left) and silver nitrate solution (right).

3.4 Synthesis of Silver Nanoparticles from Plant Extracts

The *Azolla pinnata* extract (10 mL) and prepared 1 mM $AgNO_3$ (90 mL) solution were mixed at the ratio of 1:9 and was kept in dark condition for 3 hours. The colour of $AgNO_3$ turned from colourless into brownish colour after few minutes of incubation and turned deep with time to indicate the formation of silver nanoparticles.

3.5 Characterization of Synthesized Silver Nanoparticles

3.5.1 Visual observation and UV-Vis Spectrophotometer

The reduction of silver ions was monitored by visual inspection of the solution as well as by measuring the UV-Vis spectra of the solution. The UV-Vis absorption spectrum was recorded on a HACH DR 6000 UV Vis Spectrophotometer operate at a resolution of 1 *nm*. A small amount of AgNPs sample was diluted in distilled water. Ag ions reduction was monitored by observing UV-Vis spectrum between a wavelength ranging of 200 - 700 *nm* as following to past research study on 'Green Synthesis of Silver Nanoparticles using Apple Extract' (Zainal Abidin Ali Shamala Devi Sekaran, and R. Puteh, 2016).

3.5.2 Fourier Transform Infrared

Next, the functional groups presence in AgNPs was determined using FTIR spectrometry (Hu et al., 2012). According to Sundaravadivelan et al. (2013), AgNPs will be subjected to analyse the presence of possible functional groups for the reduction of Ag+ ions resulting in the formation of AgNPs. Fourier Transmission Infra-Red (FTIR) spectroscopic analysis was carried out in the range of 400 – 4000 cm^{-1}. According to Faez Kadhim et al. (2016) scanning electron microscopy (SEM) was performed to study the shape and size of the synthesized AgNP, thin films of the silver nanoparticles were mounted on a metallic stub and an ultrathin coating of gold was deposited by low vacuum sputter coating.

3.5.3 Scanning Electron Microscopy

The SEM technique produced high resolution images of the sample surface. The deliberation of particle size and microstructure was done by high resolution SEM in UMK Kampus Jeli (model: JSM-IT100). During the process of SEM characterization, solution form of silver nanoparticles was first to be converted into dry and powder form. Then, the dry powder was mounted on a sample holder. The whole sample was analysed by scanning with a focus fine beam of electrons. The image was taken for further analysis.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Visual Observation

Figure 4.1 shows a beaker of synthesized silver nanoparticles obtain after a mixture of *Azolla pinnata* extract and 1 mM of $AgNO_3$ solution. The green colour of the *Azolla pinnata* extract can clearly be observed in Figure 4.1(A). Silver nitrate solution (1 mM) was added into *Azolla pinnata* extract by following ratio of 1: 9. A picture of the solution after 24 hours is shown in figure 4.1(B). It can be observed that the green colour of reaction mixture (silver nitrate + aqueous plant extract) was changed to the dark brownish colour after 30 minutes of reaction. The appearance of a brownish colour in the Figure 4.1.B is a clear indication of presence of silver nanoparticles in the reaction mixture.

The changing colour of the solution was due to the excitation of surface plasmon vibrations in the silver nanoparticles (Vahabi, Mansoori, & Karimi, 2011) and also indicate the reduction of Ag^+ ions to Ag^0 ions as according to (Yasin *et al.*, 2013). The bio-reduction of silver ions to silver caused changing of colour of the solution as due to the active ingredient present in the plant extract such as alcohols, phenol and protein (Abirami *et al.*, 2016).

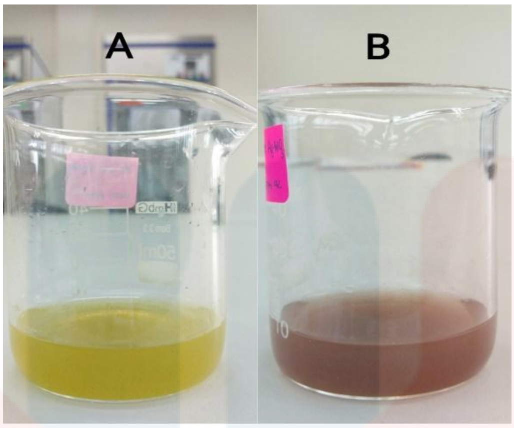

Figure 4.1: Pictures of beakers containing aqueous plant extract (A) and silver nanoparticles synthesized from Azolla pinnata extract (B).

4.2 UV-Vis Spectroscopy

Next, the reaction process was followed up by using UV-Vis spectrophotometer by analysing the excitation which is due to applied electromagnetic field of surface plasmon resonance (SPR). It is generally recognized that UV-visible spectroscopy could be used to examine size and shape controlled nanoparticles in aqueous suspension. Here, the plant extract of *Azolla pinnata* had changed the colour of silver nitrate solution from transparent to dark brown due to the reduction of Ag ions to AgNPs in within half an hour of the commencement of the reaction. These colour change arise because of the excitation of surface plasmon vibrations with the silver nanoparticles (Zhang et al., 2016). The surface plasmon resonance (SPR) peak centred near 425 nm affirmed the reduction of Ag^+ to Ag^0. In particular, the absorbance range for presence of silver nanoparticles is between 420 to 450 nm (Vahabi et al., 2011). UV- visible absorbance of reaction mixture was taken after 30 minutes of the reaction commencement which further remained constant.

UV-visible absorbance of *Azolla pinnata* plant extract also showed absorbance near 240 nm and 320 nm indicating the presence of proteins and phenols in the extract respectively (Figure 4.2). Absorption peak at around 320 nm shown in Figure 4.2

disappeared during the reaction which indicates the involvement and role of phenols in the reaction.

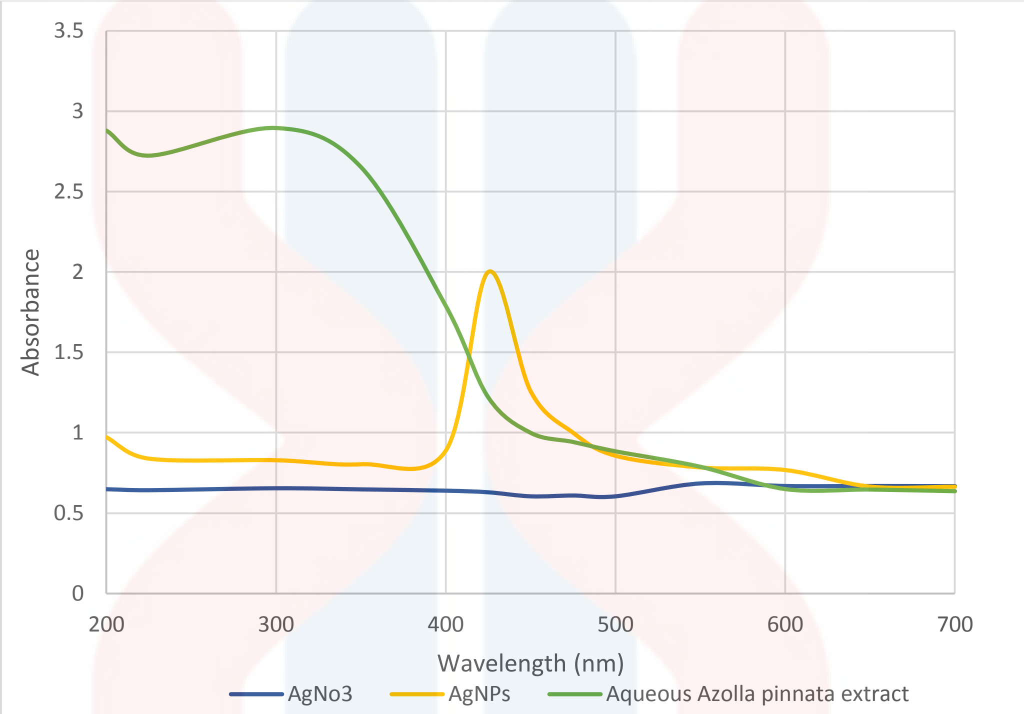

Figure 4.2 shows UV Vis spectra recorded for synthesis of silver nanoparticles using *Azolla pinnata* extract.

4.3 FTIR Spectrum

The FTIR measurements of biosynthesized silver nanoparticles were carried out to identify the possible interaction between protein and silver nanoparticles. Results of FTIR study showed absorption peaks located at about 1636 and 3395 cm^{-1} (Fig. 4.3). Absorption peak at 1636 cm^{-1} correspond to the amide I bond of proteins arising due to carbonyl stretch in proteins, and the stronger peaks at 3395 cm^{-1} are assigned to OH stretching in alcohols and phenolic compounds (Sundaravadivelan *et al.*, 2013). This groups were involved in the reduction of Ag+ to Ag^0 and stabilization of formed silver nanoparticles (Raut *et al.*, 2010). The band at 3395 cm^{-1} might also be contributed by the N-H stretching and bonded O-H groups of carboxylic acids.

In addition, the absorption peak at 1636 cm^{-1} is close to that reported for native proteins (Jyoti, Baunthiyal, & Singh, 2015). This absorbance indicate predominant surface capping species having –C=O which responsible for stabilization of silver nanoparticles. The C=O also relate to the backbone conformation. This evidence suggest that proteins are interacting with biosynthesized nanoparticles and also their secondary structure was not affected during reaction with Ag+ ions or after binding with Ag nanoparticles. These IR spectroscopic studies confirmed that carbonyl group of amino acid residues have strong binding ability with metal suggesting the formation of layer covering metal nanoparticles and acting as capping agent to prevent agglomeration and providing stability to the medium. These results confirm the presence of phenols and proteins which may act as reducing and stabilizing agents for silver nanoparticles.

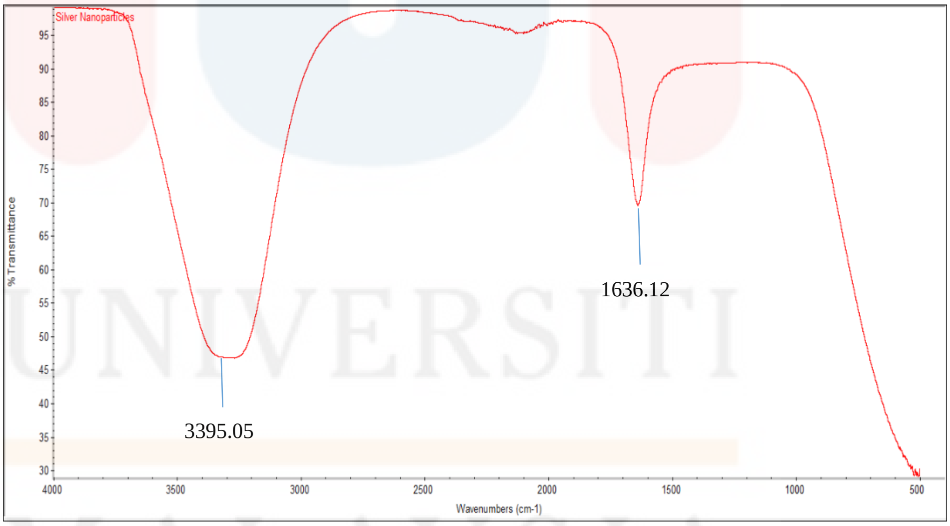

Figure 4.3: FTIR spectrum for synthesized silver nanoparticles using *Azolla pinnata* extract

4.4 Scanning Electron Microscopy (SEM)

A SEM micrograph of the dried sample of biosynthesized silver nanoparticles was presented in Figure 4.4, which shows the silver nanoparticles structure and morphology.

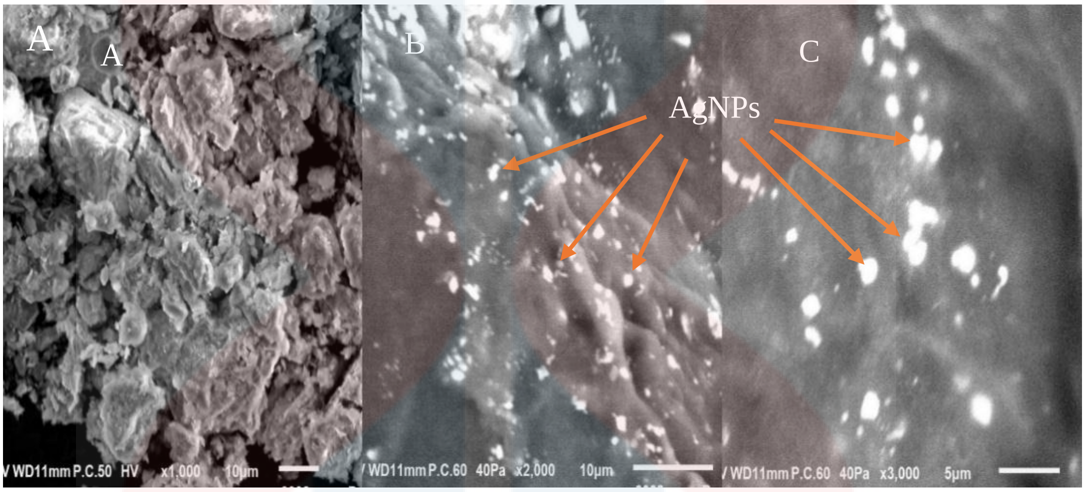

Figure 4.4: SEM photograph of the synthesized AgNPs by Azolla pinnata with magnifications of 1000× (A), 2000× (B), and 3000× (C)

The morphology of the synthesized silver nanoparticles are almost spherical. It can be seen that SEM showed image of AgNPs with different magnification which were 1000×, 2000× and 3000× respectively. The shape was spherical with agglomeration. The silver nanoparticles have been analysed that particles with average diameter of 80 nm were synthesized (not shown in picture).

Around the examine area in the first picture, one can notice the presence of objects clotted in a place. Those objects consist of tiny particles surround them. The above image (Figure 4.4) shows the results of three different magnifications. However, the structure of the observed nanoparticles was quite hard to be examined because of difficulties connected with getting higher magnification. The problem was caused by washing out the details on SEM picture which appeared as a result of sample charging. The charging evidence low electric conductivity of the medium. It should be mentioned that the examined silver nanoparticles are surrounded by a non-conducting carbon stabilizer. In my opinion, the possible reason of difficulties connected with getting higher magnification was high susceptibility of nanoparticles to aggregate into larger conglomerates.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

In this research, an approach been made in order to achieve eco-friendly way for the synthesis of silver nanoparticles using *Azolla pinnata* extract. The silver ions in aqueous solution of silver nitrate was exposed to the plant extract for a reduction process to take place. The biosynthesis of AgNPs was confirmed by the colour change of the plant extracts due to rise of surface plasmon resonance. The change of colour from colourless silver nitrate to brown colour indicate the presence of silver nanoparticles in the solution. The further characterization of synthesized silver nanoparticles was carried out by using UV-Vis spectrophotometer, Fourier Transform Infrared and also SEM. UV-Vis spectrum reveals absorbance peak at 425 nm. Phenols and flavonoids were present in the plant extract and they served as the reducing agent for synthesis of AgNPs.

SEM result also revealed the morphological structure of synthesized silver nanoparticles which was irregular sphere with size less than 80 nm. Synthesis also was found to be efficient in term of reaction time. This study proved *Azolla pinnata* extract potential as reducing agent in synthesizing silver nanoparticles. Additionally, this method provide an edge in sustainable way of utilization of plant biomolecules towards eco-friendly synthesis process. Thus, this study also have the potential to be integrate into future mosquito control program.

5.2 Recommendations

Throughout conducting this research, there are some limitations that had been identified and need to be improve in the future. Some improvements that can be done are firstly, the Soxhlet extraction method of obtaining compounds from the plant materials can be done with the use of different solvent instead of methanol which is water. The use of methanol as solvent for extraction to some extent effect the composition of silver nanoparticles produced. The use of water also could minimise the use of chemical and more towards achieving green synthesis process. Water which act as solvent also would be less hazardous to the environment and human mankind. Future research could compare the effectiveness of different solvent used in the process on extraction products. Perhaps in term of duration of extraction take place, or the size of silver nanoparticles being synthesized.

Secondly, the choosing of extraction method which is by Soxhlet extraction method. In the future, it is recommended to use maceration method instead. Though the yield of extraction is higher by Soxhlet, but maceration method is also have advantages in term of fast and low energy consuming. Maceration method also use simple setup and materials for the extraction process to occur. So, it is more convenient compared to Soxhlet extraction method.

Thirdly, it is recommended that the whole process of synthesizing silver nanoparticles would be done in a short period of time. This is because, the plant extract could not be kept for too long as it will affect its biomolecule compound. The extraction products also recommended to be produced in crude form to make sure that all the solvent are being totally remove from the plant extract. In the present work, the plant extract was only been evaporated by using rotary evaporator until it yield 50 ml.

This had gave impact on the FTIR analysis which showed the presence of alcohol in the synthesized AgNPs.

Lastly, the characterization are recommended to be further analysed by using TEM, XRD and chromatography testing on the *Azolla pinnata* extract and also synthesized silver nanoparticles. The characterization of many different machine will give more accurate result on the morphology and characteristics of the AgNPs. Thus, can be further integrate the use of *Azolla pinnata* in other field of study not only for the larvicidal but also give benefits to the human.

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APPENDICES

APPENDIX A: Collection of *Azolla pinnata* plant at Kuala Krai, Kelantan, Malaysia.

Figure D1: Collect *A. pinnata*.

Figure D2: *A. pinnata* grow in a tank.

Figure D3: Phyllotaxis and pinnately structure of *A. pinnata*

APPENDIX B: Preparation of *A. pinnata* extract.

Figure B1: Washing of plant leaves

Figure B2: Leave for air-dried

Figure B3: Dried *A. pinnata* plant.

APPENDIX C: Extraction of Plant Extract

Figure C1: Filtration of extract after Soxhlet method

Figure C2: Plant Extract after rotavap until 50 ml.