BIOSYNTHESIS OF SILVER NANOPARTICLES USING CASSAVA LEAVES AQUEOUS EXTRACT

GOPAL A/L KANANIASAN

UNIVERSITI SAINS MALAYSIA

2018

BIOSYNTHESIS OF SILVER NANOPARTICLES USING CASSAVA LEAVES AQUEOUS EXTRACT

by

GOPAL A/L KANANIASAN

Thesis submitted in partial fulfillment of the requirement

for the degree of bachelor of chemical engineering

JUNE 2018

ACKNOWLEDGEMENT

First and foremost, I would like to convey my sincere gratitude to my supervisor, Dr. Masrina Mohd Nadzir for his precious encouragement, guidance and generous support throughout this work.

I would also extend my gratitude towards all the MSc and PhD students 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. I would like to express my deepest gratitude to my beloved parents, Kananiasan A/L Gobar and Gowendamah A/P Mutturaman for their continuous love and support.

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 to the accomplishment of this project. Thank you very much.

Gopal A/L Kananiasan

June 2018

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AgNPs	Silver nanoparticles
SPR	Surface Plasmon Resonance
EDX	Energy Dispersive X-ray
DLS	Dynamic Light Scatter
SEM	Scanning electron microscopy
UV-Vis	Ultraviolet Visible
$AgNO_3$	Silver Nitrate

BIOSINTESIS PARTIKEL PERAK MENGGUNAKAN AKUAS EKSTRAK DAUN UBI KAYU

ABSTRAK

Nanopartikel perak boleh disintesis menggunakan kaedah fizikal, kimia dan biologi. Antara tiga kaedah ini, pendekatan biologikal lebih mudah, mesra persekitaran dan berkos rendah. Kajian ini memberi tumpuan kepada biosintesis nanopartikel perak menggunakan ekstrak daun ubi kayu 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 sudah disintesis akan disifatkan dengan menggunakan teknik- teknik Spektroskopi UV-Vis, Mikroskop Elektron Pengimbas, Penyerakan Cahaya Dinamik, Penyebaran Tenaga X-ray dan Analisis Keupayaan Zeta. Spektrum UV-Vis yang dihasilkan oleh sampel mengandungi nanopartikel perak menunjukkan peningkatan secara beransur-ansur pada 430 nm hingga 435 nm. Antara parameter yang dikaji adalah penggunaan kepekatan ekstrak daun ubi kayu yang berbeza, nisbah isipadu ekstrak tumbuhan kepada kepekatan argentum nitrat. Kajian tersebut juga menunjukkan julat jarak gelombang yang sama pada penyerapan maksimum. Serapan tertinggi, 2.00 didapati selepas 3 hari menggunakan 2 mM argentum nitrat bertindak balas dengan 1% ekstrak tumbuhan dalam ratio 1:5. Analisis Keupayaan Zeta memberikan nilai -15.8 mV. Purata saiz bagi nanopartikel perak adalah di antara julat 12 nm hingga 1000 nm.

BIOSYNTHESIS OF SILVER PARTICLES USING CASSAVA LEAVES AQUEOUS EXTRACT

ABSTRACT

Silver nanoparticles can be synthesized using physical, chemical and biological pathway. As compared to these three methods, biological approach is easier, more environmentally friendly and low cost. This study emphases on biosynthesis of silver nanoparticles using *Cassava* leaf extract as reducing and stabilizing agents. Aqueous silver ions, Ag^{+} when exposed to plant leaf extract were reduced to silver metal, Ag and resulted in a colour change from colourless to brown indicating the formation of silver nanoparticles. The synthesized silver nanoparticles were characterized by UV-Vis Spectroscopy, Surface Electron Microscopy (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Dynamic Light Scattering (DLS) and Zeta Potential Analysis techniques. The UV-vis spectrum of sample containing synthesized silver showed gradual increase of surface plasmon resonance peak intensity at 430 nm to 435 nm. A few parameters such as plant extract concentration, volume ratio of plant extract to concentration of silver nitrate and silver nitrate concentration have been studied and show the same range of wavelength at maximum absorption. The highest absorbance, 2.00 was obtained after 3 days using 2 mM silver nitrate react with 1 wt% plant extract in ratio of 1:5. Zeta Potential Analysis for silver nanoparticles' stability shows the value of -15.8 mV. The hydrodynamic size distribution was analyzed using DLS which gives average Z-diameter values at the range between 12 nm to 1000 nm.

CHAPTER ONE

INTRODUCTION

1.1 Research background

Nanotechnology applications are exceptionally reasonable for noble metal particles, due to their selected properties. The organic atoms experience very controlled gathering for making them reasonable for the metal nanoparticle synthesis which was observed to be dependable and environmentally friendly (Banala et al., 2015). The amalgamation of metal and semiconductor nanoparticles is an immense zone of research because of its potential applications. Nowadays, nanoparticles are considering as a fundamental building blocks for nanotechnology applications (Kumar Sur et al., 2018). Nanotechnology field has been one of a pulling in look into field in the advanced field of nanomaterial science Employments of nanoparticles and nanomaterials are incipient quickly on numerous arena. Nanoscale materials are generally arranged as materials having prearranged sections with at least one measurement less than 100 nm. The development of nanoscience has given promising results lately by crossing with unusual branches of science and framing sway on all types of life.

Metal nanoparticles, mainly the noble metals, have chiefly been inspected because of their solid optical ingestion in the noticeable region caused by the aggregate excitation of free-electron gas. Metal nanoparticles have a bigger surface zone and a shrill portion of superficial molecules. They are picking up the considerations of technologist due to the elite physicochemical physiognomies of nanoparticles, consolidating catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties (Geethalakshmi and Sarada, 2012) for their strategies of amalgamation. Recently, research on the synthesis of metal nanoparticles mostly on silver nanoparticles had a tremendous increment of interest among scientists (Elavazhagan and Arunachalam, 2011). Silver has been known, for more than 2000 years ago, as a noble metal that shows great restorative properties. There are huge emerging applications of silver nanoparticles. Several applications are in the fields of high sensitivity biomolecular detection, diagnostics, antimicrobials, therapeutics, catalysis and micro-electronics. Notwithstanding, there is still requirement for ecologically synthesis route to manufacture the silver nanoparticles. Silver nanoparticles are used to produce skin ointments and cream for the infections caused by burns and wounds in pharmaceutical fields (Durán et al., 2005).

Silver nanoparticles can be produced using numerous methods comprising physical, chemical, and biological. Normally, silver nanoparticles synthesis using chemical method is most prevalent due to the short synthesis period for large quantity production. But this method still has cons such as requires surpassing agents for the nanoparticles stabilization. Moreover, the surpassing chemicals are toxic and the by-products produced are also non-environmentally friendly. Therefore, biosynthesis or well known as green nanotechnology has gain interest of most of the scientist (Vidya et al., 2013). Many green synthesis methods for the silver nanoparticles synthesis have been described. Normally, microorganisms such as fungi, bacteria and plant extract are being used for the synthesis of nanoparticles (Bindhani et al., 2013). Biosynthesis methods have slower kinetics; this method also has great manipulation and control on the crystal growth and stabilization of nanoparticles. The ways nanoparticles synthesis that allow better control of shape and dimension for different nanotechnological uses (Satyavani et al., 2011, Mukunthan et al., 2011).

Plants stipulate an upgraded podium for nanoparticles synthesis as they are unrestricted from poisonous chemicals and give intrinsic capping specialists. In addition, usage of plant extract additionally downsizes the financial plan of microorganisms' segregation and culture media improving the cost suitable practicality over nanoparticles synthesis by microorganisms' viable feasibility over nanoparticles synthesis by microorganisms (Kannan et al., 2011).

It has been reported that synthesis of silver nanoparticles using plant extract has more advantages as compared to other biosynthesis methods which need an extremely complex procedure of upholding the microbial cultures (Ramya and Subapriya, 2012). Biosynthesis of silver nanoparticles using aqueous plant extract has a special benefit of that environmental friendly method, the plant can be get in large quantity with small capital cost, it also easily obtainable, it contains many metabolites and much securer to process (Paulkumar et al., 2014).

Cassava (*Manihot Escluenta*) is one of the most versatile crops now cultivated in Malaysia, is under *Euphorbiaceae* family also known as tapioca. The major use of cassava is in the production of tapioca flour due to its rich source of calories and isndustrial starch. In Malaysia, cassava plants are grown everywhere because it can be grown on mineral or peat soil easily without any special requirement. Cassava originated from Brazil and Paraguay. Malaysian eat cassava tubers and leaves as a source of food. Today it has been given the status of a cultigen with no wild forms of this species being known. Though young cassava leaves contain a high (40%) protein content, vitamins A, B, C, and E and minerals like iron, calcium and phosphorus, they also have a few antinutritional factors such as the cyanogenic glucosides - linamarin and lotaustralin. Young tender leaves can be used as a potherb, containing high levels of protein (8-10% F.W.). Prepared in a similar manner as spinach, care should be taken to eliminate toxic compounds during the cooking process.

Here in, report for the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of Cassava leaves.

1.2 Problem statement

There are many techniques for synthesis of silver nanoparticles. The strategies may be categorized as conservative or alternative with two diverse style which are top-down and bottom-up (Sepeur, 2008). Top down invention techniques mainly focus to the surface structure of the nanoparticles. This method has defects in the surface structure of the crystal nanoparticles and this is a remarkable constraint (Thakkar et al., 2010). Bottom up invention techniques mainly focus to the chemical and biological methods of synthesis of silver nanoparticles. Examples of conservative methods are solution, chemical or photochemical reactions in reverse micelles, thermal decomposition of different silver compounds, electrochemical, sonochemical, radiation and microwave-assisted routes (Rauwel et al., 2015). Some compound amalgamation techniques had been utilized to get equipped with noble silver metals and other metal nanoparticles. These techniques incorporate substance diminishment of metal salt precursors in arrangement, splash pyrolysis, sono-chemical, microwave-assisted, and microemulsions. All this procedure usually encompasses perilous chemicals, low compound adaptations, high energy necessities and wasteful sanitization.

Recently green science and biosynthetic procedures have concluded up being all the more attractive ways to deal with synthesis of silver nanoparticles. These original strategies use either natural microorganisms (e.g.: microscopic organisms, parasites, marine green growth, yeasts) or special alcoholic or watery plant separates. Green synthesis has various determinations of passion over traditional paths: it is practical, eco

– advantage and does not require high pressure, strength, temperature or the usage of toxic synthetic reagents (Bar et al., 2009). Plant-mediated synthesis of silver nanoparticles is more beneficial compared from the techniques that use microorganisms especially because they can be easily upgraded, are less biohazards and do exclude the complex period of creating cell cultures (Kannan et al., 2011). Today, it is conceivable to synthesize metallic nanoparticles with various shapes. *Cassava* leaves extracts contain alkaloids, flavonoids, unsaturated sterols and triterpenes, steroid glycosides, saponins and phenols. This study is focusing on the synthesis of silver nanoparticles using extract of Cassava leaves since no such study has been reported. Furthermore, characterization of the synthesized silver particles in terms of size, morphology and charges was also carried out.

1.3 Research objectives

The main intention of this research is to synthesize silver nanoparticles using cassava leaves extract via biological path and characterize the synthesized nanoparticles using analytical instruments. In general, the objectives of the present study are as follows:

I. To reduce silver ion to silver nanoparticles using cassava leaves extract.

II. 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.

III. To characterize silver nanoparticles in term of absorbance, size, shape and stability using using UV-Vis spectrophotometer, scanning electron microscopy, energy dispersive X-ray techniques, zeta potential and dynamic light scattering.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of nanoparticles

Nanotechnology is a well rising arena that encompasses the design and engineering of functional systems at the nanomolecular scale which has bigger surface area particles. It is a field of connected science concentrated on the synthesis, characterization and utilization of materials and gadgets on the nanoscale. Nanotechnology has been an opportunity to become a useful resolution for more than a few environmental and technological difficulties. By and large, nanotechnology can be characterized as the workmanship and investigation of controlling issue at the nanoscale to make innovative and extraordinary materials such as optoelectronic, magnetic, and mechanical, which diverges from bulk. Nanoparticles refer as particles with dimensions of less than 100 nm. Nanoparticles are important fundamental building blocks for nanotechnology applications. The dimensions of nanoparticles are mainly deals with nanometer units. The sizes of nanometer measurements decided the physical and concoction properties of materials experience outrageous changes, which open an extensive variety of future, however mostly effectively acknowledged applications (Santos et al., 2015). There are various sources or various chemical natures can be used to produce nanoparticles. Normally, nanoparticles have various shapes (e.g. platelets, tubes, sheet and cylinders etc.) but most commonly they are in sphere shape. Nanoparticles are classified based on their shape, size and morphology, the particles medium, the state of dispersion of the particles and most importantly, the several possible surface variations of the nanoparticles (Khodashenas and Ghorbani, 2015).

2.2 Overview of silver particles

Silver nanoparticles are one of the most important syntheses for current and future generation due to their inimitable properties. Nowadays, the research development on synthesis of silver nanoparticles has risen to a peak level due to its extreme applications on nanotechnology industry (Banne et al., 2017). There are three main methods for the synthesis of silver nanoparticles which are chemical, physical and biological method (Cholula-Díaz et al., 2018). But, most recently there are rising attention on production of nanoparticles using environmental friendly method (green synthesis). Biosynthesis has its advantages when compared to the normal methods involving chemical agents for capping purposes associated with environmental toxicity (Iravani, 2011).

2.3 Methods of nanoparticles synthesis

There are several techniques for the synthesis of silver nanoparticles. Explanations on these techniques are included in the section 2.3.1, 2.3.2, and 2.3.3.

2.3.1 Physical approach

There are several methods in physical approaches for the synthesis of silver nanoparticles such as evaporation-condensation, laser ablation, electrolysis, diffusion, plasma arcing, sputter deposition, pyrolysis and high energy ball (Kruis et al., 2000). The most important and practical methods are evaporation-condensation and laser ablation.

The physical evaporation-condensation method can be used to produce various metal nanoparticles such as silver, gold, cadmium sulphide etc (Haider and Kang, 2015).

This method vaporizes the solution into vapor using a tube furnace, and then the gas is cooled to produce crystal. There are some disadvantages of using tube furnace:

i) Tube furnace consumes great amount of energy.

ii) As the tube furnace are operated at high temperature, it raises the environmental temperature around the source materials (Goudarzi et al., 2016).

iii) More horsepower has been used which indirectly increases the operational cost.

iv) Required longer preheating time in order to achieve stable operating temperature.

This process can also be done using small ceramic heater using local heating source. It has more advantages as compared to tube furnace. The steeper temperature gradient of the heater surface makes the cooling rate of evaporated gas much more faster as compared to tube furnace. This makes the high potentials of formation of high concentration small nanoparticles.

The laser ablation method practices innumerable synthetic solvents to yield nanoparticles (Amendola et al., 2005). The requirements for pulse laser ablation process to take place are the chamber under vacuum and the presence of some inert gases (Bayazitoglu et al., 2013). Laser ablation does not use chemical reagents in the solution for the synthesis of silver nanoparticles (Amendola et al., 2005). There are several factors affects the efficiency and the characteristics of silver particles produced via ablation method. The factors are the time period of laser pulses, the wavelength of the laser intruding the metallic target, the laser fluence, the duration of the ablation and the liquid medium effectiveness, with or without the presence of surfactants (Mafuné et al., 2000). Thus, an uncontaminated colloid can be produced via this method for further usages in specific field. In plasma-arcing, the high temperatures related with the development of an arc or plasma is utilized to successfully isolate the structure types of feedstock, which rapidly re-joined at the exterior of the plasma to produce nanosized particles. As of late, huge advances have been made in the field of splash pyrolysis for the amalgamation of nanoparticles and a few expert researchers reported its flexibility in delivering particles of an assortment of mixtures, shapes, and dimensions (Korbekandi and Iravani, 2012).

2.3.2 Chemical approach

Chemical approach is well known as chemical reduction is the most communal method to produce nanoparticles. There are several methods in physical approaches for the synthesis of silver nanoparticles such as micro emulsion, electrochemical and thermal decomposition. Normally, organic and inorganic reducing agents will be used in chemical method. Some of the reducing agents are sodium borohydride ($NaBH_4$), potassium bitartrate ($KC_4H_5O_6$), trisodium citrate dihydrate ($Na_3C_6H_9O_9$), ascorbate, elemental hydrogen, Tollens reagent, N, N-dimethylformamide (DMF), and poly (ethylene glycol)-block copolymers. All these reducing agents are used to reduce the silver ion (Ag^+) to silver particles (Ag) and then, the silver nanoparticles will agglomerate to produce cluster form nanoparticles (Jana et al., 2001). Moreover, some nanoparticles will have isolated during preparation and some will be absorbed or bind onto exterior surface of other nanoparticles. Thus, to avoid these phenomena the protective agents are used to protect, stabilize and avoiding the agglomeration of nanoparticles (Iravani et al., 2014). Micro-emulsion process requires an organic solvent where the aqueous cores of the reverse micelles will disperse in it and surfactant will stabilized the nanoparticles formed. This method will produce a homogeneous reaction. There are few attempts has been done to manipulate the structure and dimension of the nanoparticles using this method (Oliveira et al., 2005).

Electrochemical method uses electric current as a source for the synthesis of nanoparticles. The mechanisms of this method are by the flow of electric current among two electrodes which is separated by an electrolyte. The nanoparticles will yield at the crossing point of electrode and electrolyte (Abou El-Nour et al., 2010). Another common chemical approach is thermal decomposition. Thermal decomposition is used to produce stable monodisperse suspensions which have the ability of self-assembly. In this method nucleation is the main occurrence for the synthesis of nanoparticles. When the precursor metal is added into a heated solution in the presence of surfactant nucleation occurs. Chemical approach path for the synthesis of nanoparticles has few pros, but there are still some drawbacks such as the usage of excessive solvents, surfactants and other chemicals which make it not environmental friendly and increase the capital cost of the production of nanoparticles.

2.3.3 Biological approach

Recently, biological synthesis of silver nanoparticles becomes the interesting technique for the researches due to the usage of biomaterials which are the natural reducing, capping, and stabilizing agents. Biological (green) synthesis method has a lot benefits as compared to physical and chemical approach. Some of the advantages are (Abou El-Nour et al., 2010):

1. It is environmental friendly and need lower capital cost method (cost effectiveness).
2. This method catalyzes the solution media at standard temperature and pressure.
3. It is a flexibility method where the process can be executed at any scale and settings.

The common types of biological materials used in this technique are bacteria, fungi, yeast and algae.

2.3.3.1 Synthesis using bacteria

Bacteria is an unicellular living organism which belonging to prokaryotes. Microorganisms will absorb the silver ion from the mixture and convert the ions into silver metal with the aid of enzyme secreted by them. They synthesis inorganic materials via two ways which are intracellular or extracellular (Anthony et al., 2014). Intracellular synthesis is by captivation of the metal ions inside the cell and reduces the ion into metal by the enzyme present inside the cell. Extracellular synthesis is by the adsorption of target ions onto surface of the cell and followed by the reduction of ion with the help of the enzyme. There are few microorganisms reports by researches for the synthesis of silver nanoparticles such as *Bacillus licheniformis* (Kalimuthu et al., 2008) and *Escherichia coli* (Gurunathan et al., 2009) etc. Bacteria has confrontation to metal from affecting their performance. This phenomenon let them to endure and cultivate in any metal ion concentration. Another important advantage of using bacteria are even the dead bacteria can synthesize nanoparticles with the presence of some of active organic components of the outer layer of cell (Singh et al., 2017). One of the problem faced bacteria is the concentration of metal ions, where at high concentration they can encourage toxicity. Few factors that affect the growth of bacteria are temperature, incubation time, oxygenation and pH but all these factors can be controlled easily so that the bacteria can synthesize the silver nanoparticles at optimum condition.

2.3.3.2 Synthesis using fungi

Fungi are another multicellular microorganism which has a rigid cell wall and can make their own food. The most popular enzyme used by fungi cell to amalgamation of nanoparticles is chitin and glucan. They produce nanoparticles through two path which are intercellular and extracellular. As from the research, extra-cellular path has more advantages over intra-cellular (Vigneshwaran et al., 2006). Nanoparticles with better dimension can be quickly produced using extracellular method due to the nucleation of particles inside the fungi cell. The method that used by fungi cell to synthesis metal nanoparticles is by the adsorption of the metal ion onto the surface of the cell and then followed by the conversion of metal ion to metal via the secretion of the enzyme from inside of the cell. Normally, fungal cell will secrete more active substances as compared to other microorganism which let them to synthesize large number of nanoparticles (Abou El-Nour et al., 2010). This is because active substances can trap great amount of metal ions and can avoid accumulation of particles during synthesis process (Narayanan and Sakthivel, 2010).

2.3.3.3 Synthesis using plant extract

Nowadays, researchers are highly interest for development of the silver nanoparticles using plant extract. The benefits of using plant extract are faster process as compared to microorganisms, easily can get the raw materials, environmental friendly and economically worth.

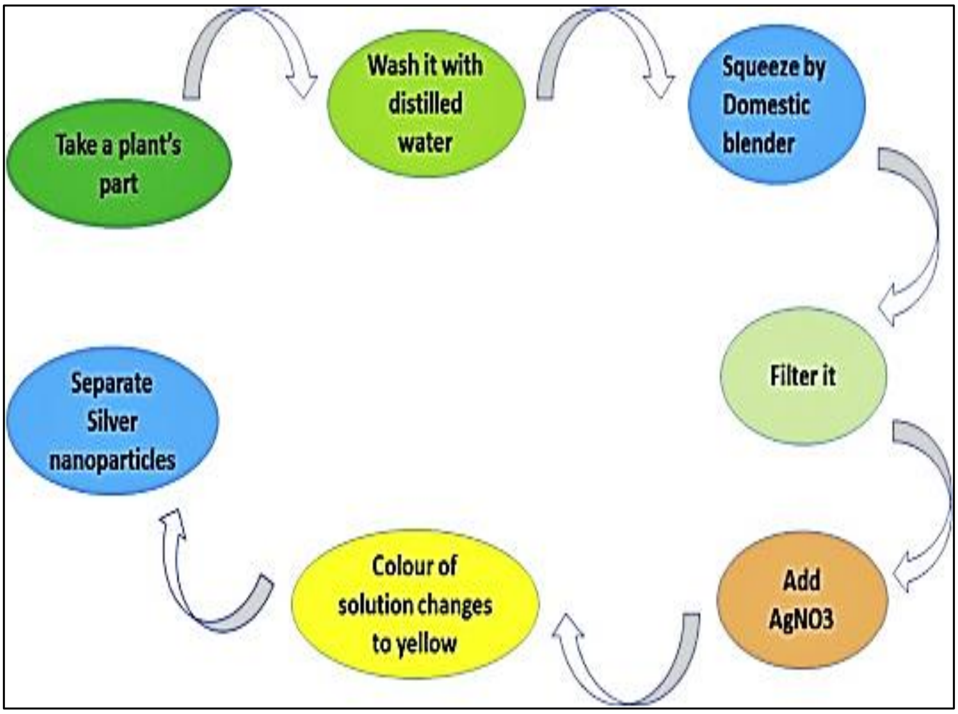

Figure 2.1 Pathway of biosynthesis of silver nanoparticles using plant extract (Ahmed et al., 2015)

The mechanism involves in this method are the reduction due to the metabolites in plant extracts. Their skills of amass high absorptions of metals was detected for all vital nutrients, such as copper (Cu), iron (Fe), zinc (Zn), and selenium, as well as non-essential metals, such as cadmium (Cd), mercury (Hg), lead (Pb), aluminum (Al), and arsenic (As) (Bharani and Namasivayam, 2017). Some of the important metabolites involves in silver nanoparticles reduction are flavonoids, terpenoids, carboxylic acids, quinones, aldehydes, ketones and amides. Some of the plants that have used for silver nanoparticles synthesis are *Acalypha indica* Linn (Krishnaraj et al., 2014), *Chenopodium album* leaf (Dwivedi and Gopal, 2010), *Hibiscus rosa sinensis* (Philip, 2010*), Calendula officinalis* (Baghizadeh et al., 2015) , *Origanum vulgare* (Sankar et al., 2013) etc.

Firstly, plant extract will be mixed with silver nitrate solution; the reduction will begin once the colour of solution start to change to brownish colour.

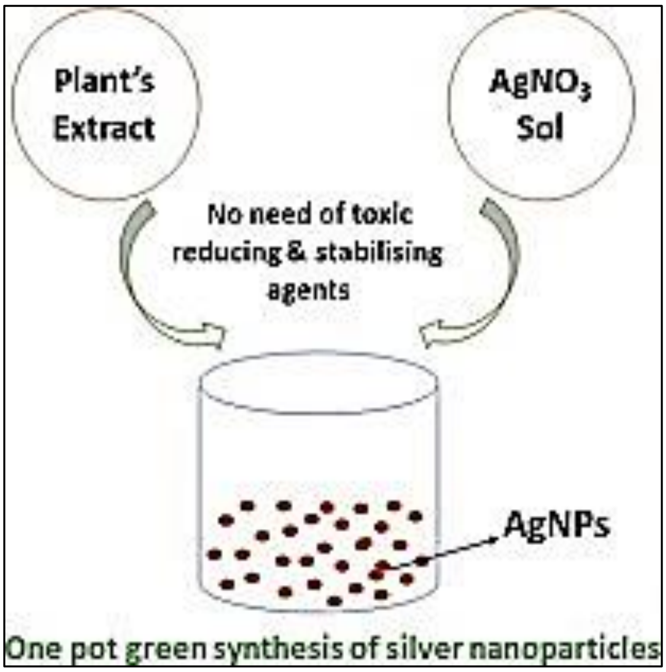

Figure 2.2 Schematic view of biogenic synthesis of silver nanoparticles (Ahmed et al., 2015)

The synthesis using plant extract depends on several factors. The silver nitrate solution will dissociate into Ag^+ and NO_3^- ions. Whereas the plant extract contains high concentration of flavonoids. The Ag^+ will adsorb onto the enzyme released by flavonoids to form a complex substrate. Then, silver nanoparticles will form in green manner (Vinković et al., 2017, Iravani, 2011).

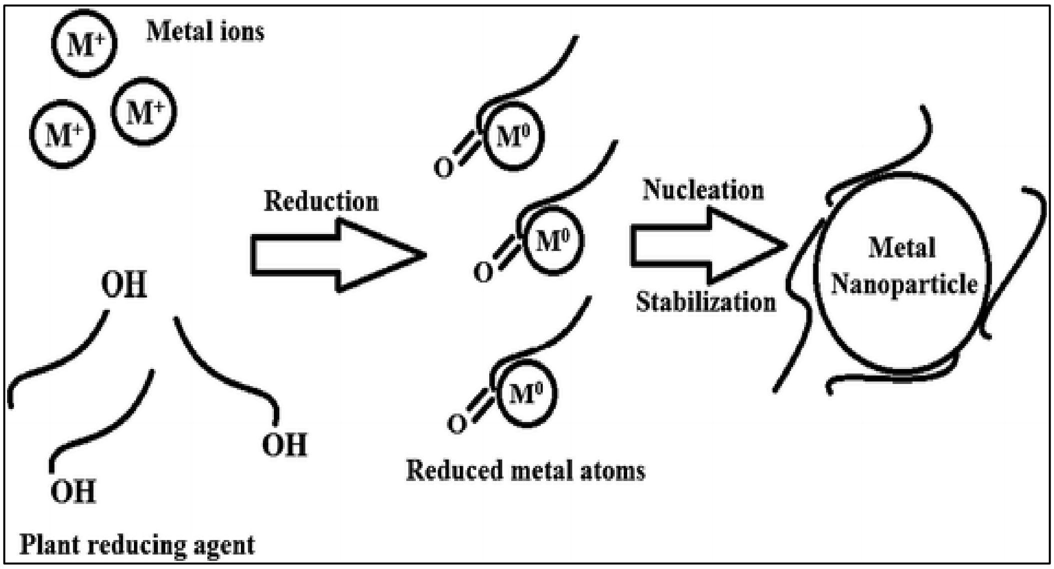

Figure 2.3 Schematic representation of a proposed mechanism of plant-mediated synthesis of metal nanoparticles (Keat et al., 2015)

2.3.4 Comparison between three approaches to produce silver nanoparticles

As from the reported research papers, the reduction is the main process occurs during the green synthesis of silver nanoparticles. Green synthesis offers several benefits as compared to physical and chemical methods. The most famous advantage is cost effective (Baghizadeh et al., 2015). Chemical pathway may affect the toxicity of the silver nanoparticles where some of the toxic material can be adsorbed on the surface of the particles. Green synthesis has no issues on toxicity of silver nanoparticles. Thus, most of the researchers prefer biosynthesis method because of its low cost of production as compared to physical and chemical pathway (Abou El-Nour et al., 2010).

As we now, most of the plants has antioxidant properties where it can be used as capping agent in synthesis of silver nanoparticles. Green synthesis provides non-hazardous nanoparticles production whereas physical method is dangerous due to the usage of furnace which may be explodes during the experiment. Moreover, chemical is toxic where the toxicity can cause health problems. In terms of energy consumption, pressure and temperature plays a vital role which may lead to undesired silver nanoparticles. Physical and chemical pathway required higher pressure and temperature whereas green synthesis must be carried out at atmospheric pressure and normally the temperature will be in the range of 25-80˚C which are lower as compared to other two methods (Korbekandi and Iravani, 2012). Green synthesis required lower temperature because of the capping agent used. This is because microorganisms cannot survive at higher and the some of the enzyme in the plants used will be denatured when heated at high temperature (Kalimuthu et al., 2008). Conclusively, biosynthesis pathway is better as compared to other two methods.

2.4 Factor affecting the synthesis of silver particles

The biosynthesis of silver nanoparticles can be improved by changing several constraints of silver nanoparticles such as concentrations of plant extract and silver nitrate solutions, concentration ratio of silver nitrate and plant extract, pH, incubation time and temperature.

2.4.1 Concentrations of plant extract and silver nitrate solutions

According to Rai et al., (2012) and Ahmad et al., (2016) concentration of plant extract and silver nitrate solutions affect the formation of silver nanoparticles. The concentration of plant extract was changed using 2%, 5% and 10%. The concentration of silver nitrate solution was kept constant at 1mM. This experiment’s purpose is to optimize the silver nanoparticles formed in several aspects. Based on the report of several researchers, as the concentrations of silver nitrate solution increases the absorption peaks intensity also increases due to the more silver ion Ag^{+} are there in the solution mixture for the reduction. Moreover, the solution will become darker in colour which result in the formation silver nanoparticles. (Iravani et al., 2014), (Ahmed et al., 2016) and (Veerasamy et al., 2011) almost get the same results for the silver nanoparticles synthesis. Conclusively, increase in the concentrations of silver nitrate solution can optimize the silver nanoparticles formations.

2.4.2 Concentration ratio of silver nitrate and plant extract

Veerasamy et al., (2011) had studied the effect of concentration ratio of silver nitrate and plant extract to maximize the yield of silver nanoparticles. The experiment was setup with different ratio of silver nitrate and plant extract of (0.5:19.5, 1.5:20, 1:19, 2:20 and 2:25). Adequate plant extract must be added for the reduction of silver ion, Ag^+ present in the silver nitrate in the solution. Thus, with best ratio maximum yield of silver nanoparticles can be achieved Ali et al (2016) using *A. absinthium L.* plants also conducted a research to determine the effect of concentration ratio of silver nitrate and plant extract to maximize the yield of silver nanoparticles. The researchers use 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 ratio of silver nitrate and plant extract. Here, they identified that as the ratio is 1:1 the SPR peak is at maximum. Whereas, there is no peaks were shown at 9:1 ratio. Thus, here they observed that as the ratio of plant extract is higher than silver nitrate concentration no silver nanoparticles were synthesized.

2.4.3 pH

pH plays a vital role in optimizing green synthesis of silver nanoparticles. Researchers has been determined that pH affects the size, shape and reaction rate (Ganaie et al., 2016). Nucleation centres plays an important role in the reduction of silver ion to silver metal. Thus, pH increases the nucleation centres and increases the reduction rate. Sheny et al., (2011) identified that the size of Ag nanoparticles produced was strongly dependent on the solution pH. They repeat the experiment using pH (2,3 and 4). From that experiment, at pH 2 big size of silver nanoparticles (35-90 nm) was formed. Furthermore, at pH 3 and 4 huge amounts of small sized silver nanoparticles were shaped. At small pH nanoparticles will aggregate and form larger sized nanoparticles somewhat than to nucleate and form small sized nanoparticles. Moreover, at higher pH more functional groups will bind onto the silver nanoparticles surface. As the pH increase, the intensity of absorbance peaks also increases (Korbekandi and Iravani, 2012).

2.4.4 Incubation time

Incubation time is the reaction time of the solution mixture was kept for gestated. Incubation time also affect the structure, shape and size of the silver nanoparticles synthesis using green synthesis pathway. According to Nazeruddin et al., (2014) synthesis of silver nanoparticles is much faster using by seed extract of *Coriandrum sativum* within 1 – 2 hr whereas 3-4 day required when bacteria are used as reducing agents. As we know, plant mediated nanoparticles synthesis is much more faster as compared to microorganisms but few of the scientists' experiential competent synthesis at high incubation time.

Sharma et al., (2011) observed that *Ocimum sanctum (Tulsi) leaf* mediated synthesis of silver nanoparticles begins within 20 minutes of the reaction. They found that as the time of the reaction increased, the intensity of absorbance peaks become more sharpen. Several scientists also reported that higher incubation time changes the structure and shape of the silver nanoparticles (Veerasamy et al., 2011).

2.4.5 Temperature

Temperature is another important factor which may influence the size, shape and extent of silver nanoparticles. Temperature also increases the nucleation centres during the synthesis. Indirectly, it also increases the reduction of the Ag^{+} into Ag metal. Through TEM images, the researchers seen nanoparticles sizes are not consistent at lower temperature (Veerasamy et al., 2011). At temperature of 50-60 ˚C, a consistent size of nanoparticles is synthesizing due to the dominations of density of spherical nanoparticles over other shapes of nanoparticles. Moreover, authors also determined that larger amount of plant extract are needed to obtain stable silver nanoparticles at lower temperature due to the lower reduction rate (Kiani and Taherkhani, 2018). In addition, bigger size and more even nanoparticles can be yield at high temperature reaction.

2.5 Application of silver nanoparticles

There are increasing trend of attentions on noble metal nanoparticles in several applications. Silver nanoparticles is one of the noble metal nanoparticles which recently have extensive awareness due to their huge number of applications such as in non-linear optics, spectrally selective coating for solar energy absorption, biolabeling, intercalation materials for electrical batteries as optical receptors, catalyst in chemical reactions and as antibacterial capacities (Zhang et al., 2013). The fixed properties of silver nanoparticles make them to be used in various applications such as in the fields of medicine, catalysis, optics, agriculture, paints, nanosensor, antifouling etc. In medical field, normal cells will always be intermingling with biological environment which become one of the main cause for the cancer. Baghbani-Arani et al., (2017) synthesized silver nanoparticles using

Artemisia tournefortiana Rchb extract and inspected their anticancer effectiveness against different cell lines including human bone marrow, leukaemia and human cervix. From this research, the scientist has proved that silver nanoparticles can be one of the best anticancer agent.

Moreover, silver nanoparticles also well known for their antimicrobial activity against pathogens. With this antimicrobial activity silver nanoparticles can be applying in preserving food and can be act as sanitizing agent for disinfecting and sterilizing food industry equipment. Antimicrobial activity was observed using different pathogenic bacteria such as *Escherichia coli* (Alshareef et al., 2017), *Salmonella typhimurium* (Williams et al., 2017), *Staphylococcus aureus* (Zheng et al., 2018) and *Bacillus cereus* (Ghiuță et al., 2018).

Catalyst is one of the substances that is important in any chemical reaction. Nanoparticles have higher surface area to volume ratio which can be used as catalyst and this nanoparticle can speed up the reaction and increase the gross revenue. According to Kumar. R et al., (2014) a batch process of heavy metal removal using silver nanoparticles. The removal of heavy metal is very fast at beginning and become slower once it reaches equilibrium. Initially, the heavy metal will have high vacant site and the silver nanoparticles has higher adsorption rate. Then, all the vacant site was adsorbed till reaches the equilibrium stage and process will become lower.

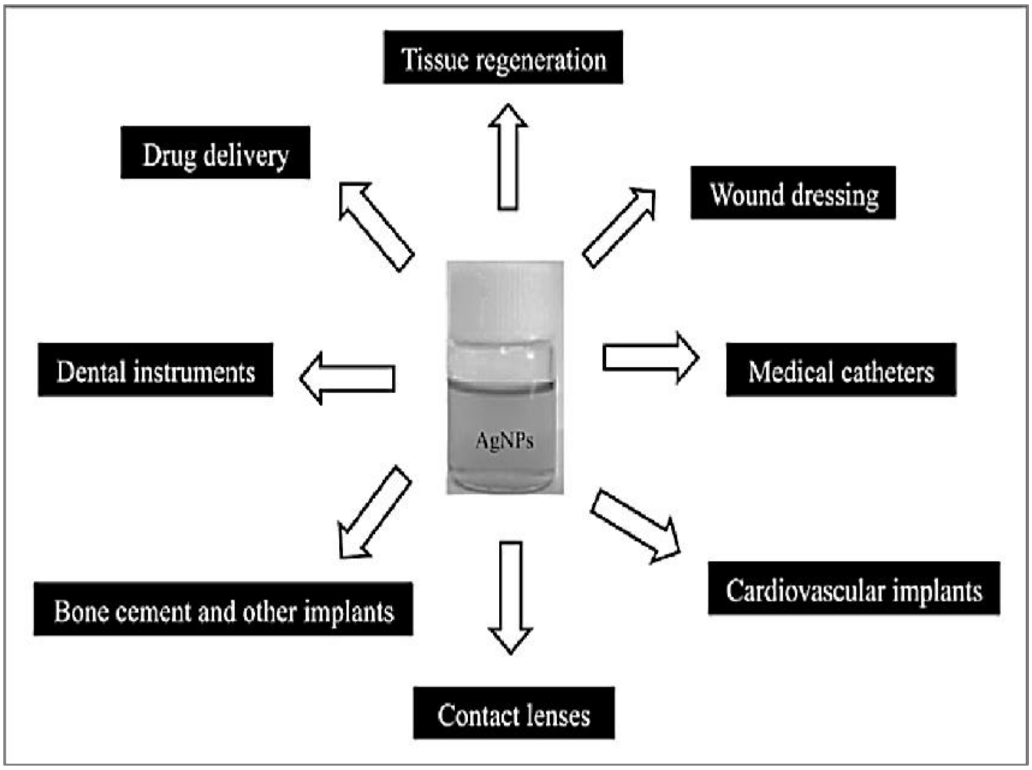

Figure 2-4 Application of silver nanoparticles (Santos et al., 2015)

2.6 Overview of Cassava leaves

Casssava leaves also known as *Manihot Escluenta Crantz* is abundantly cultivated at nonarid tropical world. Root is the main part that has been consumed by human as a food source. Nowadays, the leaves also being consumed due to the protein and vitamins contains which can provide supplement for dietary person. Young cassava leaves contain vitamins A, B, C and E, high 40 % protein content, minerals such as iron, calcium and phosphorus. There is some antinutrional value such as HCN.

Figure 2-5 Cassava leaves

CHAPTER THREE

MATERIAL AND METHOD

3.1 Materials and chemicals

The materials and chemicals used in the experiment are list in Table 3.1.

Table 3.1 List of materials and chemicals

Materials/chemicals	**Usage**
Silver Nitrate	As a precursor of silver ions
***Cassava* leaves**	To provide capping reagent and stabilizer for silver nanoparticles

3.2 List of Equipments

The equipment's used in the experiment are list in Table 3.2.

Table 3.2 List of equipment

Equipment/ Facility	**Usage**	**Brand/ Model**
Electronic balance Scale	To weigh the powder of plant	Shimadzu
Stirring Hot plate	To heat and stir samples	Fisher Scientific
Centrifuge	Separation of liquid solution from liquid	Labogene Scanspeed 1248R
Food blender	Blend the leaves until become powder	Philips
Refrigerator	To store sample at 4°C	CoolTech
Oven	To dry samples	Memmert 600, Germany
UV-Visible Spectroscopy	Analysis (absorbance and wavelength)	Thermo Scientific Genesys 20
Dynamic Light Scattering (DLS)	Analysis (size distribution)	Malvern Instrument, UK

3.3 Experimental flow chart

The preparation and characterization of Cassava leaf extract is shown in the flow chart below.

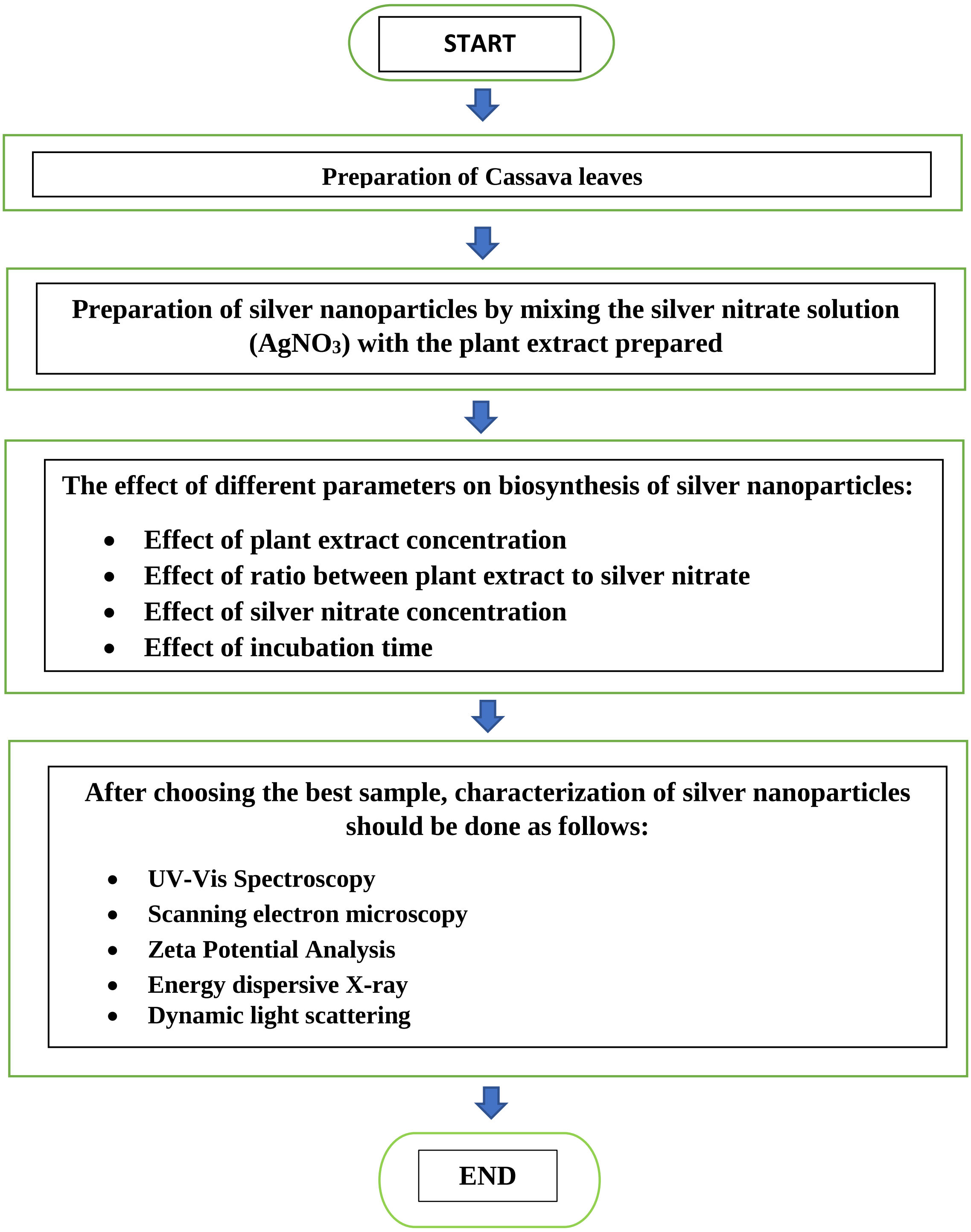

Figure 3.1 Experimental flow chart