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Using Green Synthesized Silver Nanoparticles
from *Kaempferia Galanga* Rhizomes Extract
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**COLORIMETRIC DETECTION OF HEAVY METAL IONS USING GREEN
SYNTHESIZED SILVER NANOPARTICLES FROM *Kaempferia galanga*
RHIZOMES EXTRACT**

by NURUL NADIA BINTI MUHAMMAD

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of Bachelor of Chemical Engineering**

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LIST OF ABBREVIATION

NPs	Nanoparticles
AgNPs	Silver Nanoparticles
SEM	Scanning Electron
EDX	Energy Dispersive X-ray
TEM	Transmission Electron Microscopy
DLS	Dynamic Light Scattering
ZP	Zeta Potential
LSPR	Localize Surface Plasmon Resonance
SPR	Surface Plasmon Resonance
TCHM	Traditional Chinese Herb Medicine

Ag⁺ ions

Silver ions

LIST OF SYMBOLS

Symbol	Description	Unit
mL	Millilitre	
L	Litre	
M	Molarity	mol L ⁻¹
mM	MilliMolar	
w/v	Weight/Volume	g mL ⁻¹

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**PENGESANAN KOLORIMETRIK LOGAM BERAT MENGGUNAKAN
NANOPARTIKEL PERAK YANG DISINTESIS SECARA HIJAU DARIPADA
EKSTRAK RISOM *Kaempferia Galanga***

ABSTRAK

Sintesis hijau nanopartikel perak (AgNPs) adalah bidang penyelidikan yang berkembang pesat kerana penjimatan kos dan mesra alam sekitar. Dalam kajian ini, AgNPs telah disintesis dengan pantas dengan merawat ion perak (ion Ag^+) melalui laluan mudah dan mesra alam menggunakan ekstrak rizom *Kaempferia galanga* yang boleh bertindak serentak sebagai reduktor dan penstabil. Warna larutan AgNPs diperhatikan dan spektroskopi UV-Vis digunakan untuk mengesahkan kehadiran AgNPs dalam larutan. Keputusan menunjukkan bahawa 4% (b/v) ekstrak tumbuhan, 0.01 M kepekatan perak nitrat (AgNO_3), 72 jam tempoh inkubasi dan nisbah 1:3 ekstrak tumbuhan kepada AgNO_3 adalah kepekatan yang paling sesuai di mana warna larutan berubah daripada kuning cerah kepada coklat gelap dan spektroskopi UV-Vis menunjukkan puncak dalam julat 400-500 nm yang merupakan spektrum penyerapan biasa AgNPs. Kemudian, pencirian AgNP yang disintesis daripada ekstrak rizom *K.galanga* dilakukan menggunakan Microskop Electron Pancaran (MEP) dan Sinar-X Penyebaran tenaga (EDX) di mana bentuk sfera boleh dilihat dan lebih daripada 58% berat zarah perak dikesan berada dalam larutan. AgNPs yang disintesis digunakan untuk pengesanan kolorimetrik 12 jenis ion logam berat iaitu Mn^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Fe^{3+} , Fe^{2+} , Al^{3+} , Cr^{3+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , and Cu^{2+} dengan kepekatan 1 mM dan keputusan menunjukkan perubahan warna antara keperangan hingga kehijauan untuk Fe^{2+} manakala tiada perubahan warna yang jelas untuk ion logam berat yang lain. Spektrum UV-Vis juga menunjukkan perubahan puncak yang jelas untuk Fe^{2+} ,

Fe^{3+} dan Hg^{2+} . Ujian lanjut telah dijalankan untuk mengesan sensitiviti AgNPs tersintesis terhadap ion logam berat yang dipilih dalam kepekatan yang lebih rendah. Tiada perubahan yang jelas dalam warna larutan bagi setiap ion logam berat dapat diperhatikan tetapi spektrum UV-Vis menunjukkan bahawa 0.4 mM adalah kepekatan terendah yang dapat dikesan oleh AgNPs.

**COLORIMETRIC DETECTION OF HEAVY METAL IONS USING GREEN
SYNTHESIZED SILVER NANOPARTICLES EXTRACT FROM *Kaempferia
Galanga* RHIZOMES**

ABSTRACT

Green synthesis of silver nanoparticles (AgNPs) is a vast developing area of research due to its cost-effectiveness and environmental friendliness. In this study, silver nanoparticles (AgNPs) were rapidly synthesized by treating silver ion (Ag^+ ion) through a simple and green route using *Kaempferia galanga* rhizome extract which can act simultaneously as a reductant and stabilizer. The colour of AgNPs solutions were observed and UV-Vis spectroscopy was used to validate the presence of AgNPs in the solution. The result shows that 4% (w/v) of plant extract, 0.01 M concentration of silver nitrate (AgNO_3), 72 hours incubation time and 1:3 ratio of plant extract to AgNO_3 are the most suitable concentration where the colour of solution change from pale yellow to dark brown and UV-Vis spectroscopy showed a peak in the range of 400-500 nm which is the typical absorption spectrum of AgNPs. Then, characterization of AgNPs was done using Scanning Electron Microscope (SEM) and energy dispersive X-ray (EDX) where a spherical shape can be seen and more than 58 % weight of silver particles were detected to be in the solution. The synthesized AgNPs were used for colorimetric detection of 12 types of heavy metal ions, namely Mn^{2+} , Ni^{2+} , Zn^{2+} , Co^{2+} , Fe^{3+} , Fe^{2+} , Al^{3+} , Cr^{3+} , Cd^{2+} , Pb^{2+} , Hg^{2+} , and Cu^{2+} with 1 mM concentration and the results showed a colour discoloration ranging from brownish to greenish for Fe^{2+} while no obvious colour changes for other heavy metal ions. The UV-Vis spectra also show obvious peak changes for Fe^{2+} , Fe^{3+} and Hg^{2+} . Further testing was carried out to detect the sensitivity of the synthesized

AgNPs towards the selected heavy metal ions in lower concentration. No obvious changes in colour of solution for all three heavy metal ions were observed but UV-Vis spectra shows that 0.4 mM was the lowest concentration that are able to be detect by the AgNPs.

CHAPTER ONE

INTRODUCTION

1.1 Effect of Heavy Metal on The Environment and Health

The term heavy metal is essentially associated with the sum of metals with densities above 5 g cm^{-3} and belongs to the inorganic chemical hazard (Kumar et al., 2016). Nowadays, a widespread problem is water pollution is always linked to the growth of cities, development of industries, and intensive agriculture. Heavy metals get into the environment through direct and indirect releases such as from soil degradation, quarrying activity, industrial discharges, urban runoff, municipal wastewater, insect or disease control chemicals widely used in agricultural and a variety of other factors which contribute to the contamination of heavy metals into natural ecosystems and hydro systems. Several studies have shown that there are trace amounts of heavy metals in water bodies, and that these metals influence both aquatic life and human health.

Heavy metals such as manganese (Mg), nickel (Ni), zinc (Zn), aluminum (Al), chromium (Cr) and others are toxic even at a very low concentration. Besides, these heavy metals are non-biodegradable and the conventional water treatment techniques were not effective enough to treat these harmful compounds of the carcinogenic, teratogenic, and mutagenic types in surface and groundwater bodies of fresh water (Que et al., 2018). Consequently, they have the potential to reduce the quality of drinking water. Aside from that, heavy metal exposure can lead to a variety of serious diseases, including respiratory problems, kidney pathology, neurological disorders, and cancer. For instance, chromium is carcinogenic and can cause skin lesions and respiratory issues (Mohammadi et al., 2019). The brain, kidney and the developing fetus are at the risk of damage if they are

exposed to a high concentration of metallic, organic and inorganic mercury (Alina et al., 2012). Furthermore, high copper concentrations in water may destroy biological reprocessing systems. (Zhao et al., 2009). Hence, it is very important to develop effective methods of sensing heavy metal ion contaminants so that further treatment can be done to remove these heavy metals from wastewater before releasing the treated water into the environment.

1.2 Characteristics of Nanoparticles

Nanotechnology has become a quickly developing field providing the manufactures an extensive range of different metal nanoparticles (NPs). NPs are nanomaterials with sizes ranging from 1-100 nm and have distinct chemical and physical properties. Besides, NPs display various morphological structures with shapes like rods, spherical, tube, hexagonal, amorphous and so on. According to reports, their optical properties are influenced by the size, resulting in significant optical absorption in the visible region. NPs are ideal candidates for a variety of industrial and domestic applications, including catalysis, imaging, medicinal applications, energy-based research, and environmental applications because of their distinctive size, shape, structure, and also chemical and mechanical properties which affect their reactivity, toughness, and other qualities. (Khan et al., 2019, Mousavi et al., 2017).

NPs of noble metals such as silver, gold, platinum, copper, zinc, titanium and magnesium have received considerable attention in various fields due to their unique properties in catalysis, chemical sensing, biosensing, photonics, electronics and pharmaceutical (Krithiga et al., 2015). However, silver nanoparticles (AgNPs) are the

most beneficial and popular noble metal NP because of their flexibility, ease of preparation, and low cost. Besides, silver is an inorganic antimicrobial agent capable of destroying over 650 different disease causing by microorganisms (Bhuyar et al., 2020). Moreover, silver was named “energetic” due to its capacity to be use in a range of biological purposes, including antifungal, antibacterial, antiviral, anti-infectious, wound healing, and anti-inflammatory activities at relatively low levels (Aina et al., 2020). Marimuthu et al., 2020 has proposed AgNPs to be applied in anticancer, wastewater treatment, plant growth, and crop production.

The dominant feature of metal NPs can be observed by the surface plasmon resonance (SPR) in the visible absorption spectrum. However, different NPs have different characteristics and the exact position of resonance will depends upon the particle size, shape, interparticle interaction, free electron density and the embedding medium (Krishnaraj et al., 2010). SPR is the resonant interaction between plasma and the incident light. Plasma is conduction band electron near the surface of NPs. When the frequency of the incident light matches the resonant frequency of the plasma, strong absorption will occur. This property is useful for observing electron injection and aggregation in NPs. (Sunita & Palaniswamy, 2017).

AgNPs, in particular have an outstanding localized surface plasmon resonance (LSPR) capability showing vibrant and well-defined colours and allowing for easy colour change viewing (Jeong & Kim, 2015). Furthermore, because on its broad spectrum of bactericidal and fungicidal properties and ability to interact with a variety of ligands and macromolecules in microbial cells, silver is the most commonly used metal in the creation of NPs. Additionally, improvements in the ligand chemistry of metallic NPs have created

new opportunities for the production of colorimetric sensors that can assay a variety of chemical and biomolecular species with the proper selectivity and sensitivity, which can then be recognized via visible readouts.. (Proposito et al., 2020). Besides, silver has been utilized extensively in managing microbial formation as well as a medicinal injury due to its anti-inflammatory effect (Hamouda et al., 2019).

1.3 Medicinal Plant *Kaempferia galanga*

Kaempferia galanga (Figure 1.1) or commonly known as Kencur is a highly sought-after medicinal plant found in Tropical Asia. It is widely used in India and distributed throughout China, Myanmar, Indonesia, Malaysia, and Thailand. Traditional Chinese Herb Medicine (TCHM) uses *K. galanga* to treat colds, dry coughs, toothaches, rheumatism and hypertension. Besides, it has also been commonly used as a spice due to its strong scent (Wang et al., 2021). A previous study from Asem & Laitonjam, (2014) has biologically synthesized AgNPs and functionalized AgNPs using extract of *K. galanga* rhizomes and characterized the AgNPs using UV-Vis spectroscopy, the transmission electron microscopy (TEM), and the scanning electron microscopy (SEM) analysis.

Herein, a few parameters such as concentration of plant extract, incubation time, the ratio of plant extract to silver nitrate (AgNO_3) and concentration of AgNO_3 will be varied to synthesis AgNPs. Then, colorimetric detection of heavy metal ions using green synthesized AgNPs from the extract of *K. galanga* rhizomes will be studied by varying the types and concentration of heavy metal ion solutions to observe the colour changes of solution and the absorbance by UV-Vis spectroscopy.



Figure 1.1: *Kaempferia galanga* plants in natural habit and their rhizomes (Preetha & Hemanthakumar, 2016)

1.4 Problem Statement

Toxicity caused by heavy metal ion contamination is a serious threat to both human health and the environment. Heavy metal contamination is due to the dye, coal, plastic, paper, and natural gas sectors. As a result, extra attention should be given to the selective detection of low to high levels heavy metal ion, particularly in biological systems and wastewater.

Green synthesized nanoparticles-based colorimetric detection is gaining popularity among the various types of colorimetric sensors designed for biomolecule detection. These eco-friendly colorimetric sensors have a high tendency for detecting a variety of pollutants, including dangerous metal ions. Plant extracts, bacteria, fungi, and biopolymers such as chitosan cellulose have all been used to create metal and metal oxide nanoparticles. However, plant parts such as leaves, seeds, and flowers are effective reducing and capping agents, and the functional group facilitates pollutant adhesion (Iravani et al., 2014)

Hence, this study focuses on the colorimetric detection of different types of heavy metals ions using green synthesized AgNPs from *K. galanga* rhizomes extract while varying types of heavy metal salt solutions. A few parameters which are the concentration of plant extract, the concentration of AgNO₃, incubation time and ratio of plant extract to AgNO₃ will be studied to determine the optimum condition to synthesize the AgNPs. Then, the absorbance and shifting of absorption bands will be measured at various heavy metal ion concentrations to determine the linearity, sensitivity, and selectivity of AgNPs in detecting the ions. Characterization of the AgNPs was done using Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX).

1.5 Research Objectives

1. To investigate the influence of concentration of plant extract, incubation time, the ratio of plant extract to silver nitrate and concentration of silver nitrate on the biosynthesis of AgNPs.
2. To characterize the AgNPs synthesized from *K.galanga* rhizome extract using Scanning Electron Microscope (SEM) and Energy Dispersive x-ray (EDX)
3. To investigate the colorimetric detection of heavy metal ions using green synthesized AgNPs.
4. To analyze the sensitivity of the colorimetric detection system to the different concentrations of the heavy metal ions.

CHAPTER TWO

LITERATURE REVIEW

2.1 Green synthesis Silver Nanoparticles

Several routes for synthesizing AgNPs have been developed in previous studies, which can be classified as physical, chemical, or bio-based techniques. The physical method which is the evaporation-condensation and laser ablation method have some drawbacks. For instance, synthesized NPs in an atmospheric pressure tube furnace needed a lot of space, high energy consumption as well as increase the ambient temperature and taking a long time to reach the thermal stability. Besides, chemical methods such as chemical reduction, microemulsion technique, UV-initiated photoreduction, irradiation method and a few more common approaches are non-environmentally friendly and expensive because of the usage of sodium borohydride, NaBH_4 in the process. NaBH_4 is a hazardous reducing agent and can increase environmental pollution, limiting the use of AgNPs, particularly in the clinical field. As a result, a green bio-based method for synthesizing AgNPs has been developed by replacing previous reducing agents with natural bioactive compounds (Iravani et al., 2014).

Due to the environmentally friendly, safe, cost-effective, and required renewable reducing agent, the green approach becomes a powerful aspect for modern nanotechnology studies (Azmi & Ahyat, 2015). Besides, the green synthesis of metal NPs receives greater attention compared to the conventional physical or chemical synthesis techniques due to their good stability and effective optical features called LSPR (Alzahrani, 2020). Bacteria, fungi, algae and plants are being used in a bio-based method

to synthesis the highly stable characterized NPs. Besides, inheritable, and genetic characteristics of organisms, as well as ideal environments for cell development, enzyme activity, optimal reaction conditions, and biocatalyst state selection are also needed to be taken into account. Furthermore, some critical factors, such as substrate concentration, pH, light, temperature, buffer strength, electron donor, substrate concentration, mixing speed, and incubation time, can be manipulated using this method to change the sizes and morphologies of the synthesized NPs (Iravani et al., 2014).

Moreover, some natural bioactive compounds extracted from yeasts, enzymes, fungi, microorganisms, and plants are suitable for reducing Ag^+ ion from (I) to (0) oxidation state because of the abundance of hydroxyl and amino groups found in those natural resources. However, plant extracts will be the best reducing agent for synthesizing AgNPs among these sources because they reduce the synthesis time and have a higher manufacturing scale than other natural resources (Azmi & Ahyat, 2015). Indeed, plant extracts-mediated green synthesis is now more favorable than other biological systems since it does not require culture, aseptic conditions, or maintenance. Besides, plant-based NPs synthesis is proven to be very cost-effective, making it a viable and cost-effective option for large-scale production (Iravani et al., 2014).

Furthermore, the plant extract-mediated synthesis is a user-friendly and has low-biohazard as it contains phytochemicals such as terpenoids, flavonoids, and phenol derivatives, as well as plant enzymes such as hydrogenases and reductases, which function as both reducing and stabilizing agents, reducing metal salts and preventing NP aggregation (Kumar et al., 2016). Moreover, the cost of microorganisms isolation and culture media also can be reduced when using plant extract (Krithiga et al., 2015). As a

result, various plants are now being extracted for use as a reducing agent in the production of AgNPs.

2.2 Colorimetric detection using the silver nanoparticles method

In recent times, there have been numerous reports on heavy metal ion detection methods. Table 2.1 shows several methods proposed by a few researchers to detect heavy metal ions in water. However, most heavy metal detection procedures in biological and environmental samples demand the application of expensive instruments and chemicals. Furthermore, majority of the methods listed below are time-consuming, have poor enrichment factors, and are difficult to use (Tian et al., 2019). Therefore, it is necessary to develop a more practical method for detecting the presence of heavy metals in a straightforward and fast manner.

Colorimetric detection based on AgNPs is being selected among all NPs due to their strong distance-dependent optical characteristics or called LSPR and extraordinarily high extinction coefficient. The colorimetric detection method is carried out based on the colour change of AgNPs colloids after reacting with metal ions (Zhao & Li, 2019). Compared with other noble metals, AgNPs are favorable due to the sensitivity to electromagnetic waves in the visible light spectrum where the radiation induces a surface polarization of the nanoparticle. The electron begins to oscillate in resonance if the particle size is similar to the dimension of the evanescent wave's penetration layer and hence causes a strong absorption at that specific wavelength. This is why the size and form of nanoparticles have a significant impact on the spectrum obtained (Alberti et al., 2021). Furthermore, colorimetric detection of AgNPs is the best method to utilize because it is

simple to manufacture, versatile, and inexpensive. AgNPs exhibiting optical and plasmonic effects have been used as colorimetric probes for the detection of heavy metals such as Cu^{2+} , Pb^{2+} , Co^{2+} , Mn^{2+} , Ar^{2+} , Hg^{2+} , and Ag^{2+} ions (Narayanan & Han, 2017).

Table 2.1: Several methods proposed to detect heavy metal ions in water

Method of Detection	Heavy Metal Detected	References
Atomic absorption spectroscopy	Nickel (II) ion, Ni^{2+} Manganese (II) ion, Mn^{2+}	Mirabi. (2019) Narayanan & Han. (2017)
Chromogenic sensors	Copper (II) ion, Cu^{2+} Cobalt (II) ion, Co^{2+}	Chuen et al. (2021)
Chemo dosimeter-functionalized sensor	Mercury (II) ion, Hg^{2+}	Samanta et al. (2017)
Cold vapor atomic fluorescence spectroscopy (CVAFS)	Manganese (II) ion, Mn^{2+}	Narayanan & Han. (2017)
Differential pulse anodic stripping Voltammetry (DPASV)	Cadmium (II) ion, Cd^{2+}	Wu et al. (2015)
Fluorescent chemo-sensor	Nickel (II) ion, Ni^{2+} Ferric (III) ion, Fe^{3+} Magnesium (II) ion, Mg^{2+}	Lian et al. (2020)
Ionic liquid and ionic liquid modified sorbents	Lead (II) ion, Pb^{2+} Aluminum (II) ion, Al^{2+} Cadmium (II), ion, Cd^{2+}	Tian et al., 2019

The colorimetric approach, which can be seen with the naked eye, is a great on-site approach for instant detection of target heavy metal ions due to its smooth production and portability on-site. The principle of colorimetric detection is based on the unique LPSR of AgNPs and their ability to aggregate. Any heavy metal that can be detected by AgNPs will cause the colour of the solution to change and SPR bands to shift. This visual detection is based on the well-known metal-ligand coordination, in which the metal and the ligand, respectively, operate as electrical acceptors and donors. (Sung et al., 2013).

Besides, colorimetric detection of metal ions is possible because AgNPs have variable sizes and distance-dependent optical properties with high extinction coefficients in the visible range. Furthermore, as AgNPs are formed and bind with metal ions, the inter-particle distance-dependent absorption wavelength and colour change of the solution can be observed. This is because the SPR and colorimetric properties of AgNPs are tightly linked and heavily influenced by particle size (Khwannimit et al., 2019). As the size, shape, and structure will affect the performance of the AgNPs, the AgNPs synthesized from *K. galanga* rhizome extract need to be determined and characterized.

2.3 Silver nanoparticles from *Kaempferia galanga* rhizome extract

AgNPs are usually manipulated with stabilizing and capping agents to prevent them from aggregating (Hyder et al., 2022). Hence, green synthesis of AgNPs from *K. galanga* rhizomes extract was employed in this study and it served as a reductant and stabilizer for the silver nitrate solution (AgNO_3). Phytochemical studies by Wang et al., (2021) have identified numerous chemical substances of *K.galanga* mainly terpenoids, phenolics, flavonoids and diarylheptanoids. These biomolecules are said to be responsible

for the reduction of silver ions to AgNPs as they act as reducing as well as stabilizing agents (Jain & Mehata, 2017). Some parameters such as reaction time, temperature, pH, the ratio between plant extract and AgNO₃, and concentration of plant extract and AgNO₃ are reported to affect the AgNPs synthesis.

2.3.1 Effect of plant extract concentration

The high concentrations of plant extract are expected to decrease more Ag⁺ ions to AgNPs as it has more phenolic in the antioxidant groups. Sunita & Palaniswamy, (2017) uses five different concentration of *Aegle marmelos* fruit pulp extract to study the effect of the concentration of plant extract on the synthesis of AgNPs. They reported increasing in the amount of AgNPs formed. The plasmon peak increase with increasing of fruit pulp extract concentration where sample with 10 mL fruit pulp extract shows absorbance higher than 0.966. Besides, there are also blue shift in wavelength at 432 nm, 430 nm, 428 nm, 427 nm, 427 nm and 427 nm for 2 mL, 4 mL, 6 mL, 8 mL and 10mL respectively which indicates the reduction in the particle size.. Masum et al., (2019) reported that a strong and sharper absorption band of SPR spectra can be observed when using a higher concentration of plant extract. This is being explained by the number of the functional biological group that is responsible for the stabilization of NPs.

2.3.2 Effect of Silver Nitrate concentration

Besides the plant extract concentration, the concentration of the precursor which is AgNO₃ and incubation time also gives a significant effect on the synthesis of AgNPs. Sunita & Palaniswamy, (2017) has varied the concentration of AgNO₃ from 0.001 M to 0.01 M and reported that 0.01 M is the most suitable concentration where a better SPR was obtained. A few literatures reported that an increase in Ag⁺ ion intensity can speed up

the process of achieving the SPR. At this rate, the ideal bell shape graph can be observed from UV-Vis in the wavelength range of 400-490 nm which indicates the formation of AgNPs. Besides, Ibrahim, (2015) synthesized AgNPs using banana peel extract as a reducing agent and variation concentrations of AgNO₃ are being used. He reported a change in colour intensity of the solution from yellowish-brown to light reddish brown and darker shades of reddish brown can be seen with increasing AgNO₃ concentration. Additionally, SPR was said to become more distinct with increasing concentration of AgNO₃.

A study by Azmi & Ahyat, (2015) using different concentration of AgNO₃ which is 0.001 M, 0.01 M and 0.1 M found that AgNPs with 0.01 M of AgNO₃ gives the highest intensity of absorbance as shown in Figure 2.1. Besides, as depicted in Figure 2.2, they found out that the absorption increases linearly with reaction time at a specific wavelength. This is due to more incubation time for Ag⁺ ions to be reduced by plant extract to form AgNPs.

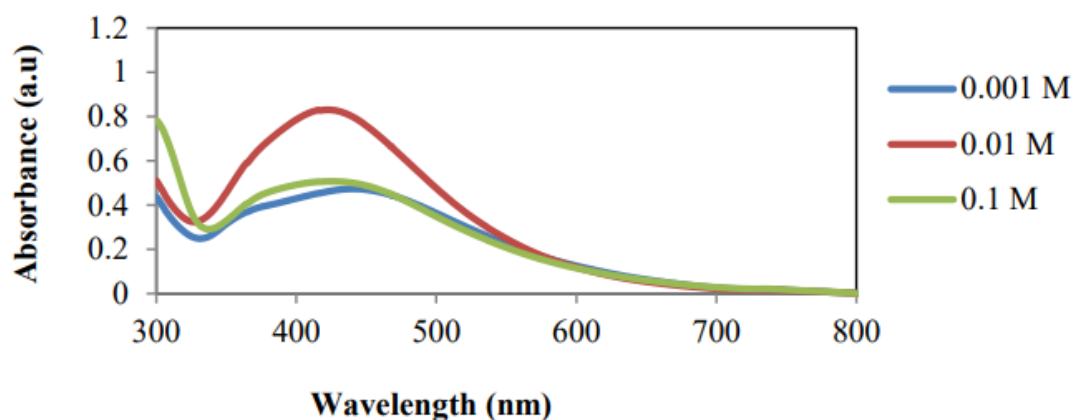


Figure 2.1: UV-Vis spectra of AgNPs in different AgNO₃ concentrations after 48 hours (Azmi & Ahyat, 2015)

2.3.3 Effect of incubation time

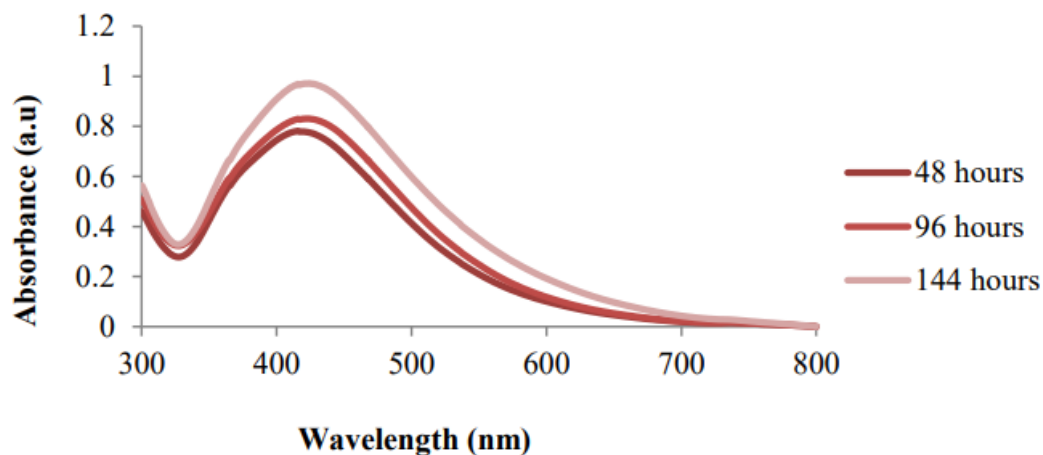


Figure 2.2: UV-Vis spectra of AgNPs in different incubation times (Azmi & Ahyat, 2015)

The colour changes of the prepared solution were observed to see the formation of AgNPs, which was due to electron transitions of SPR in the solutions. He was observed that the colour intensity of AgNPs solution changed from colourless to light brown at 48 hours to brown at 96 hours and finally to dark brown after 144 hours when the AgNO_3 is added with *Alpinia galanga* rhizome extract as shown in Figure 2.3. It is revealed that Ag^+ ions were reduced and stable AgNPs were formed. The excitation of surface plasmon vibrations in AgNPs resulted in a yellowish brown colour in the aqueous solution (Azmi & Ahyat, 2015).

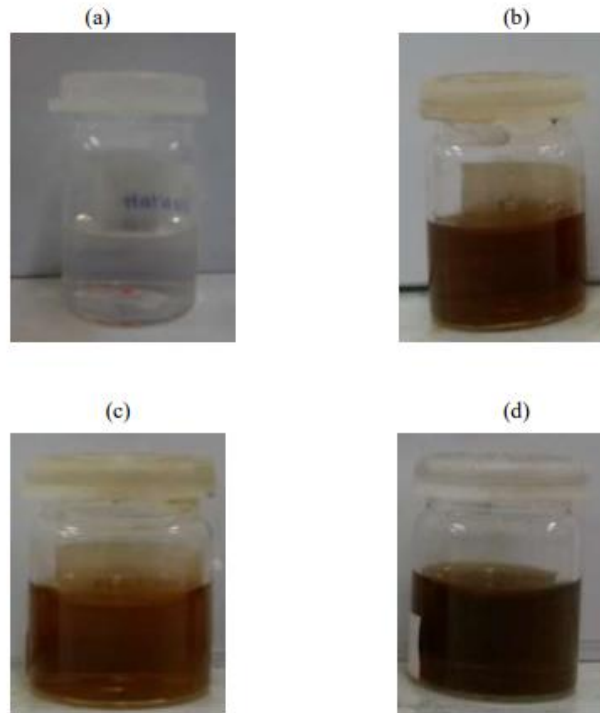


Figure 2.3: a) AgNO_3 solution before addition of *A. galanga* extract. AgNO_3 solution after addition of extract at b) 48 hours, c) 96 hours and d) 144 hours (Azmi & Ahyat, 2015)

2.3.4 Effect of Ratio of Plant Extract to Silver Nitrate

Besides, the ratio of plant extract to AgNO_3 is important in the synthesis of AgNPs. According to the literature, an excess of Ag^+ ion is required for the better synthesis of AgNPs. The effect of changing the volume of *T. peruviana* aqueous leaf extract to AgNO_3 was studied by Oluwaniyi et al., (2016) where they varied the ratio from 1:4, 2:3, 3:2, and 4:1 4:1. Excellent SPR on UV-Vis was recorded at a 1:4 ratio, indicating that the leaf extract can stabilize the NPs at 460 nm plasmon resonance. Other volume ratios did not produce distinct SPR characteristics of AgNPs in the visible UV-Vis region.

2.4 Effect of concentration of heavy metal ions solution

In this study, the concentration of heavy metal ions was varied to see the ability and also the minimum detection of heavy metal ions by AgNPs synthesized from *K. galanga* rhizome extract. The detectable concentration of heavy metal ions for visual detection was measured by adding different concentrations of heavy metal ions to an AgNPs suspension and observing colour changes in the solution, then tracking the change in absorbance in the UV-Vis spectra of the solution. Heavy metal salt solution that was used in this study are manganese (II) chloride (MnCl_2), nickel (II) chloride (NiCl_2), zinc (II) chloride (ZnCl_2), cobalt (II) chloride (CoCl_2), ferric chloride (FeCl_3), ferrous chloride (FeCl_2), aluminum chloride (AlCl_3), chromium nitrate ($\text{Cr}(\text{NO}_3)_3$), cadmium chloride (CdCl_2), lead chloride (PbCl_2), mercuric chloride (HgCl_2) and copper chloride (CuCl_2). The colour of a suspension of green synthesized AgNPs is expected to change when an equal quantity of heavy metal salt solution is added. Different heavy metal salt will give different effects on the suspension of green synthesized AgNPs.

Roy et al., (2015) prepared AgNPs from an AgNO_3 solution and leaf extract of *Dahlia pinnata*, the AgNPs solution shows discoloration after the addition of Hg^{2+} selectively. However, when other metal ions (Cd^{2+} , Cr^{3+} , Zn^{2+} , and Pb^{2+}) were added to the solution, no distinctive colour changes were observed other than broadening and a little shift of the absorbance peaks, as shown in Figure 2.6. The addition of HgCl_2 to the AgNPs solution change the light brown solution to colourless almost instantaneously, establishing the selective colorimetric detection of Hg^{2+} (Lin et al., 2010).

This result is explained by the electrochemical differences between the ions which are Hg^{2+} and Ag^+ . Ag^+ has the reduction potential of +0.80 V ($\text{Ag}^+ + e = \text{Ag}$) whereas the standard reduction potential for Hg^{2+} is +0.92 V ($2\text{Hg}^{2+} + 2e = \text{Hg}_2$) (Ravi et al., 2013). According to the electrochemical series, the higher the metals' reduction potential, the better their oxidizing powers. As a result, Hg^{2+} can oxidize colloidal silver and generate Ag^+ ions during contact, causing the solution to become discoloured. The higher reduction potential of the metals signifies better oxidizing abilities (Zhang & Ming Li, 2012). The UV-Vis spectra demonstrate that as the concentration of Hg^{2+} increased, the absorbance peak fell progressively and eventually vanished, as seen in Figure 2.7 (Roy et al., 2015).

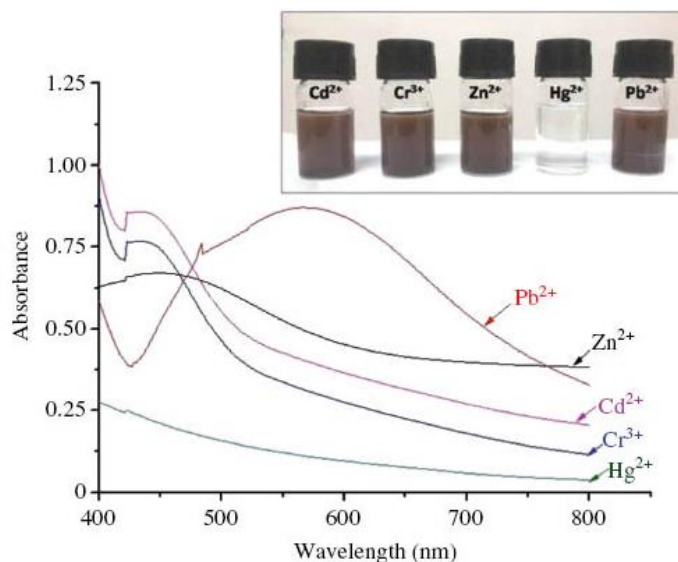


Figure 2.4: UV-Vis spectra of AgNPs solution with different metal ions; the inset figure shows the selective colorimetric sensing of Hg^{2+} ion in an aqueous solution (Roy et al., 2015)

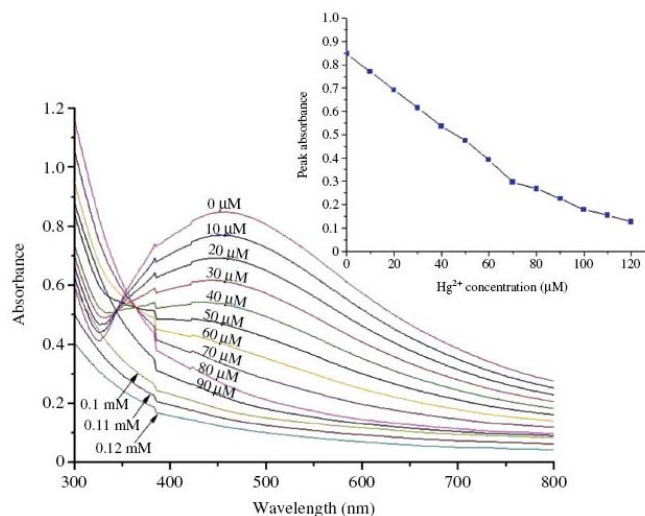


Figure 2.5: The variation of peak absorbance with Hg²⁺ concentration (Roy et al., 2015)

Barman & Bankura, (2016) observe the corresponding changes of absorption coefficient by adding different concentrations of Cu(NO₃)₂ solution which is from 0 – 75 µM and in the functionalized AgNPs. The UV-Vis spectra show that when Cu (NO₃)₂ solution was added, a new peak at around 720 nm was discovered in addition to the peak at 400 nm, as shown in Figure 2.8. More interestingly, as the concentration of Cu²⁺ ion in solution increased, the absorption coefficient of the 400 nm peak decreased, whereas the absorption coefficient of the 720 nm peak gradually increased. This could be because the SPR of functionalized AgNPs at 400 and 720 nm is related to the amount of dispersed and aggregated AgNPs. From their findings, a calibration curve of E720/400 and concentration of Cu²⁺ were plotted to detect and estimate quantitatively the presence of Cu²⁺ in the sample at µM level. From that, it is suggest that this method can be used to detect the presence of Cu²⁺ in solution with a minimum detectable limit of 25 µM

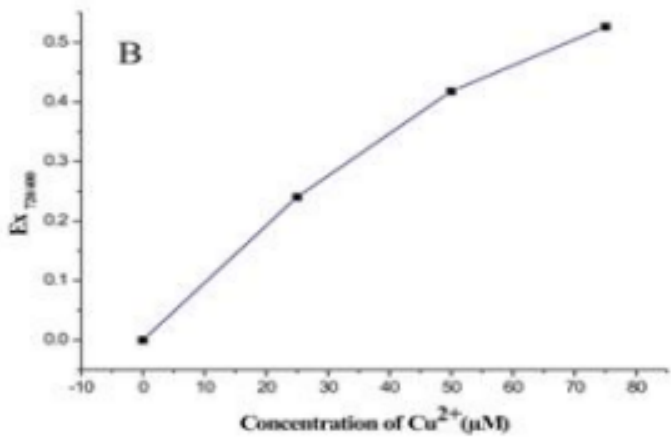
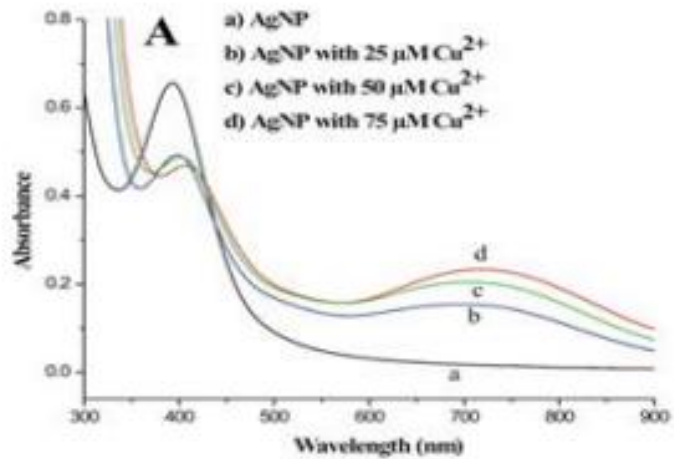


Figure 2.6: UV-Visible spectra of (A) AgNPs with varying concentration of Cu^{2+} ion (B) calibration curve between absorption ratios ($E_{720/400}$) versus concentration of Cu^{2+} ion (Barman & Bankura, 2016)

CHAPTER THREE

MATERIALS AND METHOD

This chapter explained the materials, equipment used and experimental procedures in this research. Each subsection described the list of materials and equipment followed by the experimental flowchart, detailed experimental methodology including setups and investigation steps of the parameters. Lastly, the detailed procedure of the experiment is described.

3.1 List of materials and chemicals

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

Table 3.1: List of materials and chemicals used

Materials/Chemicals	Supplier	Purpose
Silver nitrate, AgNO ₃	Bendon Laboratory Chemical	As a precursor of silver ion
<i>Kaempferia galanga</i> rhizome extract	Local herbs vendor	To reduce Ag ⁺ ions to AgNPs
Metal salt (MnCl ₂ , NiCl ₂ , CoCl ₂ , FeCl ₃ , FeCl ₂ , AlCl ₃ , Cr (NO ₃) ₃ , CdCl ₂ , PbCl ₂ , HgCl ₂ , CuCl ₂)	Bendosen Laboratory Chemical	As metal ions solutions

3.2 List of equipment

The list of equipment used in this study is listed in Table 3.2:

Table 3.2: List of equipment used

Equipment	Brand	Purpose
Electronic balance scale	Shimadzu	Weight the mass of <i>K.galanga</i> extract powder, AgNO ₃ and all metal powders
Drying Oven	Memmert	Drying the samples
Hot plate stirrer	Fisher Scientific	Stir the samples
Refrigerator	CoolTech	Store the sample
Centrifuge	Labogene Scan speed 1248R	Separation of liquid solution from impurities
Ultraviolet-visible spectroscopy (UV-Vis spectroscopy)	Thermo Scientific Genesys-20	Analysis of absorbance and wavelength of AgNPs
Scanning Electron Microscope (SEM)	Micromeritics, Model ASA 2020	Analysis of the morphology of particles
Energy Dispersive X-ray (EDX)	Quanta FEG 450	Analysis of the composition of the element in the sample

3.3 Overall flow of research

The proposed research methodology is shown in Figure 3.1

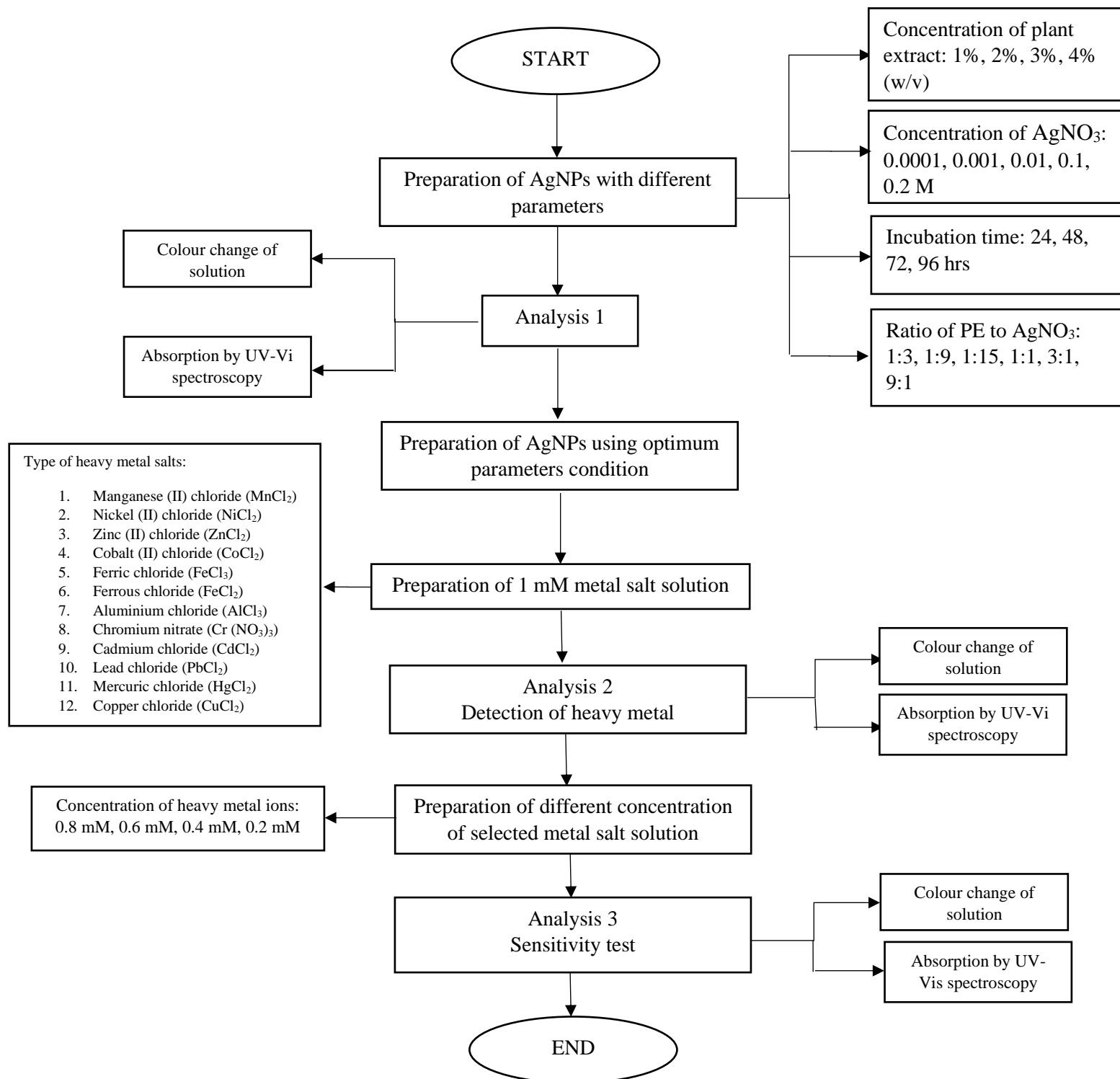


Figure 3.1: Overall experiment flowchart

3.4 Preparation of silver nanoparticles from *Kaempferia galanga* rhizome extract

There are four parameters studied in this research which include the concentration of plant extract, the concentration of AgNO₃, the ratio of plant extract to AgNO₃ and incubation time. The experimental procedures for each parameter are explained in detail as followed. Each set of parameters was repeated at least three times.

3.4.1 Effect of concentration of plant extract

The first parameter that is being studied in this research is the effect of plant extract concentration on the synthesis of AgNPs. The *K.galanga* rhizome extract was bought from a local herb vendor. The concentration of plant extract was varied at 1%, 2%, 3%, 4% (w/v). Here, the concentration of AgNO₃, incubation time and also ratio of plant extract to AgNO₃ are kept constant at 0.01 M, 48 hours and 1:3 respectively. First, to produce the plant extract, 1, 2, 3 and 4 g of *K. galanga* rhizome powder was weighted using a weighing machine separately and added into Erlenmeyer flasks containing 100 mL deionized water to make 1%, 2%, 3%, 4% (w/v) plant extract respectively. A suitable size magnetic stir bar was then inserted into the solutions before placing the solution on the magnetic stirrer. The flask was then covered using a piece of aluminum foil.

The solutions were then placed on a magnetic stirrer and started to stir for 20 minutes at 750 rpm in room temperature. After 20 minutes, the solutions were then filtered using Whatman filter paper No 1 then further filtered using a syringe filter to remove all the impurities and insoluble powder. Then, the plant extract solutions are stored in the refrigerator for further use.

After AgNO₃ was prepared, 30 mL of 0.01 M AgNO₃ was added into 4 different 100 mL reagent bottles. Then, 10 mL of different concentration plant extracts were added to make the ratio of plant extract to AgNO₃ 1:3. The bottles are then swirled a few times to let them mix. The colour of the mixture was observed before leaving it for 48 hours. The AgNPs were gradually obtained during the incubation period. Throughout the reduction process, all solutions were kept at room temperature and in a dark place to avoid any photochemical reactions. After 48 hours, the colour changes of the mixture were observed and noted. Analysis using UV-Vi spectrophotometer was done to evaluate the production of AgNPs. All steps are repeated for three times to make sure same results were observed.

3.4.2 Effect of concentration of Silver Nitrate

Five different concentrations of AgNO₃ which are 0.0001 M, 0.001 M, 0.01 M, 0.1 M, and 0.2 M were used in this research to study the effect of AgNO₃ concentration to the formation of AgNPs. For this experiment, the concentration of plant extract, incubation time and the ratio of plant extract to AgNO₃ were kept constant. For the preparation of 0.01 M AgNO₃ solution, 0.849 g of AgNO₃ was weighted using a weighing machine and transferred into a 1 L beaker containing 500 mL distilled water. The solution was then stirred until all AgNO₃ was soluble in the water. The same procedure as explained in section 3.4.1 was repeated by using different concentrations of AgNO₃. The equation of molarity was used to calculate the mass of AgNO₃ needed for a specific concentration.

Equation 1

$$m = M \times V \times 169.87$$