BIOSYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT OF *COLUBRINA ASIATICA* AND ITS CHARACTERIZATION.

NOR EZANEY BINTI IBRAHIM

UNIVERSITI SAINS MALAYSIA

2017

BIOSYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT OF *COLUBRINA ASIATICA* AND ITS CHARACTERIZATION.

by

NOR EZANEY BINTI IBRAHIM

Thesis submitted in partial fulfilment of the requirement

for the degree of Bachelor of Chemical Engineering

June 2017

ACKNOWLEDGEMENT

In the name of Allah SWT, the most gracious and most merciful, I would like to express my gratitude to Allah SWT, as with His willing I able to complete my final year project on time. All praise belong to Him.

First and foremost, I would like to convey my sincere gratitude to my supervisor, Dr. Masrina for his precious encouragement, guidance and generous support throughout this work. Thank you for your criticism and advice, I can give better performance in this project. Thank you also to help me in improving my scientific writing and for her efforts in reviewing my thesis despite her time constraints. Not forget, special appreciation to Mrs. Siti Farhana and Mrs Siew Mun for their precious advices, guides and encouragement. They really helped me a lot in solving all the problems arise during conducting my experiments.

I would also extend my gratitude towards all my colleagues for their kindness cooperation and helping hands in guiding me carrying out the lab experiment. They are willing to sacrifice their time in guiding and helping me throughout the experiment besides sharing their valuable knowledge.

Apart from that, I would also like to thank all SCE staffs for their kindness cooperation and helping hands. Indeed their willingness in sharing ideas, knowledge and skills are deeply appreciated.

Once again, I would like to thank all the people, including those whom I might have missed out and my friends who have helped me directly or indirectly. Their contributions are very much appreciated. Thank you very much.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOL

		Unit
λ	wavelength	nm
ζ	Zeta potential	mV

LIST OF ABBREVIATIONS

Ag^{+}	Silver ions
$AgNO_3$	Silver nitrate
AgNPs	Silver nanoparticles
CdS	Cadmium sulphide
DLS	Dynamic Light Scattering
FTIR	Fourier Transform Infrared
GNP	Gold nanoparticles
hr	hour
KBr	Potassium bromide
NO_3^{-}	Nitrate ions
PbS	Lead sulphide
PE	Plant extract
SPR	Surface Plasma Resonance
TEM	Transmission Electron Microscope
UV-Vis	Ultra-violet Visible

BIO-SINTESIS ZARAH-ZARAH NANO PERAK MENGGUNAKAN EKSTRAK TUMBUHAN *COLUBRINA ASIATICA* DAN KECIRIANNYA.

ABSTRAK

Bio-sintesis zarah-zarah nano perak menggunakan ekstrak tumbuhan (ET) telah mendapat sambutan yang banyak pada kebelakangan ini, umumnya disebabkan keberadaannya yang berterusan dan pelbagai kehadiran komponen metabolit penurun. Melalui kajian ini, kami telah mengkaji kemampuan menurun ekstrak berair ET *Colubrina asiatica* dalam menghasilkan zarah-zarah nano perak. Penghasilan optimum zarah-zarah nano telah dikaji menggunakan analisis UV-Vis, Mikroskop Electron Pancaran (MEP), Spektroskopi Infrared Transformasi Fourier (SITF) dan Penyerakan Cahaya Dinamik (PCD). Pelbagai parameter yang mempengaruhi penghasilan zarah-zarah nano perak telah dikaji seperti kepekatan ET, kepekatan nitrat perak, nisbah ET kepada nisbah nitrat perak serta tempoh pengeraman. Ekstrak tumbuhan ini mampu menurunkan ion-ion perak kepada zarah-zarah nano perak dalam masa 24 jam, dibuktikan dengan perubahan warna larutan daripada kuning pucat kepada coklat kekuningan. Analisis UV-Vis menunjukkan ciri-ciri puncak permukaan resonan plasma (PRP) pada panjang gelombang 445 nm. Mikroskop Elektron Pancaran (MEP) mengesahkan bentuk sfera pada zarah-zarah nano tersebut. Spektra STIF membuktikan wujud pelbagai kumpulan berfungsi biomolekul yang memerangkap zarah-zarah nano. Purata saiz bagi zarah-zarah nano ialah 97 nm seperti yang dibuktikan oleh PCD. Kajian ini mempersembahkan siasatan terperinci tentang sifat berlainan ekstrak tumbuhan dan nitrat perak untuk menghasilkan zarah-zarah nano yang stabil secara biologi.

BIOSYNTHESIS OF SILVER NANOPARTICLES USING PLANT EXTRACT OF *COLUBRINA ASIATICA* AND ITS CHARACTERIZATION.

ABSTRACT

Biosynthesis of silver nanoparticles (AgNPs) using plant extract (PE) has gained great interest recently, mainly due to their tremendous availability and various arrays of reducing metabolites. In this study, we investigated the reduction ability of aqueous extract of *Colubrina asiatica* in order to synthesize AgNPs. Optimal synthesis of AgNPs was investigated using UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), Fourier Transform Infrared (FTIR) and Dynamic Light Scattering (DLS). Different parameters which include concentration of PE solution, concentration of silver nitrate ($AgNO_3$), ratio of PE to the $AgNO_3$ solution and incubation period that affect the synthesis of AgNPs was investigated. *Colubrina asiatica* can reduces silver ions to silver nanoparticles within 24 hr as indicated by the color changes from pale yellow to yellowish brown. The UV-Vis spectrum of AgNPs revealed a characteristic Surface Plasmon Resonance (SPR) peak at 445 nm. Transmission electron microscope (TEM) confirmed the spherical nature and the crystallinity of nanoparticles. Fourier Transform Infrared (FTIR) spectra prove that various functional groups of biomolecules capping the nanoparticles. The average size of nanoparticles was 97 nm as determined by DLS. This study presents a comprehensive investigation of the differential behavioural of PE and $AgNO_3$ to synthesize biologically stable AgNPs.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Metallic nanoparticles (NP) possess noteworthy antioxidant properties due to their large surface area to volume ratio which attract researchers due to development of microbial resistant against metal ions, antibiotics and development of resistant strains (Ikram, et al., 2016). Among all metallic nanoparticles, AgNPs has gained strong interest for its special properties of chemical stability, good conductivity, antibacterial, antifungal and anti-inflammatory characteristics which fits in various field of applications. Traditionally, silver is used to control body infection and prevent food spoilage. It is used to heal wound and treatment of ulcer. Todays, silver have been used as microbial agent, wound dressing material, bones and teeth cement and also water purifier.

Nanoparticles display various morphological structures with shapes like rods, spherical, tube, hollow sphere and platelets. Nanoparticles can be prepared generally by variety of chemical and physical methods, yet the production methods are usually expensive and using high radiation and highly concentrated reductants and stabilizing agents that are harmful to environment and human health. Thus, biological approaches are applied to fill the dots, as in applying green approaches by using biological agents such as microorganisms and PE (Kuppusamy, et al., 2016).

However, microbe mediated synthesis is not of industrial feasibility since it required high aseptic conditions and maintenance. Therefore, the use of plant, or PE are more favourable since it eliminates elaborated process of culturing and can be scale-up to large-scale nanoparticles synthesis (Ikram, et al., 2016). Plants have antioxidant or reducing properties that responsible for reduction of metal compound in their respective nanoparticles. Plant extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids that is responsible for the reductions of ionic nanoparticles into bulk metallic nanoparticles formation. This metabolites constantly involved in the redox reduction to synthesize eco-friendly nanoparticles. (Kuppusamy, et al, 2016).

The present study focuses on synthesis of AgNPs using leaves extract of *Colubrina asiatica* and its characterization. *Colubrina asiatica* (Figure 1.1) is known as lather bush or in Malaysia is called peria pantai/laut and belongs to family of Rhamnaceae. This plant is evergreen shrub that most commonly adopts a scrambling or climbing habit with stems that can be 6 metres or more long and 6 cm in diameter. The PE contains alkaloids, flavonoids, unsaturated sterols and triterpenes, steroid glycosides, anthraquinones, saponins, tannins and phenols. The leaves contain two saponins and glycosides. This plant is believed to have the potential to control blood pressure and cure diabetes (Fern, 2017).

Other benefits of the leaves of *Colubrina asiatica* includes the ability to produce foam in water and thus, have long been used as a substitute for soap in the rural areas in some countries. Leaves and fruits are used as fish poison. In the Maldives, leaves are used to alleviate inflammations and boils. In order to alleviate painful swellings, leaves are crushed and juice is rubbed on the affected body. Young stems are cut into pieces and boiled in water, which is drunk to alleviate stomach disorders. Medicinal oil is prepared from seeds along with other ingredients, which is used to treat rheumatism and numbness in adults and also in treating weak legs in children (Fao, 2016).

Figure 1.1 Leaf of *Colubrina asiatica.*

1.2 Problem statement

Silver nanoparticles can be prepared using various methods. There are physical, chemical and biological method. Physical and chemical methods are not eco-friendly since they use high radiation and concentrated reductants and stabilizing agents that give harmful effect to environment and human health. Thus, the greener alternative is by using biological method. Biological method to synthesize AgNPs is a single step bio-reduction method that utilizes less energy (Kuppusamy, et al., 2016). The advantages of green synthesis over chemical and physical methods is it being environmentally friendly, cost-effective and boundless availability of raw materials and also no need to use high temperature, pressure and, energy and toxic chemicals (Dhuper et al., 2012).

Biological method comprises of using microorganism including bacteria, fungi and plant. This is due to their antioxidant and reducing properties that play crucial role for the reduction of metal compounds. However, among various biological methods, microbe mediated synthesis are not industry feasible mainly due to the requirements of highly aseptic environments and their maintenance (Kalishwaralal et al., 2010). Hence the uses of plant for this purpose are applicable due to the ease of improvement and greener synthesize method.

This study is focusing on synthesize of AgNPs using PE of *Colubrina asiatica* and its characterization. *Colubrina asiatica* leaves are alterative and cooling. This PE contain alkaloids, flavonoids, unsaturated sterols and triterpenes, steroid glycosides, anthraquinones, saponins, tannins and phenols. Especially, the leaves contain two saponins and glycosides. Till to date, there is no report of synthesis of AgNPs utilizing an aqueous leaf extracts of *Colubrina asiatica*.

Other than that, this research aim to determine the characteristics of AgNPs using this plant. The characteristics includes the shape, size and morphology, charges surrounding the particles and phytochemical components in PE.

1.3 Objectives

The aim of this research is to investigate the biological synthesis AgNPs using PE of *Colubrina asiatica* leaf extract, the characterization of the synthesized nanoparticles. This study is conducted according to the following specific objectives:

i. To synthesize the AgNPs using the extract of leaves of *Colubrina asiatica*.

ii. To investigate the effect of PE concentration, ratio of PE to $AgNO_3$ solution, concentration of $AgNO_3$ and incubation period.

iii. To characterize the synthesized AgNPs based on shape, size and morphology, charges surrounding particles, and phytochemical components exist in PE using different formulation.

1.4 Scope of study

In this experiment, PE is utilized to reduce silver ions to AgNPs. The preparation of AgNPs was done via biological synthesis by mixing the PE solution to $AgNO_3$. The different parameters were experimented such as concentration of PE, ratio of PE to $AgNO_3$, concentration of $AgNO_3$ and incubation period to determine the parameters that gave better performance. The performance was studied by carry out UV-Vis analysis. The synthesized AgNPs was characterized in terms of absorption spectra, particles size, shape and morphology, surface charges and capping biomolecules surrounding the particles.

1.5 Organization of thesis

The following are the contents for each chapter in this study.

Chapter 1 introduces the synthesis of AgNPs using PE of *Colubrina asiatica*, problem statement, research objectives and organization of thesis.

Chapter 2 discusses the literature review of this study which includes metal and its application, types of AgNPs synthesis, factors influencing the synthesis of AgNPs and antimicrobial properties of AgNPs and its mechanism.

Chapter 3 covers the materials and details of methodology. It discusses on the description of equipment and materials used, experimental procedure and description of factors affecting the reduction of silver ions process.

Chapter 4 refers to the experimental results and discussion of the data obtained. Further elaboration on the effect of different parameters on performance of synthesized AgNPs is provided in this chapter.

Chapter 5 concludes all the findings obtained in this study. Recommendations are also included as well.

CHAPTER TWO

LITERATURE REVIEW

2.1 Metal and its application

The metallic nanoparticles possess great antioxidant properties due to their large surface area to volume ratio, (Kuppusamy, et al., 2013) which led the great interest from researchers due to the development of microbial resistant strains. The common metallic nanoparticles comprise of silver, gold, palladium, iron, platinum, copper, zinc oxide and selenium. The most studied metallic NP in literature are gold and silver. Figure 2.1 shows the metal nanoparticles and their applications. In the past, gold not only known as symbol of power and wealth, but it was also used to improve health. Silver is used traditionally to control body infection and prevent food spoilage and also used as wound healing agents and ulcer treatment (Singth et al., 2015). The large surface area to volume ratio possessed by metallic nanoparticles exhibits impressive antibacterial properties, lead to growing microbial resistances against metal ions, antibiotics and the advancement of resistant strains (Kuppusamy, et al., 2013).

Among all the metallic nanoparticles, AgNPs garner a lot of attention due to its unique properties such as chemical stability, good conductivity, antibacterial, antifungal and anti-inflammatory properties that can be assimilated into various products such as composite fibres, cryogenic superconducting materials, cosmetic products, food industry and electronic components.

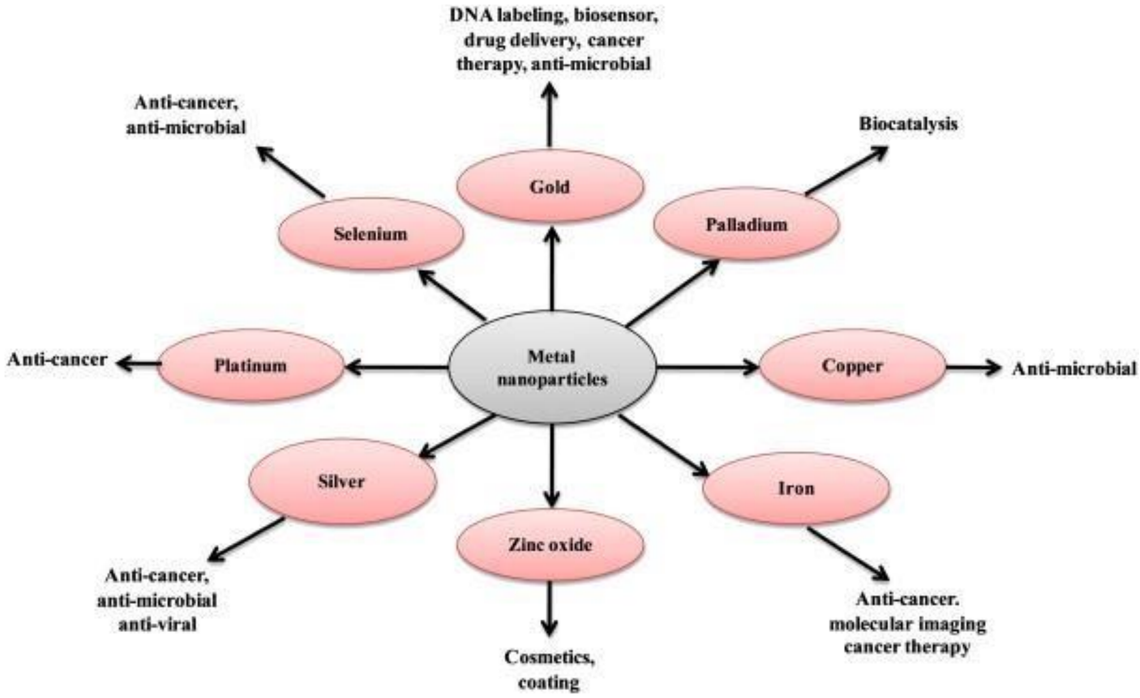

Figure 2.1 Types of metal nanoparticles and their applications in biotechnology. (Mittala, et al., 2013).

2.2 Types of silver nanoparticles synthesis

Various methods had been used in order to synthesis nanoparticles. The common ones are physical, chemical and biological methods. There are two alternative approaches for synthesis of metallic nanoparticles which are the bottom up and the top-down approachs.

Bottom-up, or self-assembly, refers to the construction of a structure atom-by-atom, molecule-by-molecule, or cluster-by-cluster (Quang et al., 2013;Iravani et al., 2014). In this approach, initially the nanostructured building blocks as in nanoparticles are formed and, subsequently, assembled into the final material using chemical or biological procedures for synthesis. In this approach, the most common method is chemical reduction (Quang, et al., 2013). A distinct advantage of the bottom-up approach is the large possibility in obtaining metallic nanoparticles with comparatively lesser defects and more homogeneous chemical composition. Due to toxicity of chemicals used, the idea of green route to synthesis silver NP is established.

In the top-down approach, a suitable starting material is reduced in size using physical or chemical means (Samberg et al., 2010). A distinct disadvantage of the top-down approach is the imperfection of the surface structure. Such defects in the surface structure could led to the possible impact on physical properties and surface chemistry of the metallic nanoparticles due to the high aspect ratio (Astruc and Daniel, 2004). The summary of different approaches of AgNPs is shown in Figure 2.2.

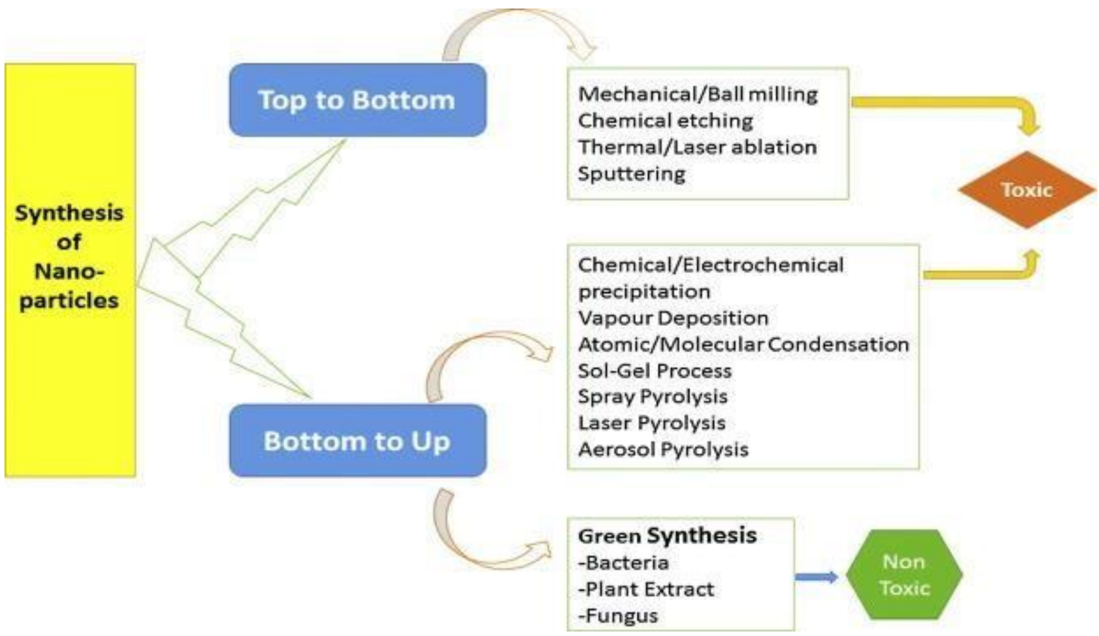

Figure 2.2 Different approaches of synthesis of silver nanoparticles (Ikram, et al., 2016).

2.2.1 Physical and chemical method

Physical methods that are commonly used include plasma arcing, ball milling thermal evaporate, spray pyrolysis, ultrathin films, pulse layer desorption, lithographic techniques, sputter deposition, layer by layer growth, molecular beam epistaxis and diffusion flame synthesis of particles. The chemical methods also includes the electrodeposition, sol-gel process, chemical solution deposition, chemical vapor deposition, soft chemical method and wet chemical method (Kuppusamy et al., 2016).

Several methods including the commonly used attrition and pyrolysis could be used for physical synthesis of metallic nanoparticles. In attrition, macroscale or microscale particles are ground by a size-reducing mechanism which is ball mill that consist of ordinary or planetary structure. The resulting particles are subsequently air classified to recover oxidized nanoparticles. The factors affecting the properties of synthesized nanoparticles are the material and time of milling as well as the atmospheric medium. In pyrolysis, an organic precursor either liquid or gas is forced through an orifice at high pressure and subsequently burned. However, synthesized nanoparticles production rate is quite low and most importantly by using physical procedures, the expense is very high. Further drawback of the physical approaches is large consumption of energy in order to maintain the high pressure and temperature used in the synthesis procedures. Since most bioprocesses occur under normal air pressure and temperature, therefore it save great number of energy consumption. (Thakkar, et al., 2009)

The traditional and most widely used methods for synthesis of metallic nanoparticles is the wet-chemical procedures. Nanoparticles is grown in a liquid medium containing various reactants in certain reducing agents such as hydrazine (Li et al., 2000), sodium borohydride (Tan et al., 2003), potassium bitartrate, glucose (Qiao et al., 2005), methoxypolyethylene glycol (Kim et al., 2007), or cetyltrimethlyammonium bromide, CTAB (Khan et al., 2011).

In order to prevent the agglomeration of metallic nanoparticles, a stabilizing agent such as sodium dodecyl benzyl sulfate or polyvinyl pyrrolidone was also added to the reaction mixture. Chemical methods are low-cost for high volume; however, the possibility of synthesized nanoparticles could be contaminated by precursor chemicals, use of toxic solvents, and generation of hazardous by-products was high. (Mallick , 2009)

2.2.2 Biological synthesis of silver nanoparticles.

Biological method mainly comprises of the uses of microorganisms such as bacteria, fungi, actinomycetes, and algae along with PE to reduce the metal ions to their respective particles.

2.2.3 Comparison of methods of metal synthesis

In order to reduce the expenses in downstream processing of the synthesized nanomaterials and to improve the application of nanoparticles, the scientific community targeted the bio-resources. Hence, biological method to synthesize AgNPs has gained a lot of interest nowadays due to their greener characteristics, affordable cost, scale up convenience and the exception of the use of high temperature, pressure, energy and toxic chemicals (Kalishwaralal, et al., 2010

2.3 Biosynthesis using microorganism

The aims for the use of microbe-mediated synthesis are the possibilities to develop clean, non-toxic, and environmental friendly method. Microorganisms aim to target ions and capture it. Nanoparticles will be biosynthesized, then metal ions will be converted to elemental metal atoms through enzymes that is generated through cell activities. The position of nanoparticles formed will determine whether it is intracellular and/or extracellular nanoparticles synthesis.

In extracellular nanoparticles synthesis, microorganism will trap ions on the surface of cells in the presence of enzyme in order to reduce it to metal atom. Meanwhile, for intracellular nanoparticles synthesis, ions will be transported into the microbial cell in order to synthesize nanoparticles in the presence of enzyme (Shankar, et al., 2016).

Marine microbes play several important roles in synthesis of nano-based drugs for human life improvement, which has potential to synthesize nanoparticles. This is for the fact that the marine microbes exist in the sea bottom, over millions of years in the past for reducing the vast amount of inorganic elements deep in the sea. Moreover, nanoparticles synthesized by microorganisms tends to be stabilized by peptides presence such as phytochelatins, thus aggregation could be prevented. The synthesis of nanoparticles using marine resources will satisfy the need for safe, stable and eco-friendly particles since, it involves varied marine ecosystem that is abundant and in this method harmful solvents are absent and downstream processing steps is reduced which would minimize the cost for their synthesis (Singh et al., 2015).

Actinomycetes are the microorganisms that carry important characteristics as fungi and prokaryotes such as bacteria. Even though they are also classified as prokaryotes, they were originally designated as ray fungi. Focus on actinomycetes has primarily targeted on their exceptional ability to produce secondary metabolites such as antibiotics. Langmuir et al. (2010) have studied the formation of monodisperse gold nanoparticles (GNP) by *Thermomonospora* sp. Shankar et al. (2016) has said that the nanoparticles formation had been influenced by the extreme biological conditions such as alkaline and slightly elevated temperature.

Phyconanotechnology has also become one of the prominent fields of research in nanoparticles synthesis. Algae are being used as a "bio-factory" for synthesis of metallic nanoparticles. Among different genre of bio-reductants, seaweeds have most garner interest due to their high metal uptake capacity, low cost and macroscopic structure. Biological units from marine resources consist of typical nanostructures such as diatoms and sponges. Several marine-derived species have been reported to synthesize different nanoparticles such as gold, silver, cadmium, silicon–germanium and lead (Shankar, et al., 2016).

Microbe mediated synthesis is impracticable in industry due to the demanding highly aseptic conditions and their maintenance. Hence, PE for the silver particles biosynthesis are beneficial in comparison to microorganisms as it is easily improved, utilize less biohazard and elaborate process of maintaining cell cultures along with absence of toxic chemicals, natural capping agents for stabilization of silver particles as well as cost-saving (Kalishwaralal et al., 2010).

2.4 Biosynthesis using plant extract

Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles. The source of the PE is known to influence the characteristics of the nanoparticles (Kumar & Yadav, 2009). This is because different extracts contain different concentrations and combinations of organic reducing agents. (Mukunthan & Balaji, 2012) Typically, a PE-mediated bio-reduction involves mixing the aqueous extract with an aqueous solution of the relevant metal salt. The reaction occurs at room temperature and is normally complete within a few minutes.

Plant biomass could be an alternative to chemical and physical methods for the synthesis of nanoparticles in a greener manner. The plant metabolites are usually employed in these synthetic methods in the form of concentrated aqueous extracts of fruits (Kumar, et al., 2017; Is, 2016; Annadurai, et al., 2013), seeds (Iqbal et al., 2016; Dhand et al., 2016; Misra et al., 2009), barks (Nayak et al., 2016; Rajeshkumar., 2016), chili peppers (Resendez, et al., 2013; Prasad and Anal, 2011) and leaves (Song and Kim, 2009) with high levels of antioxidant polyphenols. There are more reports on the plant mediated synthesis of nanoparticles due to its availability and abundance. Yet, most of these studies were reported that the nanoparticles synthesized were agglomerated and unstable due to higher concentration of phytochemicals in the extract, which plays a key role in the reduction and stabilization of nanoparticles.

Different types of metallic nanoparticles undergo different type of bio-reduction mechanisms. The similarity is they will produce their respective metallic nanoparticles along with byproducts. For the silver metal, the proposed mechanism is the reaction between silver nitrate and PE to produce AgNPs and byproducts. The proposed mechanism is stated as in Equation 1 (Raichur et al., 2010).

$$Ag^{+}NO_{3}^{-} + plant\ extract \rightarrow AgNPs + byproduct \qquad (2.1)$$

Basically, metal nanoparticles can be produced in various shapes and sizes either by using whole plant or certain parts of plant such as stem, root, fruit, seed, callus, peel, leaves, and flowers. Figure 2.4 shows the probable chemical constituents present in the PE responsible for the bio-reduction of metal ions, their growth and stabilization.

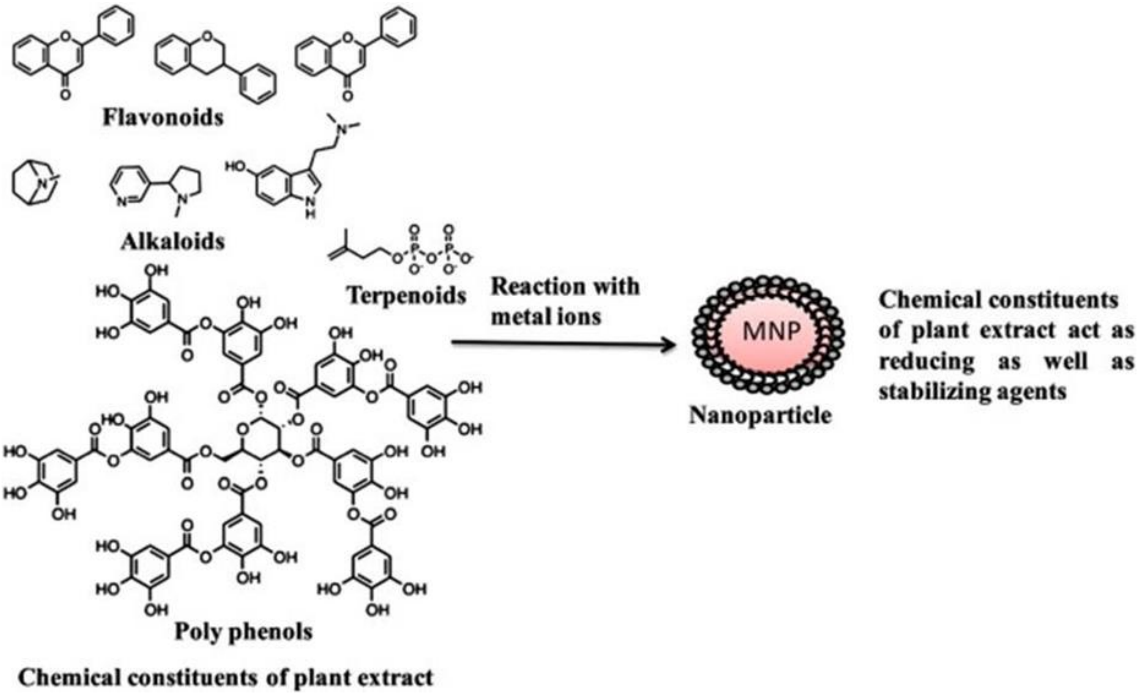

Figure 2.3 Possible chemical constituents of plant extract responsible for the bio-reduction of metal ions (Dubey, et al., 2009; Huang, et al., 2007).

2.5 Factors influencing the synthesis of silver particles.

The synthesis of AgNPs can be improved by altering the experimental parameter of AgNPs such as concentration of PE, concentration of $AgNO_3$, incubation period, pH and temperature.

2.5.1 Concentrations of plant extract and silver nitrate

Umoren et al. (2014) and Ikram et al. (2015) studied the effect of concentration of $AgNO_3$ solution and PE to optimize the stable AgNPs synthesized. The concentration of $AgNO_3$ solution were manipulated and concentration of PE were maintained in order to observe the effect of concentration of salt whereas to study the effect of concentration of PE, their concentration were varied in the form of ratio percentage by maintaining the concentration of salt of 1 µM. It is reported that the intensity of absorption peaks constantly increase with the increase concentration of $AgNO_3$ salt and concentration of respective PE. The results obtained by Ikram et al (2015) is close with the result that is reported by *Cochlospermum religiosum* extract (Anusuya and Sathiyabama., 2014) and *Pithophorace dogonia* extract (Sinha et al., 2014). A variation in the biological material and metal salt concentration is known to influence the nanoparticle synthesis.

2.5.2 Incubation period

By obtaining the optimum concentration condition of the AgNPs produced, the incubation period were observed at different time intervals such as every one hour, which was tested in the UV-vis spectra. Increase in the intensity of absorption peak was observed with constant intervals of time and also the color intensity of mixture were increase with duration of time. The increase in the absorbance along with color intensity could be ascribed to an increase in the number of AgNPs with time which is reported by Bhaisa and D’Souza (2006) and is agreed by Kuppusamy, et al. (2016).

2.5.3 pH

Ibrahim (2015) studied the effect of pH to obtain the optimum stability of AgNPs synthesized. At pH of 2.0, no colour change was observed. The highest colour intensity was observed at 4.5. The absence of colour change was agreed before by Roopan et al. (2013) by reporting that at pH 2.0 no colour change present and at pH

11.0, highly monodispersed nanoparticles were present at average size of 23±2 nm. Pimprikar et al (2009) had found that the different in the color obtained over the range of pH could be ascribed to a variation in the dissociation constants (pKa) of functional groups on the biomass that are involved which is later reported by Kuppusamy, et al., (2016).

2.5.4 Temperature

The temperature also affected the process of silver reduction. At higher incubation temperature (60, 80 and 100 °C) dark reddish brown color and more intense SPR peaks were revealed. From the research of Ibrahim (2015), at room temperature of 30°C the color change took 10 min to develop, while by heating the reaction mixtures the reduction process was faster and the reddish brown color was developed within 5 min. The maximum SPR peak intensity was detected at 100 °C. The increase in reaction temperature, UV spectra show sharp narrow peaks at lower wavelength region (412 nm at 100 °C), which indicate the formation of smaller nanoparticles, whereas, at lower reaction temperature, the peaks observed at higher wavelength regions (440 nm at 30 °C) which clearly indicates increase in AgNPs size. It is a well-known fact that when the temperature is increased, the reactants are consumed rapidly leading to the formation of smaller nanoparticles which is agreed by Park et al (2007) and Kuppusamy, et al (2016).

2.6 Antimicrobial properties of silver nanoparticles and its mechanism

Silver has been in used since long in the state of metallic silver for the treatment of burns, wounds and several bacterial infections. But use of these silver compounds has been declined remarkably due to the appearance of several antibiotics.

Metallic silver in the form of AgNPs has made an important role as a potential antimicrobial agent. The use of AgNPs have improved with diverse medical applications such as silver based dressings, silver coated medicinal devices, such as nanogels, and nanolotions (Dykman and Khlebtsov, 2012). In recent years a major challenge for the health care industry is the resistance to antimicrobial agents by pathogenic bacteria. The combined effects of AgNPs with the antibacterial activity of antibiotics have been studied by Rai et al. (2009). The report told about synthesis of silver nanoparticles by a reduction of aqueous Ag^{+} ion using the culture supernatants of *Klebsiella pneumoniae*. From the report, It shows the antibacterial activities of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin were increased in the presence of AgNPs against both test strains. The highest enhancing effects were observed for vancomycin, amoxicillin, and penicillin G against *S. aureus*.

Similarly, the AgNPs synthesized by biological method possesses great use in medical field for their efficient antimicrobial function which is efficient antimicrobial activity against pathogenic bacteria. Moreover, AgNPs plays a major role in the field of nanotechnology and nanomedicine. Shahverdi et al. (2013) had reported the antimicrobial activity of the AgNPs against *Staphlococcus aureus (S. aureus), Pseudomonas aeruginosa, Escherichia coli (E. coli)* and *Klebsiella pneumoniae*. They found that the highest antimicrobial activity for AgNPs synthesized by *Solanum tricobatum, Ocimum tenuiflorum* extracts was found against *S. aureus* (30 mm) and *E. coli* (30 mm) respectively. For Krishnaraj et al. (2010), they found that there is the presence of change in membrane permeability and respiration activity of bacterial cells treated with AgNPs.

The factor that affect the antimicrobial properties of AgNPs are size and environmental condition such as pH, size, and ionic strength, and also capping agent. To ensure that the silver exhibits the antimicrobial properties, one must make sure that it in its deionized form (Ag^{+}) as in its ionized form, silver is inert but when it come in contacts with moisture, it release silver ions (Klueh et. al., 2000).

CHAPTER 3

MATERIALS AND METHODS

3.1 List of materials and chemicals

The materials and chemicals used in this study are listed as in Table 3.1.

Table 3.1 List of materials and chemical used.

Materials/ Chemicals	Purpose
Silver nitrate`	As a precursors of silver ions.
Colubrina asiatica leaves extract	To reduce silver ions to AgNPs.

3.2 List of equipment

The list of equipment used in this study are listed as in Table 3.2.

Table 3.2 List of equipment.

Equipment/ Facility	Usage	Brand/ Model
Centrifuge	Separation of liquid solution from liquid.	Labogene Scanspeed1248R
Electronic balance scale	Weigh the mass of powder	Shimadzu
Food processor	Form leaves powder	Philips
Oven	Dry samples	Memmert 600, Germany
Refrigerator	Store sample for further use.	CoolTech
Stirring Hot Plate	Heat and stir samples.	Fisher Scientific
Dynamic Light Scattering (DLS)	Analysis (size and zeta potential of synthesized AgNPs)	Malvern Instrument, UK
Fourier Transform Infrared (FTIR)	Analysis (capping molecules in synthesized AgNPs)	Shimadzu (IR-Prestige-21)
Transmission Electron Microscope (TEM)	Analysis (size and shape of synthesized AgNPs)	Philips CM12
UV-Visible Spectrometer	Analysis (absorbance and wavelength)	Thermo Scientific Genesys-20

3.3 Overall flow of research

The preparation and characterization of AgNPs is shown in Figure 3.1.

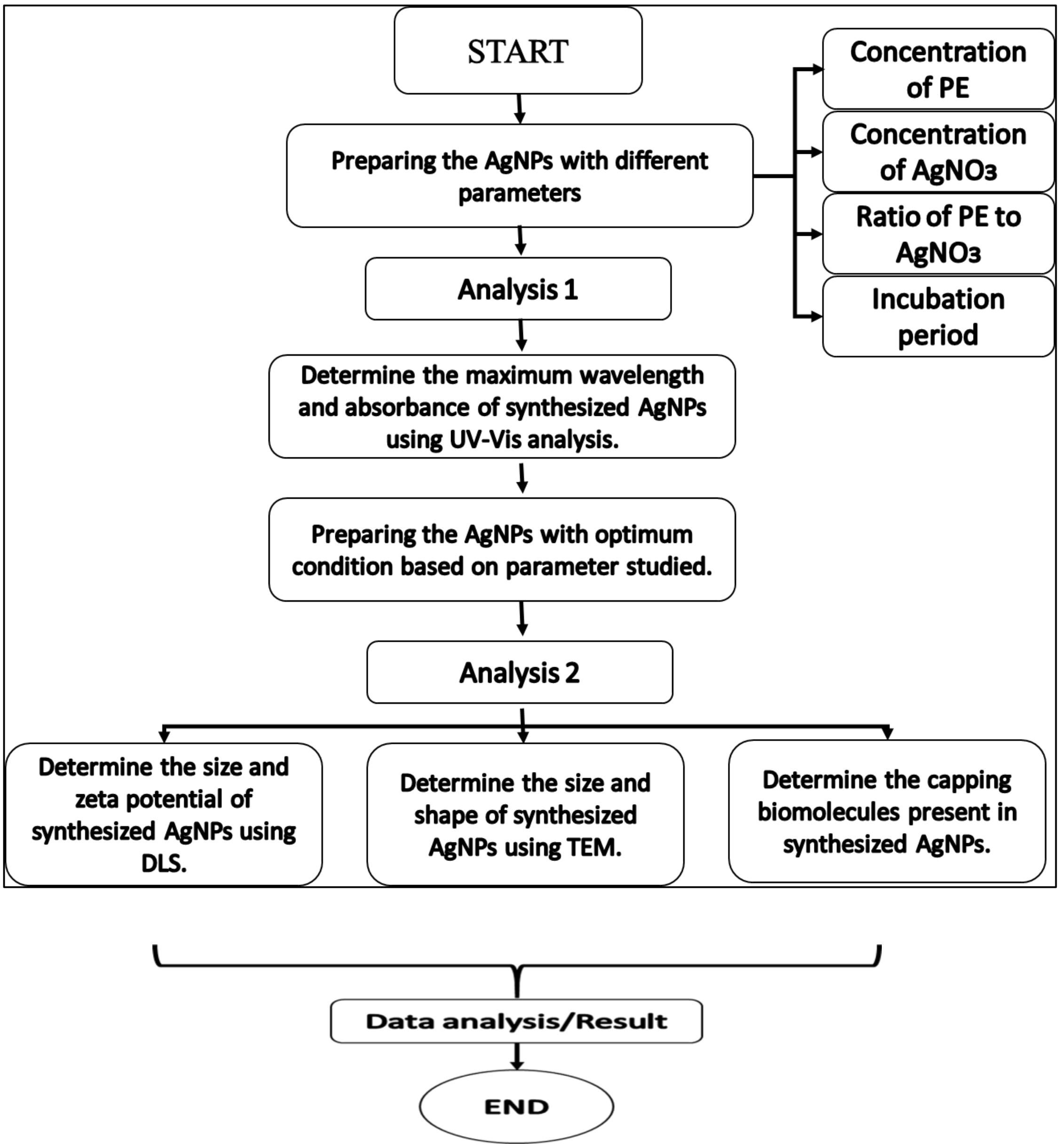

Figure 3.1 Flowchart of flow of research.

3.3 Preparation of plant extract of *Colubrina asiatica.*

The *Colubrina asiatica* leaf extract were collected from a Pantai 7 beach located in Tumpat, Kelantan, Malaysia. The leaves were checked to ensure that only the fresh one is selected. The leaves were surface cleaned with running tap water thrice to ensure all debris were removed. Leaves were then washed thoroughly with distilled water to remove any remaining dirt. The wet leaves were put into oven at 60°C to be dried. The leaves were weighted every one hour until the constant weight were achieved. This step is to ensure that the leaves were totally dry. Took out the dry leaves from the oven and kept in a place to reduce the temperature to the room temperature. After that, the leaves were blended using food processor until they became powder and were sieved with size of 20 mesh. 500 g of the leaves powder were weighted to produce PE.

To prepare 10% PE, first the 50 ml deionized water was filled into 100 ml beaker and then the beaker were placed on the hot plate. Boil the water and wait until the temperature of water reached 65°C. Later, the leaves powder were put into the boiling water and were stirred at 1200 rpm using stirrer for 30 minutes. Ensure the solution were maintained at 65°C. The solution was kept in place to reduce the temperature to the room temperature. Then, the solution was filtered first using masiline cloth. After that, it was filtered using Whatman paper no 1 and stored at 4°C

3.4 Preparation of silver nanoparticles of *Colubrina asiatica* plant extract.

0.169 g of $AgNO_3$ were dissolved in 1000 ml distilled water in a 1000 ml beaker and were stirred continuously at 1200 rpm using hot plate until it completely soluble to obtain 1 mM concentration of salt solution. The PE and $AgNO_3$ solution were mixed together in bottle using the ratio of PE to $AgNO_3$ solution of 2:298. The colour change in the mixture were observed every 2 hr and the time taken for the colour to change were noted.

3.5 Characterization of silver nanoparticles

The synthesized AgNPs was characterized in terms of absorption spectra, size, shape and morphology, surface charges and capping biomolecules surrounding the particles.

3.5.1 UV-Vis Spectrometer

Analysis of UV-Vis of AgNPs was used to evaluate the absorption spectra of AgNPs synthesized at varied concentration of salt and PE, ratio of PE to $AgNO_3$ and at different time intervals in the absorption wavelength of 300-700 nm. Based on report from Obait et al. (2015) and Ikram et al. (2016), the expected wavelength will be in the range of 400-450 mm.

To study the effect of PE concentration to synthesis of AgNPs, the PE concentration was varied with 2%, 10% and 20% PE. The other parameters which were concentration of $AgNO_3$ solution, ratio of PE to $AgNO_3$ and time were kept constant.

After the best concentration of PE was obtained, the study was continued by investigating the effect of ratio of PE to $AgNO_3$. The ratio of PE to $AgNO_3$ studied were 2:298, 3:297, 5:295, 6:294 and 8:292 while keeping the other three parameters fixed.

Later, by using best concentration of PE and ratio of PE to $AgNO_3$ and constant incubation period, the effect of concentration of $AgNO_3$ was studied. The 1 mM, 2 mM, 3 mM, 4 mM and 5 mM concentration of $AgNO_3$ was prepared to determine the best concentration of $AgNO_3$ for synthesis of AgNPs.

The effect of incubation period was studied by varying the time to 0 hr, 2 hr, 4 hr, 6 hr, 24 hr, 48 hr and 72 hr with best concentration of PE, ratio of PE to AgNPs, and concentration of $AgNO_3$ as studied.

3.5.2 Fourier Transform Infrared (FTIR)

Fourier Transform Infrared (FTIR) analysis is used to identify the capping reagents and molecules that is responsible for reduction of metal ions. For the AgNPs as sample, we need to remove any free biomass residue by centrifuged at 1000 rpm for 10 minutes and the resulting suspension was redispersed in 2 ml sterile distilled water. The centrifuging and redispersing process were repeated 3 times. Therefore, the purified suspension was freeze dried to obtain dried powder. The dried pellet were grinded with KBr pellets and were subjected to Fourier Transform Infrared Spectroscopy (FTIR) analysis from 50 scans at a resolution of 4cm^{-1} in the transmission mode (4000-400 cm^{-1}).

3.5.3 Zeta Potential Analysis

The prepared sample of AgNPs was dispersed in deionized water followed by ultra-sonification for 5 minutes. After sonification solution was filtered and centrifuged from 15 minutes at 25˚C with 5000 rpm and the supernatant were collected. The supernatant was diluted for 4 to 5 times. The particle distribution was studied in a computer controlled particle size analyser.

3.5.4 Dynamic Light Scattering (DLS)

Analysis of DLS is used to measure narrow particle size distribution especially in the range of 2-500 nm. Particles of the samples that is suspended within liquid will undergo Brownian motion in which the smaller the particles, hence the faster the motion.

3.5.5 Transmission Electron Microscopy (TEM)

For Transmission Electron Microscope (TEM), it was used to observe the size, shape and morphology of synthesized particles, a drop of aqueous AgNPs sample was loaded on carbon coated copper grid and the excess solution was removed by tissue paper and were allowed to dry at room temperature for overnight. The samples were viewed and the micrographs were obtained using TEM operating at accelerating voltage of 80 kV.