GREEN SYNTHESIS OF SILVER PARTICLES AT ROOM TEMPERATURE USING *GYNURA PROCUMBENS* AQUEOUS EXTRACT

KHAIRUNISA BINTI HAT

UNIVERSITI SAINS MALAYSIA

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by

KHAIRUNISA BINTI HAT

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First and foremost, praise be to Allah SWT, with His blessing I am successfully completed my thesis titled as ‘Biosynthesis of silver nanoparticles using plant extract’.

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LIST OF ABBREVIATIONS

AgNPs	Silver nanoparticles
SPR	Surface Plasmon Resonance
PVD	Physical Vapor Deposition
FTIR	Fourier Transform Infrared
TEM	Transmission electron microscopy
UV	Ultraviolet
PE	Plant extract
$AgNO_3$	Silver Nitrate

SINTESIS HIJAU TENTANG PARTIKEL PERAK PADA SUHU BILIK MENGGUNAKAN EKSTRAK GYNURA PROCUMBENS

ABSTRAK

Kajian ini memberi tumpuan kepada biosintesis nanopartikel perak menggunakan ekstrak Gynura procumbens sebagai agen penurunan dan penstabilan. Apabila ion perak akueus terdedah kepada ekstrak daun, ia akan diturunkan dan menghasilkan perubahan warna menandakan pembentukan nanopartikel perak. Partikel perak yang usdah disintesis akan disifatkan dengan menggunakan Spektroskopi UV-Vis, Spektroskopi Infra-merah Penjelmaan Fourier, Mikroskop Elektron Pancaran dan Analisis Keupayaan Zeta teknik. Spektrum UV-Vis yang dihasilkan oleh media akueus mengandungi nanopartikel perak sebagai fungsi masa tindak balas menunjukkan peningkatan secara beransur-ansur pada ketumpatan puncak Permukaan Gema Plasmon pada 449 nm hingga 452 nm. Beberapa parameter, sebagai contoh ratio isipadu ekstrak tumbuhann kepada argentum nitrat, kepekatan dan kepekatan argentum nitrat juga dikaji dan menunjukkan julat jarak gelombang yang sama pada penyerapan maksimum. Absorbans tertinggi, 1.75 didapati selepas 4 hari menggunakan 12 mM argentum nitrat bertindak balas dengan 5% ekstrak tumbuhan dalam ratio 1:50. Analisis Keupayaan Zeta membuktikan kestabilan nanopartikel perak dengan mempunyai nilai -40mV . Spektroskopi Infra-merah Penjelmaan Fourier menunjukkan bahawa nanopartikel perak dilitupi oleh pelbagai molekul protein. Mikroskop Elektron Pancaran memastikan bahawa nanopartikel adalah berbentuk sfera.

GREEN SYNTHESIS OF SILVER PARTICLES AT ROOM TEMPERATURE USING GYNURA PROCUMBENS AQUEOUS EXTRACT

ABSTRACT

This study focuses on biosynthesis of silver nanoparticles using *Gynura Procumbens* leaf extract as reducing and stabilizing agents. Aqueous silver ions when exposed to plant leaf extract were reduced and resulted in a colour change indicating the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-Vis Spectroscopy, Fourier Transform Infra-red Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM) and Zeta Potential Analysis techniques. The UV-Vis spectrum of aqueous medium containing silver nanoparticles as a function of time of reaction showed gradually increasing surface Plasmon resonance peak intensity at 449 nm to 452 nm of silver. A few parameters for examples volume ratio of plant extract to silver nitrate, plant extract concentration, silver nitrate concentration are also have been studied and shows the same range of wavelength at maximum absorption. The highest absorbance, 1.75 was obtained after 4 days using 12mM silver nitrate react with 5% plant extract in ratio of 1:50. Zeta Potential Analysis proves silver nanoparticles's stability by having the value -40mV. FTIR spectroscopy analysis showed that silver nanoparticles are capped by various protein molecules. Transmission Electron Microscopy confirmed the spherical shape of nanoparticles.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Nanotechnology is a crucial field of modern research where the design, synthesis, and manipulation of particles structure techniques are the main steps in this field. Nanotechnology is rapidly gaining demand in many areas such as health care, cosmetics, food and feed, environmental health and many more.

Silver nanoparticles (AgNPs) are of interest because of the unique properties for examples optical, electrical, and magnetic properties that contribute in antimicrobial applications, biosensor materials, composite fibers, cryogenic superconducting materials, cosmetic products, and electronic components. The main factor that influence the unique properties are the shape and the size of AgNPs.

Several physical, chemical and biological methods have been utilized to produce AgNPs. The most popular approaches are chemical reductions using a variety of organic and inorganic reducing agents, electrochemical techniques and physicochemical reduction. In the other hand, the safest route is the biosynthesis using microbes or plant extract and this technique is widely used for the synthesis of AgNPs (Panigrahi, 2013).

Among the various methods of nanoparticle synthesis, biological methods using plant extract is a much promising because of its effectiveness, flexibility and environment friendly approach. Considering the diverse biological applications of AgNPs, it is necessary to explore various plant extracts that can be used for AgNPs production.

The thesis presents an insight on preparation of AgNPs by using green synthesis approaches focusing on utilization of plant extract which could be obtained in Malaysia. The potentials and limitations of the above techniques are mentioned in the next pages.

1.2 Problem statement

Due to the rises of many problem associated with the physical and chemical approach, green synthesis using microbes and plant extract are getting high interest for the production of silver nanoparticles. Compared to using microbes, utilization of plant extract offers less problem. Hence, biosynthesis using plant extract is chosen.

Gynura procumbens is chosen because it is rich with chemical components that is important in enhancing and stabilizing the process and reduction of silver ions to silver nanoparticles. The chemical component involves are flavonoids and also other types of polyphenols. The plant also well known as a plant that cure many types of crucial disease such as high blood pressure, diabetic and high cholesterol level in the body.

This study highlight on the factors affecting the synthesis, for examples ratio of plant extract to silver nitrate solution, concentration of silver nitrate solution used and the concentration of plant extract. The ratio used in the experiment are 1:150, 1:100 and 1:50 while the concentration of silver nitrate used are 4 mM, 8 mM and 12 mM. The concentrations of plant extract are 5% and 20%. Huge ratios are chosen compared to preliminary studies done by others researches because the plant extract used showed negative result when only small ratios are used. Previous researches used many range of ratio for examples 1:1, 1:2, 1:3 and 1:4 (Espanti, 2016). For the concentration of silver nitrate, Shankar (2017) used 1 mM, 2mM, 3mM, 4 mM and 5 mM. In addition to the studied, the sizes, shapes and particles distribution are also have been investigated as they influence in producing silver nanoparticles with desired physical and chemical qualities.

1.3 Research objectives

The research is a study about utilizing plant extract for producing the silver nanoparticles and its characteristics. To complete the study there are a few of objectives need to be fulfilled:

1. To reduce silver ion to silver nanoparticles using *Gynura procumbens* extract.
2. To investigate the effect of ratio between plant extract to silver nitrate solution, silver nitrate concentration, plant extract concentration and time on the biosynthesis of silver nanoparticles.
3. To characterize silver nanoparticles in term of absorbance, size, shape and stability
4. To identify biomolecules surrounds the silver nanoparticles responsible as the capping reagent

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of silver particles

Nanoparticles denoted as very special particles due to its small size below than 100nm. All nanoparticles regardless of their chemical constituents, have surface area to volume ratios that are extremely high. Many of the physical properties of the nanoparticles such as solubility and stability are dominated by the nature of the nanoparticle surface (nanoComp, 2017). Table 2.1 shows the influences of diameter of the particles on the surface area to volume ratio.

Table 2.1 Influences of particles diameter on area to volume ratio (nanoComp, 2017)

Nanosphere Diameter (nm)	Surface area (nm^2)	Volume (nm^3)	Ratio Surface Area:Volume
10	314	523	0.60
20	1260	4190	0.30
30	2830	14100	0.20
40	5030	33500	0.15
50	7850	65500	0.12
60	11300	113000	0.10
70	15400	180000	0.09
80	20100	268000	0.08
90	25400	382000	0.07
100	31400	523600	0.06

Silver nanoparticles have various sizes and shapes depending on the fabrication method. Figure 2.1, 2.2 & 2.3 show images of nanoparticles obtained using TEM image. Silver nanoparticles are also sensitive to light (especially ultraviolet light) and should be kept in dark area. Highly acidic or basic solutions can also increase the dissolution rate of the nanoparticles into an ionic form that can plate onto the sides of the container or re-deposit onto existing nanoparticles changing the average diameter and size distribution.

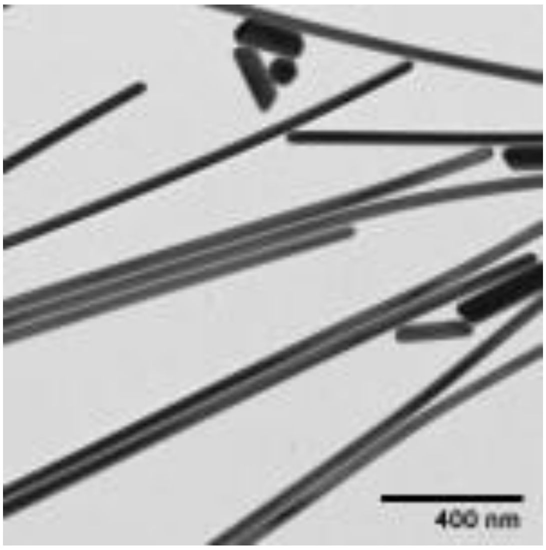

Figure 2.1: silver nanowires (nanoComp, 2017)

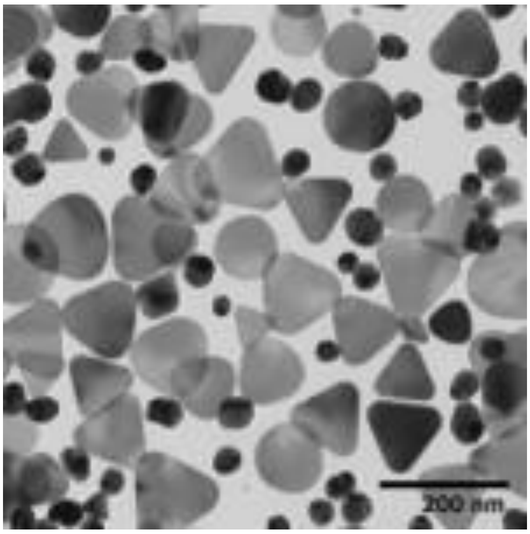

Figure 2.2 silver nanoplates (nanoComp, 2017)

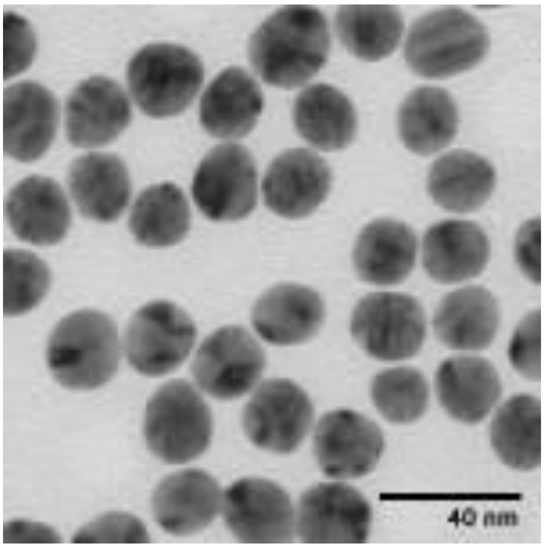

Figure 2.3 silver nanospheres (nanoComp, 2017)

2.2 Physical, Chemical and Biosynthesis Approach

There are several techniques for the production of silver nanoparticles. Explanation on these techniques are included in the section 2.2.1, 2.2.2, and 2.2.3.

2.2.1 Physical approach

The two common physical approaches for the synthesis of silver nanoparticles are the Physical Vapor Deposition (PVD) and the Laser Ablation.

1) Physical Vapor Deposition(PVD)

The physical vapor deposition method will produce a thin film of materials having size in the range of few nanometers to several micrometers. It has three main steps (John, 2017) :

i) Vaporization of the material from a solid source assisted by high temperature vacuum or gaseous plasma.

ii) Transportation of the vapor in vacuum or partial vacuum to the substrate surface.

iii) Condensation onto the substrate to generate thin films. Formation of the thin film and particle by nucleation and growth (Lee, 2009).

After undergo thermal vaporization/sublimation, the liquid/solid form of raw material will change its state to saturation vapor and will be transported to the substrate surface. Rapid condensation make the sizes to nanoparticles. The state of materials involve in this technique is illustrated in Figure 2.4.

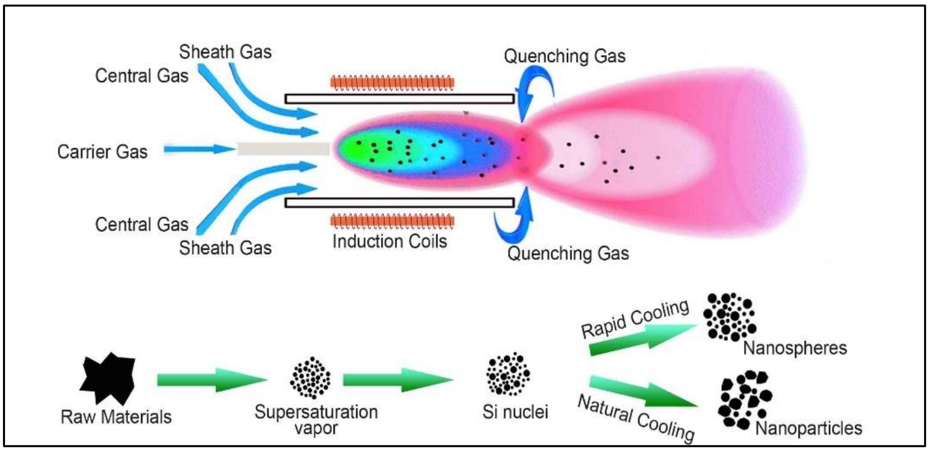

Figure 2.4 State of particles in Physical Vapor Deposition

The two most common PVD processes are thermal evaporation and sputtering. Thermal evaporation is a deposition technique that relies on vaporization of source material by heating the material using appropriate methods in vacuum.

Firstly, the material source will be heat up to desired high temperature in vacuum either by thermal or beam methods. Vapor produced form this process will be transferred to target by passing through vacuum area. Vapor will be turn into liquid by condensation and form thin film (Lofgran, 2013).

Two difference types for thermal evaporation are heating evaporation and electron beam evaporation. Heating evaporation heating material to supply the heat for the process while in electron beam evaporation, electrons are bombarded to the target material. The process is illustrated in Figure 2.5.

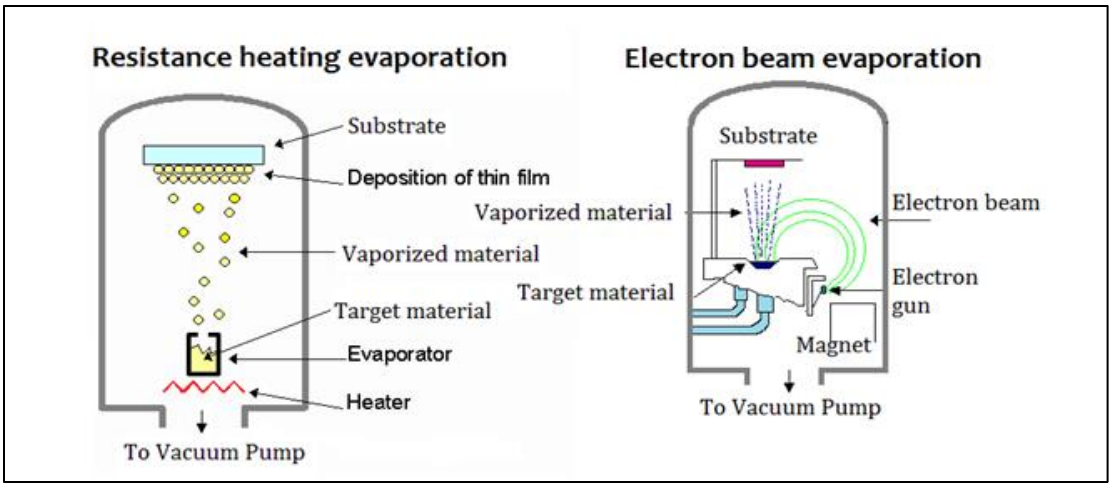

Figure 2.5 Process in resistance heat and electron beam evaporation (Hivatec, 2016)

Sputtering is a plasma-assisted technique that creates a vapor from the source target through bombardment with accelerated gaseous ions, typically Argon (Hsu, 2011) (Adam, 2004)

First step is to generate the argan ions. The argan ions will be directed toward target material. Consequently, the ions that have been targetted will sputter atoms the target material. The atoms that have been sputtered will be transferred to the substrate by passing through vacuum region. The atoms will form thin film by condensation process. For better understanding, the process is illustrated in Figure 2.6 below.

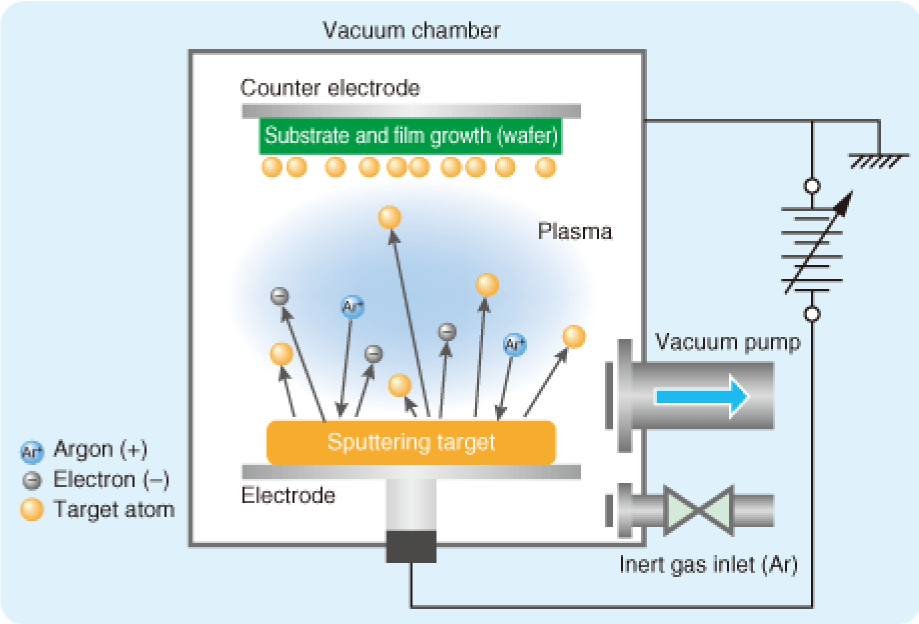

Figure 2.6 Process occur in sputtering (Michael, 2010)

In both evaporation and sputtering, the resulting vapor phase is subsequently deposited onto the desired substrate through a condensation mechanism.

2) Laser Ablation

Firstly, a pulsed laser beam is directed towards target material that located in the solvent. This result the target material to absorb the energy and cause them to vaporize and condense in solvent and produce nanoparticles (Wagener, 2011). The three steps above are illustrated in Figure 2.7 below.

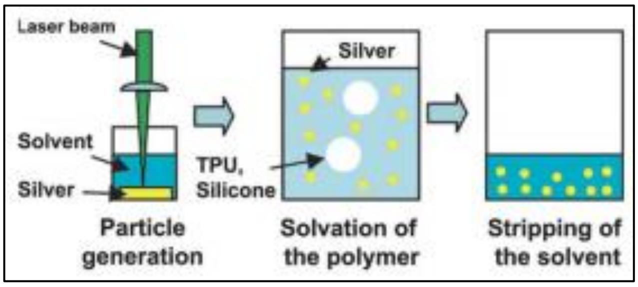

Figure 2.7 Process occur in laser ablation

A few factors influence the process including wavelength of laser. The smaller the wavelength, the smaller diameter of particles produced (Tsuji, 2002). The other factor is the duration of the laser pulse, ablation time duration, the liquid medium and the presence of surfactant.

2.2.2 Chemical Approach

There are many techniques used in chemical synthesis of nanoparticles but chemical reduction is the one of the most common one. There are many reducing agents used, for examples sodium citrate, ascorbate, sodium borohydride, elemental hydrogen, polyol process and tollens reagent.

Main function of reducing agents is to reduce the silver ion (Ag^+) to silver particles (Ag). Time passed and caused the silver particles that have been formed to agglomerate and form clusters. Here, the protective agents are used to stabilize the particles and avoid agglomeration (Muller, 2014). In the other hand, the presence of surfactants comprising functionalities for examples thiols, amines, acids and alcohols for interactions with particles surfaces can stabilize particle growth and protect from agglomeration.

2.2.3 Biological Approach

Biological synthesis of silver nanoparticles can be conducted either by using bacteria, fungi and plant extract.

2.2.3.1 Synthesis using bacteria

Bacteria owns ability to convert metal ions to metal particles and develop main advantages which is zero hazard to the surrounding. Some bacterial species have developed the ability to resort to specific defense mechanisms to defeat stresses like toxicity of heavy metal ions or metals. From the observation made by Haefeli (1984) prove that some of them could survive and grow even at high metal ion concentrations (e.g., *Pseudomonas stutzeri and Pseudomonas aeruginosa*).

Other benefits in using bacteria are fast growing, inexpensive to cultivate and easy to manipulate. Growth conditions such as temperature, oxygenation and incubation time can be easily controlled. In a study by He et al. (2007) it was found that varying the pH of the growth medium during incubation period, resulted in the nanoparticles with different sizes and shapes. Controlling such properties are important, as varying sizes of nanoparticles are required for different applications.

2.2.3.2 Synthesis using fungi

Ability of fungi to produce larger amounts of proteins enabled it to produce more silver nanoparticles compared to bacteria. Protein act as capping agents in order to stabilized the silver nanoparticles and prevent agglomeration. Fungi also have high binding capacity with metal ions in intracellular region. They are easy to culture on solid substrate fermentation and can grow on the surface of inorganic substrate during culture which leads to efficient distribution of metals as catalyst (Pradhan, 2013).

To synthesis silver nanoparticles using fungi, the fungal extract and silver are mixed and colour changes are observed. The general steps involve for the synthesizing process are illustrated in Figure 2.8:

Figure 2.8 Synthesize silver particles using fungi (Kannan, 2014)

2.2.3.3 Synthesis using plant extract

The reasons for the widely used of plant to produce silver nanoparticles are economical, eco-friendly and presence of abundant phytochemicals. The major phytochemicals responsible for the spontaneous reduction of ions are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides. A lot of plants come from different kingdoms and families are investigated for examples, *Ocimum tenuiflorum, Solanum tricobatum, Syzygium cumini, Centella asiatica , Citrus sinensis* (Logeswari, 2015)*, Lantana camara* and *Gynura Procumbens* has not been chosen yet to be studied.

After plant extract solution have been mixed with the silver nitrate solution, reduction of silver ions (Ag^+) will occur in a certain time depend on the type of plant used and also other factors. After silver nanoparticles formed with the help of any phytochemicals presence, the nanoparticles tend to accumulate and increase in size. Here, capping agent is crucial to prevent the accumulation of nanoparticles. The process is illustrated in Figure 2.9.

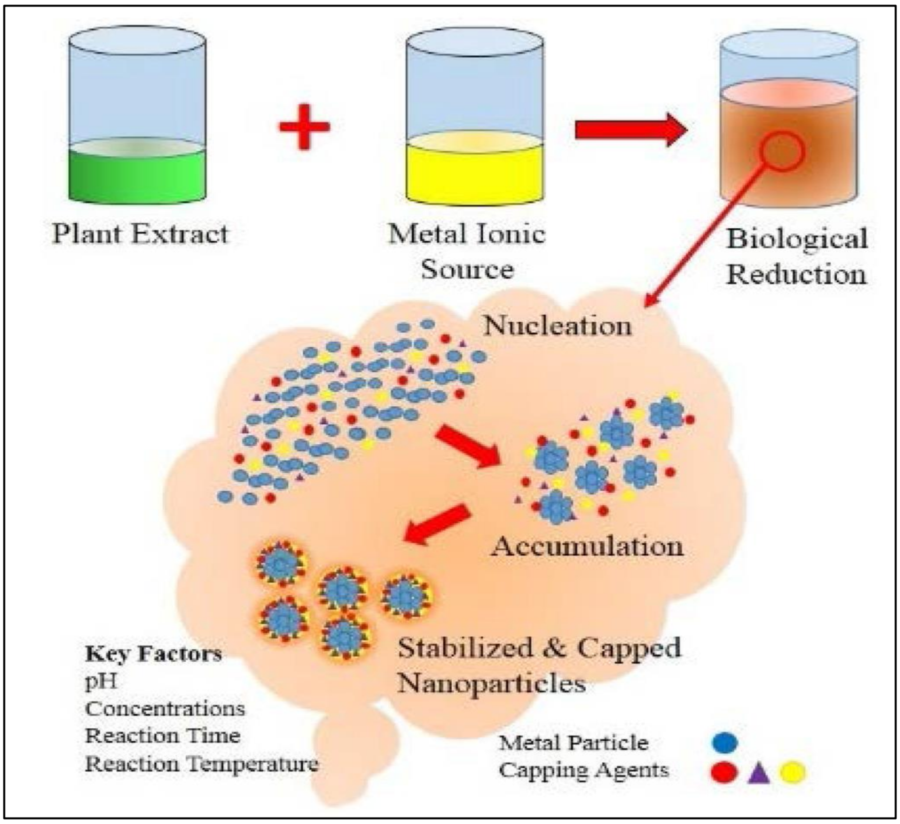

Figure 2.9 Synthesis using plant extract (Shah, 2015)

$AgNO_3$ solution dissociate into Ag^+ and NO_3^-. Plant leaf contains high level of poly-phenols (flavonoids). Phenolic compound has hydroxyl and ketonic groups which are able to bind to metals and reduce the metal salt and provide stability against agglomeration. Plant extract also provide protein and enzyme to the Ag^+ ions. Combination of the ions with the enzyme will form the enzyme substrate complex. After enzymatic reaction has been completed, silver nanoparticles will be released. The silver nanoparticle produce will combine with the protein released from the plant extract and form protein capped silver nanoparticles. This is crucial to prevent agglomeration problem.

Figure 2.10 shows some illustration about that process. (A) shows the structures of some polyphenol and flavonoid biomolecules that might be involved in the reduction and stabilization of AgNPs. (B) is the redox reaction showing mechanism of reduction of Ag^+ into AgNPs by using quercetin (flavonoid family) and (C) shows the mechanism on how the capping of AgNPs by plant active compounds (Kumar, 2013).

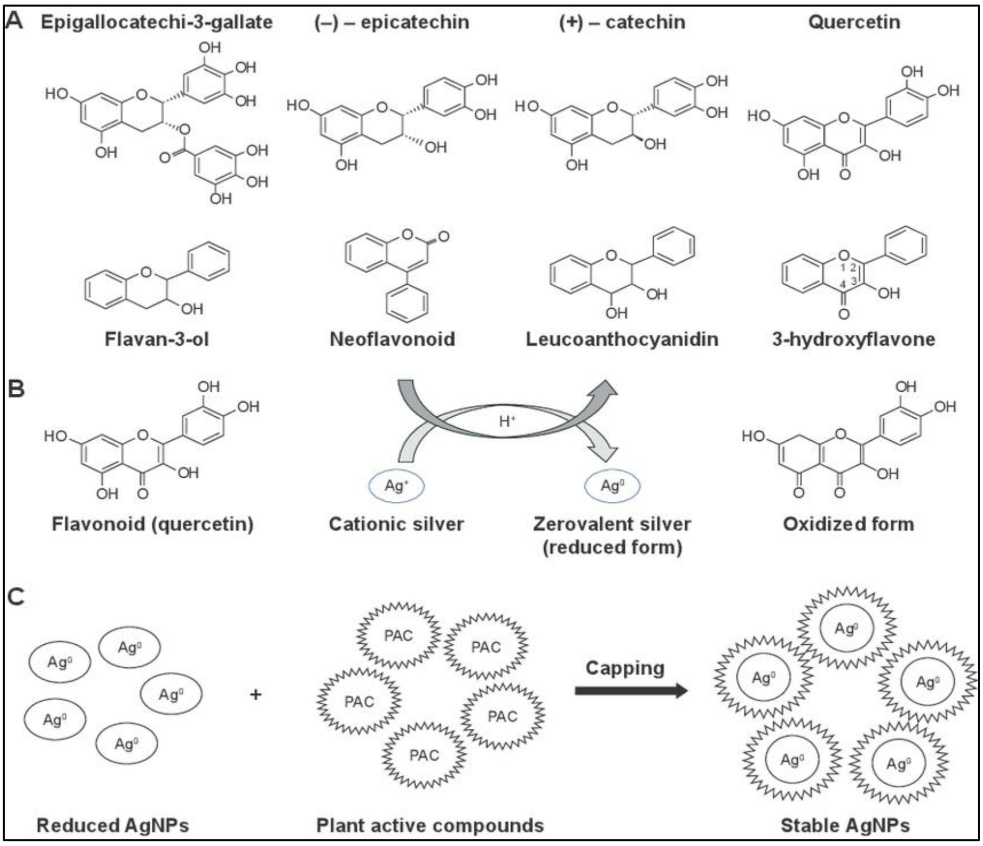

Figure 2.10 Detailed process in synthesis of silver nanoparticles using plant extract (Bastami, 2006) *(*Kumar, 2013*)*

2.2.3.4 Comparison between bacteria, fungi and plant extract

The AgNPs synthesis by microbes is strenuous compared to the use of plant extracts and biopolymers as reducing and capping agents mainly due to the difficulty in growth, culture maintenance, and inoculums size standardization. Several fungal and bacterial species have been successfully used in the synthesis (Srikar, 2016).

One of the most important parameter for the synthesis of AgNPs is temperature, where temperatures up to 100˚C were used by many researchers for AgNP synthesis using bio-polymers and plant extracts. On the other hand, the use of mesophilic microorganism restricted the reaction temperature to 40˚C (Srikar, 2016). At higher temperature the mesophilic microorganism dies due to the inactivation of their vital enzymes. This limitation makes using plant extract as a better choice for the biosynthesis of silver nanoparticles.

2.2.4 Comparison between three approaches to produce silver nanoparticles

Comparing the three main aspects in synthesis of silver particles which are safety, energy consumption and economical issues, it is known that physical synthesis may be hazardous to the surrounding due to usage of furnace which required high temperature. It creates more trouble if there is any leakage or malfunction of furnace which can lead to explosion. For chemical synthesis, utilization of hazardous chemical may affect the health of the user since they are exposed to the chemicals not only by touch but also through inhalation. However, this is not the case with regards to biosynthesis as it offers zero problem whether from health or safety issues. It also does not require any hazard chemical or specialized equipment.

In term of energy consumption, the physical synthesis may use high energy to maintain high temperature at atmospheric pressure. On the other hand, chemical and biosynthesis consumed less energy due to less temperature and pressure involved in the process.

Last but not least, the main aspect that will be the first thing considered by industries is the cost. Physical approach involves huge and expensive equipment, for example furnace while chemical approach require procedure that need much cost, for example laser irradiation and gamma irradiation. As for biosynthesis, the raw material especially plant extract can be obtained easily at minimal cost. The comparison between the three synthesizing strategies are summarized in Table 2.2.

Table 2. 2 Comparison between three approaches in producing silver particles

	Physical	Chemical	Biosynthesis
Environmental, health & safety issues	Increasing the temperature in the furnace may increase the surrounding temperature (Hussein, 2016)	Involve toxic chemicals for examples sodium borohydride which is flammable, extremely hazardous in case of skin contact (ScienceLab.com, 2005).	Eco-friendly technique (Kandasamy Selvama, 2017) (Godishala, 2015)
Energy consumption	May use tube furnace at atmospheric pressure, hence a lot of energy requires to raise the temperature	Less energy consumption	No need to use high pressure or high temperature, hence less energy consumption
Economical issues	Require expensive equipment	Require high cost for example in laser irradiation	Raw material for examples plant are abundant and most of them does not require any cost

2.3 Factor affecting the synthesis of silver particles

There is some parameters that may influence the synthesis of silver nanoparticles. For examples, pH, time, concentration of silver nanoparticles and temperature.

2.3.1 pH

The size of the silver nanoparticles was highly dependent on the pH of the reaction system (Torresdey, 2004). At high pH, smaller size silver nanoparticles were obtained compared to low pH. The difference in pH contributed to the difference in the reduction rate of the precursor. In addition to the inverse proportionality between the size and the pH value it is clear that increasing the pH value enables the obtainment of spherical nanoparticles while at low pH, rods and triangular particle shapes were formed (Aljarrah, 2014). This is may cause by poor balance between nucleation and growth processes.

According to Ibrahim (2015), the colour also changes when pH is increases. At pH 2 until 4, there was no colour changes, indicating no formation of silver nanoparticles. When pH is increasing, the colour start to become darker until monodisperse nanoparticles were obtained at pH 11. This statement is also supported by Kodialbail (2015). But another thing to be noted here is, very high pH (> 11) was associated with the drawback of formation of agglomerated and unstable AgNPs (Tagada, 2013)

To carry out the experiment, some amount of weak acid or alkali need to be included into the mixed solution to vary the pH. Examples of acid and alkali that commonly used are 0.1 N HNO_3 , 0.1 N HCl and 0.1 N NaOH.

2.3.2 Temperature

The temperature increase (30˚C - 90˚C) caused the rate of AgNPs synthesis increase (El-Rafieb, 2011). According to Fayaza (2009), the higher temperature will lead to the smaller size of nanoparticles. The same hypothesis was stated in articles by Sumithraa(2016) where the grain size of the leaves is below 90 nm when elevated temperature as applied and grain size were between 90 nm to 170 nm when carried out under room temperature.

2.3.3 Concentration of salt

More concentrated silver nitrate solution will produce darker mixed solution. The colour indicates that more silver ion is reduced to silver particles. The Surface Peak Resonance (SPR) peaks also became distinct when the concentration of metal salt is used and exhibit the smaller diameter of the particles (Ibrahim, 2015).

2.3.4 Incubation time

Reported by Ibrahim (2015) and also Kora (2010) in two different articles, the longer time given for the reaction to occur will cause higher amount of absorbance produced. The increase in absorbance along with colour intensity could be related to the higher amount of silver nanoparticles present in a certain period of time.

2.4 Application of silver nanoparticles

As the particles having obvious function which is antibacterial agent, the silver nanoparticles are widely used in any sector that required to disinfecting techniques in their processes. For examples, disinfecting medical devices (Li, 2008) and home appliances to water treatment (Bosettia, 2001). Turning to another different application, the silver nanocomposite fibers and also the cotton fiber were prepared containing silver nanoparticles incorporated inside the main materials. As the result, the materials performed high defend mechanism against *Escherichia coli* (Yeo, 2003)

Besides that, silver nanoparticles also perform well in catalytic application. Köhler (2007) found that bleaching of the organic dyes using potassium peroxodisulphate in aqueous solution at room temperature is enhanced strongly by the application of silver nanoparticles. Reduction of 4-nitrophenol with $NaBH_4$ in alkaline aqueous solutions also catalyzed by silver nanoparticles supported halloysite nanotubes (Liu, 2009).

Other special properties perform by these tiny particles is optical characteristic. There is growing interest in utilizing the optical properties of silver nanoparticles as the functional component in various products and sensors (Oldenburg, 2008). The strong interaction of the silver nanoparticles with light occurs happen due to the conduction electrons on the metal surface while having a collective oscillation when excited by light at specific wavelengths.

Known as a surface plasmon resonance (SPR), this oscillation results in unusually strong scattering and absorption properties. In fact, silver nanoparticles can have effective extinction (scattering + absorption) cross sections up to ten times larger than their physical cross section. The strong scattering cross section allows for sub 100nm nanoparticles to be easily visualized with a conventional microscope. When 60 nm silver nanoparticles are illuminated with white light they appear as bright blue point source scatters under a dark field microscope. The bright blue color is due to an SPR that is peaked at a 450nm wavelength.

A unique property of spherical silver nanoparticles is that this SPR peak wavelength can be tuned from 400 nm (violet light) to 530 nm (green light) by changing the particle size and the local refractive index near the particle surface. Even larger shifts of the SPR peak wavelength out into the infrared region of the electromagnetic spectrum can be achieved by producing silver nanoparticles with rod or plate shapes. Figure 2.11 illustrated surface plasmon resonance where the free electrons in the metal nanoparticle are driven into oscillation due to a strong coupling with a specific wavelength of incident light and at the right picture shows that dark field microscopy image of 60 nm silver nanoparticles.

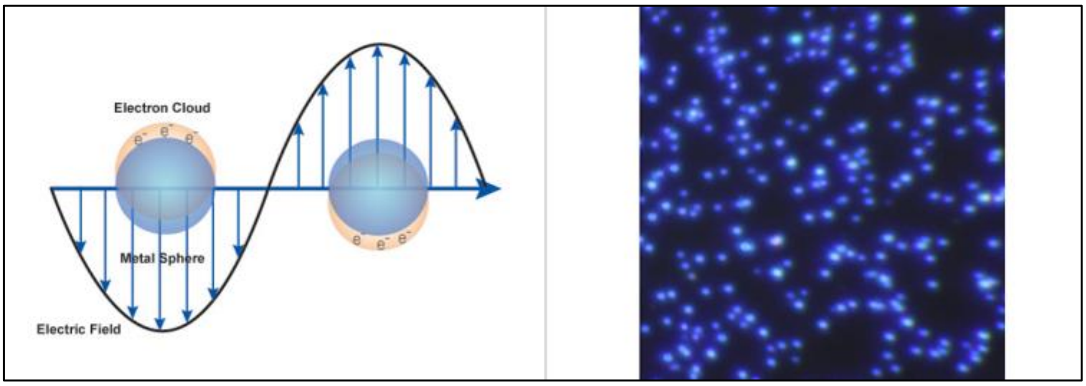

Figure 2.11 Surface Plasmon Resonance (Oldernburg, 2017)

2.5 Overview of *Gynura procumbens*

Gynura procumbens that also known as Sambung Nyawa is the herbaceous plant that can be used to reduce high level of cholesterol, lower the blood pressure, cure diabetic (Bastami, 2006) and cancer (Zhang, 2000). Chemical contents of Sambung Nyawa leaf are flavonoids (7, 3, 4 trihydroxy-flavone), quercetin glycosides, fenoleat acid (from kafeat acid, coumaric penta, penta-hydroxy benzoate and vanillic acid) triterpenoids (steroids), alkaloids, saponins, tannins, and antineoplastic substances (rahsiaherbal, 2010). Its green and wide oval leaves have sharp upper end with sow tooth shaped edges. In Malaysia, it is always eaten raw with rice besides from consuming it in juice form.

Figure 2.12 *Gynura Procumbens* Leaves