COLORIMETRIC DETECTION OF HEAVY METAL IONS USING GREEN SYNTHESISED SILVER NANOPARTICLES FROM *Curcuma xanthorrhiza* EXTRACT

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by

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LIST OF SYMBOLS

Symbol	Description	Unit
ρ	Density of the fluid	kg/m^3
u	Velocity of nanoparticles	m/s
d	Diameter of nanoparticles	m
μ	Viscosity of the fluid	kg/m.s
Re	Reynolds number	
σ	Standard deviation of y-intercept	
°C	Degree Celcius	
min	Minute	
hr	Hour	
mg	Milligram	
wt.%	Weight percent	

LIST OF ABBREVIATIONS

Symbol	Description
AgNPs	Silver nanoparticles
$AgNO_3$	Silver nitrate
AuNPs	Gold nanoparticles
DLS	Dynamic Light Scattering
EDS	Energy Dispersive Spectroscopy
EDX	Energy Dispersive X-Ray
LBG	Locus bean gum
LOD	Limit of detection
SEM	Scanning electron microscope
SPR	Surface plasmon resonance
TEM	Transmission electron microscope
UV-Vis	Ultraviolet-Visible
XRD	X-ray diffractometer

PENGESANAN KOLORIMETRI ION-ION LOGAM BERAT MENGGUNAKAN NANOPARTIKEL PERAK DISINTESIS HIJAU DARIPADA EKSTRAK *Curcuma xanthorrhiza*

ABSTRAK

Curcuma xanthorrhiza juga dikenali sebagai temulawak adalah herba perubatan yang boleh didapati secara meluas di Asia Tenggara. *C. xanthorrhiza* terkenal kerana sebatian bioaktif and fitokimia yang mempunyai fungsi yang tersendiri. Dalam penyelidikan ini, sebatian bioaktif *C. xanthorrhiza* digunakan untuk menangkap dan menurunkan ion perak menjadi nanopartikel perak (AgNPs). Kesan pH ke atas sintesis hijau AgNPs dikaji dalam keadaan optimum 8 % berat kepekatan ekstrak rizom, 1:5 nisbah ekstrak rizom kepada larutan perak nitrat ($AgNO_3$) dan 72 jam masa pengeraman seperti yang dijalankan oleh kerja terdahulu. Kesan pH dari 5 hingga 11 telah dikaji dan puncak keamatan penyerapan tertinggi dicatatkan pada pH 11. Morfologi AgNPs yang telah disintesis secara keseluruhannya adalah sfera dan purata saiz zarah yang disintesis pada pH 11 ialah 218.245 ± 0.123 nm. Kandungan perak yang telah direkodkan menggunakan analisis EDX ialah 76 %. Analisis ini mengesahkan ion perak telah diturunkan kepada AgNPs. AgNPs yang dibiosintesis menggunakan parameter optimum telah digunakan untuk pengesanan kolorimetri ion logam berat (Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, Cr^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, and Cu^{2+}) pada kepekatan 1 mM. AgNPs dapat mengesan ion Hg^{2+} dengan penyahwarnaan ketara daripada coklat kepada tidak berwarna melalui pemerhatian mata kasar. Puncak penyerapan pada panjang gelombang 410 nm telah hilang. Hal ini menunjukkan pengurangan AgNPs. Analisis kepekaan telah dilakukan dan had pengesanan (LOD) bagi logam untuk ion Hg^{2+} ialah 0.75 mM. Sebaliknya, AgNPs juga dapat mengesan ion Fe^{2+}, Fe^{3+} dan Al^{3+}, tetapi dengan peningkatan puncak penyerapan. Warna berubah

dari coklat kepada coklat gelap utuk ion Fe^{2+} dan kepada kuning untuk ion Fe^{3+} dan Al^{3+}. LOD untuk ion Fe^{2+}, Fe^{3+}, Al^{3+} ialah 0.431 mM, 0.688 mM, dan 0.847 mM, masing-masing.

COLORIMETRIC DETECTION OF HEAVY METAL IONS USING GREEN SYNTHESISED SILVER NANOPARTICLES FROM *Curcuma xanthorrhiza* EXTRACT

ABSTRACT

Curcuma xanthorrhiza also known as temulawak is a medicinal herb which can be found widely in Southeast Asia. *C. xanthorrhiza* is well-known because of its distinct bioactive and phytochemical compound. In this research, the bioactive compound of *C. xanthorrhiza* was used to capture and reduce silver ions into silver nanoparticles (AgNPs). Effect of pH on green synthesis of AgNPs was studied under optimal condition of 8 wt% rhizome extract concentration, 1:5 rhizome extract to silver nitrate ($AgNO_3$) solution ratio and 72 hours incubation time as conducted by previous work. The effect of pH from 5 to 11 was studied and the highest absorbance intensity peak recorded was at pH 11. The morphology of the synthesised AgNPs is spherical in overall, and the average particle size synthesised at this pH is 218.245$\pm$0.123 nm. The recorded silver content using EDX analysis was 76 %. This further confirmed silver ions were reduced to AgNPs. The AgNPs biosynthesised using the optimum parameters were used for colorimetric detection of 1 mM of heavy metal ions (Mn^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Al^{3+}, Cr^{2+}, Cd^{2+}, Pb^{2+}, Hg^{2+}, and Cu^{2+}). The AgNPs able to detect Hg^{2+} ion with a significant decolorization from brown to colorless observed using the naked eye. The absorbance peak at wavelength 410 nm had disappeared, indicating reduction of AgNPs. Sensitivity analysis was performed to determine the limit of detection (LOD) of the detected heavy metal ions. The LOD for Hg^{2+} ion is 0.342 mM. Conversely, AgNPs would also be able to detect Fe^{2+}, Fe^{3+}, Al^{3+} ions, but with an increase of absorbance peak. The color changed from brown to dark brown

and yellow, respectively. The LOD for Fe^{2+}, Fe^{3+}, Al^{3+} ions are 0.431 mM, 0.688 mM, and 0.847 mM, respectively.

CHAPTER 1.

INTRODUCTION

Chapter 1 gives the overview of this research and the significance of development of green synthesis silver nanoparticles in colorimetric detection of heavy metal ion. In overall, this chapter summarise the research background of the synthesis of silver nanoparticles and green synthesis of silver nanoparticles, the problem statement, and the objectives of this final year project.

1.1 Research Background

Heavy metal is a high atomic weight element that the metalloids property toxic to environment and humans (Tchounwou *et al.*, 2012). Heavy metal is formed naturally during the Earth's formation. However, heavy metal consumption has skyrocketed, as a result, metallic compounds are now becoming more and more prevalent in both the terrestrial environment and the aquatic environment (Gautam *et al.*, 2016). Anthropogenic activity such as metal mining, smelting, and foundries are found to be the main cause of heavy metal pollution, especially water pollution. Therefore, in order to response to this issue, water treatment is particularly important to reduce the amounts of pollutants in the water. Water treatment is coupled with pollutants detection to always trace the contaminant concentration. Therefore, the sensitive and precise detection of these heavy metals in biological and environmental samples without interference from other metal ions is crucial (Jamal *et al.*, 2021)

Silver nanoparticles (AgNPs) are currently one of the most promising research fields and is one of the most commercial nanomaterials (Chand, 2019). In general, nanoparticles are tiny materials having size ranges from 1 to 100 nm (Khan *et al.*, 2019). Metallic nanoparticles are attractive due to their unique properties that could not be achieved by bulk material. Nanoparticles have a larger surface area per unit volume which exhibits higher surface energy and consecutively promotes surface reactivity (Oluwafemi *et al.*, 2019), and nanoscale size (Khan *et al.*, 2019). It is reported that the application of nanoparticles varies from biosensors to optics, to the catalyst, to environmental remediation, and anti-microbes studies (Chand, 2019; Vanaamudan *et al.*, 2016).

The application of AgNPs, in particular, is getting more attention in colorimetric detection due to the excellent properties in surface plasmon resonance (SPR) and utilization in fluorescence (Tripathy *et al.*, 2013). Many have been published on selective colorimetric detections of heavy metal ions using AgNPs as probe sensors over the past two decades (Oluwafemi *et al.*, 2019). However, the synthesis of AgNP raises the environmental issues either from bottom-up or top-down approaches (Khan *et al.*, 2019; Sakly *et al.*, 2017). The destructive approach is employed in top-down synthesis; it involves the size reduction using mechanical means such as milling, grinding, and physical vapor deposition (Khan *et al.*, 2019). Hence, studies on the green-mediated synthesis of nanoparticles have been investigated due to being facile, nontoxic, naturally abundant, and cost-effective (Manjari *et al.*, 2020). Green synthesis of AgNPs is a bottom-up approach where the silver ion in the silver nitrate serves as the fundamental building block. Here, the silver ion is reduced by the plant's extract to atom which then nucleate in small clusters that grow into particles (Kumar *et al.*, 2020).

In this study, *Curcuma xanthorrhiza* extract was used for the green synthesis of AgNPs. *C. xanthorrhiza,* also known as temulawak, is a medicinal plant in the family Zingiberaceae (Gale *et al.*, 2007). It is found in abundance in Indonesia, Malaysia, and Thailand, as well as in China and India (Mishra *et al.*, 2018). This plant has distinct bioactive and phytochemical compounds, including xanthorrhizol, curcuminoids, phelandrene, camphor, tumerol, sineol, borneo, and flavonoids (Rohman *et al.*, 2020). These compounds serve as reducing and capping agents in the green synthesis of the nanoparticles. The capping agents not only increase the protection of the nanoparticle core but also provide suitable functionalization to interact specifically with heavy metal ions (Prosposito *et al.*, 2020).

C. xanthorrhiza has been used to produce environmentally friendly gold nanoparticles (AuNPs) by bio-reduction of $HAuCl_4$ and applied in the degradation of Congo red dye (Khairiza *et al.*, 2020). Besides producing metallic nanoparticles, the curcuminoids from *C. xanthorrhiza* also have been used to produce solid lipid nanoparticles to improve the solubility in water (Ambarsari *et al.*, 2014). **Figure 1.1** below shows the aerial, flower, rhizome part, and rhizome powder of *C. xanthorrhiza*.

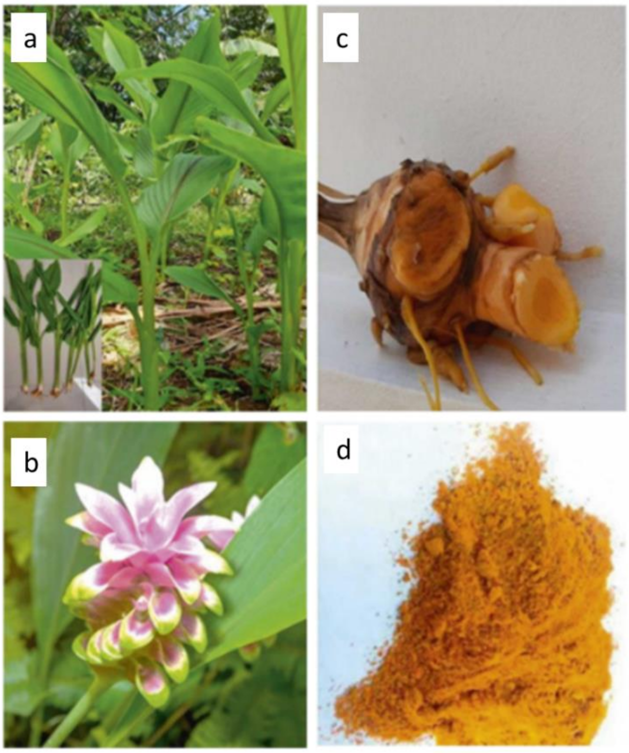


Figure 1.1 *Curcuma xanthorrhiza* Roxb. a) aerial parts, b) flower, c) rhizome, and d) rhizome powder (Rahmat *et al.*, 2021).

There are rooms to explore the uses of *C. xanthorrhiza* for the green synthesis of AgNPs as there are limited reports on the usage of this plant in this field. Although successful biosynthesis of AgNPs using *C. xanthorrhiza* extract has been reported (Nathan, 2021), the influence and the effect of pH on the green synthesis of AgNPs from *C. xanthorrhiza* extract is still unknown. Therefore, the motivation of this study is to fill in the research gap, which is to understand the role of pH on the green synthesis of AgNPs using *C. xanthorrhiza* extract and its colorimetric detection application towards heavy metal ions.

1.2 Problem Statement

Heavy metal ion pollution is still a serious issue in industrial emission due to the adverse effects causing towards life and environment. Heavy metal unable to be broken and are nonbiodegradable (Briffa *et al.*, 2020). It will bioaccumulate in organism system and causes biological and physiological problem. Therefore, to develop an immediate detection of heavy metal content is indeed crucial. Conventionally, the specific water contaminants determination is conducted in variety methods such as using inductively coupled plasma mass spectrometry, high performance liquid chromatography, atomic fluorescence spectroscopy and flame atomic absorption spectroscopy (Prosposito *et al.*, 2020). This conventional heavy metal ion detection is too time consuming and requires expensive analytical equipment. Thus, it is critical to have a simpler and cheaper heavy metal ion detection strategy. Silver nanoparticles have been proven as an effective colorimetric sensor to few heavy metal ions as stated in the literature review. The interactions between some of the heavy metal ions and nanoparticles would reduce the intensity of the coloured solution. This indicates successful detection of heavy metal ion using nanoparticle via colorimetric assay. The traditional synthesis of metal nanoparticles could create a destructive effect such as large amount of waste generation and the use of toxic chemicals and organic solvent. Therefore, green synthesis has received much attention as an alternative way to promote more sustainable and eco-friendlier approach for synthesizing metal nanoparticles. In this research, *C. xanthorrhiza* extract has been proposed to be used as the reducing and capping agent to synthesis AgNPs. Silver nanoparticles that exist in a brownish-yellow solution turn out to be a good colour indicator. It will turn colourless when in contact with heavy metal ions. Up to now, the role of pH on the green synthesis of AgNPs from *C. xanthorrhiza* has not been reported. As a result, it is worthwhile to complete the missing parameter puzzle. Hence, this research will be studying the effect of pH using *C. xanthorrhiza* rhizome extract biosynthesis of AgNPs, and the application of the synthesised AgNPs on the colorimetric detection of heavy metal ions.

1.3 Research Objectives

The objectives of this research are:

i. To investigate the effect of pH using *C. xanthorrhiza* extract to biosynthesis AgNPs.

ii. To develop heavy metal ions colorimetric detection using green synthesised AgNPs

CHAPTER 2.

LITERATURE REVIEW

Previous chapter reveals the overview of AgNPs and the selection of *C. xanthorrhiza* rhizome extract as a reducing and capping agent to synthesis AgNPs. Chapter 2 presents the physics of nanoparticles from credible scientific records and references that are related to this final year project. This chapter covers the overview of AgNPs, green synthesis of AgNPs, factor influencing the green synthesis such as concentration of plant extract, concentration of silver nitrate, incubation time, and pH. Besides, this chapter also reviews some application of AgNPs in colorimetric detection of heavy metal ion and discloses the knowledge gap.

2.1 Silver Nanoparticles

Nanoparticles are generally defined as particulate dispersions that have a size in the range of 1 to 100 nm (Khan *et al.*, 2019; Mohanraj and Chen, 2007). At this nanoscale size, particles behave differently from the macro size approach. It comes to an extent that a low Reynolds number has been imposed on nanoparticles. This observation indicates that the viscous force is dominating the inertial force. The motion of nanoparticles in water is very majestic, slow, and regular (Purcell, 1977). Therefore, external forces would easily alter the Brownian motion of nanoparticles especially metallic based on magnetic and electrostatic interactions (Li and Rothberg, 2004). The ratio of inertial forces to the viscous forces or Reynolds number can be calculated using Equation 2.1.

$$Re = \frac{\rho u d}{\mu} \qquad (2.1)$$

Here, ρ is the fluid density, u is the particle velocity, d is the particle diameter, and μ is the fluid viscosity. The viscous force dominates the inertial force when the magnitude of denominator in Equation 2.1 is much larger than numerator. This behaviour signifies the cause of Brownian motion. This distinct characteristic exhibit SPR which only observable in nanoscales. In the context of colorimetric detection, it is important to observe the color changes based on the optical properties. AgNPs in this case has been chosen for the colorimetric detection of the heavy metal ion. AgNPs has received much attention due to the raising demand in several fields such as catalyst, biosensing, antibacterial, antioxidant, and anticancer (Kumar *et al.*, 2017). AgNPs is selected over the gold nanoparticles (AuNPs) due to their stronger SPR (Solati and Dorranian, 2015). The selection of AgNPs over AuNPs is also justified by the plasmon resonance of AgNPs appearing at a shorter wavelength (420 nm), which in turn allow a higher degree of freedom in tuning plasmon resonance frequency (Yong *et al.*, 2006). The metal surface of AuNPs has larger reflectivity that leads to a loss in the absorption intensity of gold compared to silver (Solati and Dorranian, 2015). In addition, AgNPs has a sharper extinction bands and higher extinction coefficients (Badi'ah *et al.*, 2019). This made AuNPs less attractive in selection of nanoparticles.

2.2 Green synthesis of Silver Nanoparticles

There are three foremost condition for the nanoparticles synthesis align with the "Green Chemistry", the selection of green or environmental-friendly solvent, a good reducing agent, and a harmless material for stabilization (Jadoun *et al.*, 2021). In general, the synthesis of AgNPs could be categorised into two methods, which are top-down and bottom-up approaches. Green synthesis or biosynthesis falls in the bottom-up method as the nanoparticles are synthesised from atomic and molecular basis. It is a biobased strategy that utilizing plant extract to act as reducing and capping agents to reduce silver ion into nanoparticles (Nadzir *et al.*, 2019). The parameters such as temperature, pH, plant extract concentration, and incubation time could control the stability, shape, and size of nanoparticles (Jadoun *et al.*, 2021). The green synthesis mechanism of metal nanoparticles using plant extract can be separated into three phases (Makarov *et al.*, 2014). It starts with the activation phase which both reduction of metal ions and nucleation of the reduced metal ions. Then, the coagulation of small adjacent nanoparticles into a larger size during the growth phase. In this phase, it is thermodynamically stable as the nucleation and reduction continuously occur. Lastly, termination phase which decide the final shape formed from the nanoparticles. The schematic diagram for the synthesis of metal nanoparticles is shown in **Figure 2.1**.

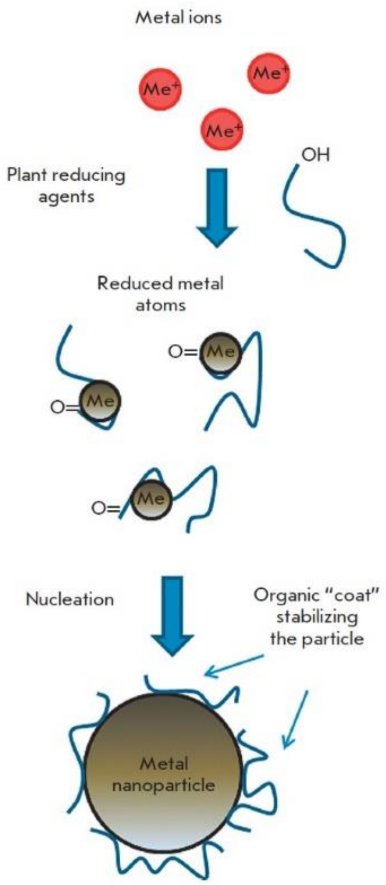


Figure 2.1 Schematic diagram representation of metal nanoparticles synthesis in a plant extract (Makarov *et al.*, 2014).

Green synthesis of AgNPs using different plant extracts were studied by numerous research and their synthesis parameter were summarized in **Table 2.1**. Dubey *et al*., (2009) conducted research on the use of *Eucalyptus hybrida* leaf extract in the extracellular biosynthesis of AgNPs. $AgNO_3$ solvent was used as the silver source in a 48-hour reaction. The estimated AgNPs size from full width at half maximum using Scherrer formula is 50 to 150 nm. Flavanoid and terpenoid are the bioactive compounds responsible to stabilize the nanoparticles (Shankar *et al.*, 2003). The shape of the AgNPs is mainly made up of cubical shape.

Table 2.1 Overview of green synthesis of AgNPs from different plant extract.

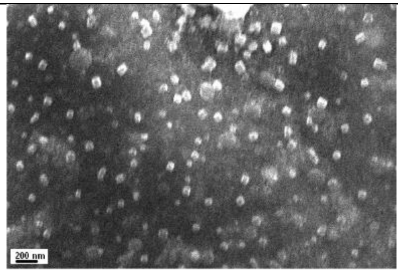
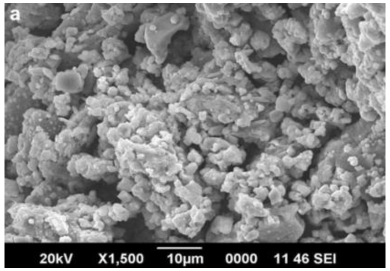
Plant	**pH**	**Reaction time (hours)**	**Average size (nm)**	**Shape**	**Image of AgNPs**	**Reference**
Eucalyptus hybrida leaf	-	48	50 – 150	Cubical		(Dubey *et al.*, 2009)
Nelumbo lucifera leaf extract	-	8	25 – 80	Spherical, triangle, truncated triangles, and decahedral		(Santhoshkumar *et al.*, 2011)

Table 2.1 (Continued)

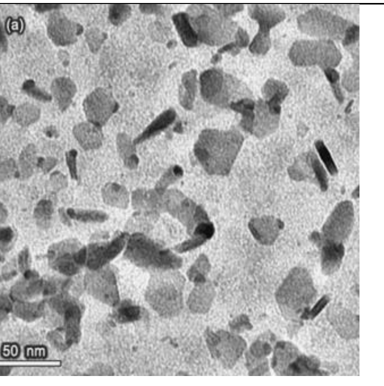
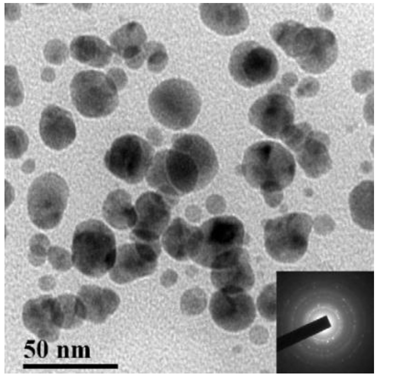
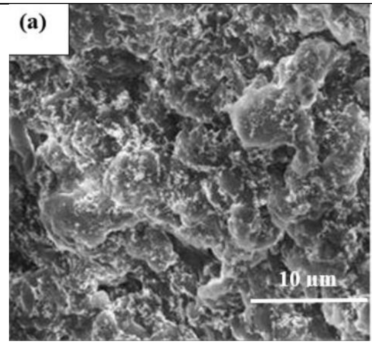
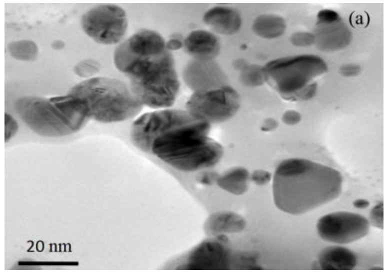
Plant	pH	Reaction time (hours)	Average size (nm)	Shape	Image of AgNPs	Reference
Hibiscus rosa sinensis	6.8	1.5	13	Spherical		(Philip, 2010)
Jatropha curcas seed extract	-	-	15 – 50	Spherical		(Bar *et al.*, 2009)

Table 2.1 (Continued)

Plant	pH	Reaction time (hours)	Average size (nm)	Shape	Image of AgNPs	Reference
Citrus macrocarpa peel extract	-	48	235	-		(Jie *et al.*, 2020)
Elaeis Guineensis leaf extract	5-10	7	55 – 85	Polydisperse and irregular		(Ramli *et al.*, 2015)

The characteristic of the AgNPs are influenced by the type of the plant extract and the synthesis parameters. Santhoshkumar *et al*., (2011) conducted research using *Nelumbo lucifera* leaf extract to synthesis AgNPs. The reaction time between the $AgNO_3$ and the plant extract was for 8 hours. Through transmission electron microscope (TEM) observation, the dominant shape of AgNPs is made up of spherical, triangle, truncated triangles, and decahedral with the dimension of 25 to 80 nm.

Philip, (2010) attempted the use of *Hibiscus rosa sinensis* to synthesis AgNPs. In this study, the pH of the solution is adjusted to 6.8 to initialize the reduction of silver ions. The formed AgNPs were mainly spherical with an average size of 13 nm. The role of pH in the green synthesis affects the size formation, thus, results in a broad SPR band.

Bar *et al*., (2009) used *Jatropha curcas* seed extract for green synthesis of AgNPs. In this study, the synthesised AgNPs were mainly spherical with a diameter of about 20 nm. Higher concentration of $AgNO_3$ solution result in larger and rocky shaped particles with diameter 30 to 50 nm.

Jie *et al*., (2020) studied the green synthesis of AgNPs using *Citrus macrocarpa* peel extract. The effect of incubation time was studied by varying the incubation time from 0 to 72 hours. Longer incubation time led to longer reaction time to reduce the silver ion into nanoparticles. This conclusion is justified by the observation of the peak of the Ultraviolet-Visible (UV-vis) spectrum. 100 to 500 nm particle diameter was observed in the study. This measurement might include the biomolecules size that attached on the surface of AgNPs.

Ramli *et al*., (2015) reported the use of *Elaeis Guineensis* extract to synthesis AgNPs. The plant extract and $AgNO_3$ were left to react for 7 hours. The effect of pH on the synthesis of AgNPs was studied at different pH (5, 6, 7, 8, 9, and 10). Agglomeration of AgNPs was observed at lower pH due to nucleation occurs at acidic environment. In alkali condition, large amount of AgNPs were observed with small surface area due to the bioavailability of functional groups in the *E. Guineensis* extract. Thus, alkaline pH is favourable to synthesis AgNPs with smaller size and larger amount. The AgNPs synthesised in the study are polydisperse and has irregular shape with the size ranging from 55 to 85 nm. The overview of green synthesised AgNPs using different plant extract is given in **Table 2.1**. $AgNO_3$ solution was used as the silver source in all these experiments.

2.3 Factor influencing the synthesis of silver nanoparticles

Some of the parameters affecting the green synthesis of AgNPs includes the incubation time, temperature, and the extract concentration and $AgNO_3$ concentration (Jie *et al.*, 2020). In this section, plant extract concentration, silver nitrate concentration, ratio of silver nitrate to the plant extract, incubation time, and the effect of pH are being discussed.

2.3.1 Effect of the plant extract concentration on the green synthesis

Plant extract concentration is one of the parameters that affect the green synthesis of AgNPs. Plant extract in this context acts as the reducing, capping, and stabilizing agent to capture silver ions into nanoparticles. However, it is not necessary that the higher plant extract concentration would yield better quality of nanoparticles. In this section, several optimal plant extract concentrations from different literature studies are discussed. The summary of effect of plant extract concentration from different studies is tabulated in **Table 2.2**.

Table 2.2 Overview of the green synthesis of AgNPs under the effect of plant extract concentration.

Plant extract	**Concentration of plant extract (wt.%)**	**Important findings**	**References**
Locus bean gum polysaccharide	0.1, 0.2, 0.3, 0.4	• Absorption peak was observed at 424 nm wavelength • The peak is blue shifted with increasing concentration of plant extract which indicates the reduction in particle size • No obvious peak was observed at 0.3 wr%, and 0.4 wt% • The peak is found to decrease with increased concentration of plant extract • Higher concentration of plant extract provides a greater embedment of nanoparticles in polymer matrix • The optimal extract concentration is 0.1 wt%	(Tagad *et al.*, 2013)

Table 2.2 (Continued)

Plant extract	Concentration of plant extract (wt.%)	Important findings	References
C. microcarpa peel extract	2, 5, 10	• The color intensity increased from pale yellow to deep brown with the increase of concentration of plant extract which indicates the formation of AgNPs • 5 wt% of peel extract concentration resulted in maximum absorption • The absorption peak was observed around 450 nm • Significant decrease of absorption peak was noticed when the concentration increased to 10 wt%. This could be because excess peel extract results in agglomeration of AgNPs to a larger particle size • Larger size reduces the absorbance due to decrease in particle density	(Jie *et al.*, 2020)

Table 2.2 (Continued)

Plant extract	Concentration of plant extract (wt.%)	Important findings	References
Aloe vera leaves extract	5, 10, and 15 % (Volume ratio)	• Higher extract volume ratio results in the increase of the absorbance because more formation of AgNPs caused by availability of more functional groups in the leaf extract • The best extract vol.% for the synthesis of AgNPs in this project is 15 vol.%	(Moosa *et al.*, 2015)
Lawsonia inermis leaf extract	0.1, 0.2, 0.3, and 0.4 ml plant extract	• Absorbance increased as the concentration of leaf extract increase which might be due to higher reduction of $AgNO_3$ • The optimal reduction concentration for this leaf extract is 0.4 ml with 50 ml of $AgNO_3$ solution • Further increase plant concentration would lead to blueshift in SPR, hence, reduce the particle size of AgNPs	(Daniel *et al.*, 2013)

Table 2.2 (Continued)

Plant extract	**Concentration of plant extract (wt.%)**	**Important findings**	**References**
Olive leaf extracts	0.5, 1, 3, 4 and 5 ml plant extract	• Color changed from pale yellow to yellowish brown to deep brown with the increased of extract concentration due to the excitation of surface plasmon vibration • The SPR of AgNPs is around 440 to 458 nm • Increase in extract concentration decrease the particle size	(Khalil *et al.*, 2014)

Tagad *et al*., (2013) manipulated the concentration of plant extract from 0.1 to 0.4 wt.% using *Locus bean gum* (LBG) polysaccharide. A peak was observed at wavelength 424 nm and 418 nm when the plant extract concentration was 0.1 and 0.2 wt.%, respectively. The blue shift of the wavelength was explained by the reduction in the particle size. However, there is no obvious peak observed at the concentration of 0.3 and 0.4 wt.% in their research. Theoretically the absorption intensity was expected to increase with the increase in concentration of plant extract. This result was explained by the nature of LGB which made up from viscous polymer matrix that capped nanoparticles strongly at higher extract concentration. In addition, high concentration of extract might reduce the movement and diffusion of the silver ion to be attached to functional groups from extract. Therefore, the amount of AgNPs present was reduced. The optimal concentration of the plant extract in their research is 0.1 wt.%.

In Jie *et al*., (2020) research, plant extract concentration of 2, 5, and 10 wt.% was manipulated to evaluate the effect of *C. microcarpa* peel extract on the green synthesis. The color of the solution changed from pale yellow to yellowish brown and deep brown with the increased of plant extract concentration. The color change indicates the formation of AgNPs and the increase in intensity was caused by the increase amount of AgNPs. Higher plant extract concentration would promote more reduction of silver ions in the aqueous solution. The optimum plant extract concentration in this research is 5 wt.% with a maximum absorption wavelength at 450 nm. Nevertheless, the absorbance intensity decreased when the extract concentration increased to 10 wt.%. This might be due to excess peel extract led to agglomeration of AgNPs and become a larger particle size. Larger particle size would lead to decrease in particle density and hence decrease in absorbance.

Through the study conducted by Moosa *et al*., (2015), aloe vera plant leaves extract was used as reducing agent to cap the silver ions in green synthesis of AgNPs. The plant extract concentration used in this research is ranging from 5, 10, and 15 % volume ratio of leaves extract to the $AgNO_3$ solution. The SPR band for all range of volume ratio was centered at 477 nm wavelength. Similarly, the absorbance was increased with the increased of extract volume. The average diameter of the AgNPs synthesised in this research was found to be 68.31 nm, 60.27 nm, and 51.64 nm at 5, 10, and 15 ml of plant extract, respectively. The increased in plant extract concentration indicate the increased in the functional group that would improve the rate of formation of AgNPs and reduce the size of AgNPs. 15 % extract volume was found to be the best for the synthesis of AgNPs in their research.

Daniel *et al*., (2013) studied the effect of plant extract concentration to the green synthesis of AgNPs using *Lawsonia inermis* leaf extract. The extract quantity was varied from 0.1, 0.2, 0.3 and 0.4 ml with the same $AgNO_3$ (50 ml of 1 mM) solution. The absorbance increased with the increased of the concentration of leaf extract. For *Lawsonia inermis* leaf extract, it was found that 0.4 ml leaf extract has a better reduction of $AgNO_3$. Further increased in the extract concentration would cause blue shift in SPR which indicates reduction in particle size of AgNPs. The result from this research agrees with the results presented by the other researchers. **Figure 2.2** below illustrates the effect of plant extract concentration on the green synthesis of AgNPs studied by Tagad *et al*., (2013).

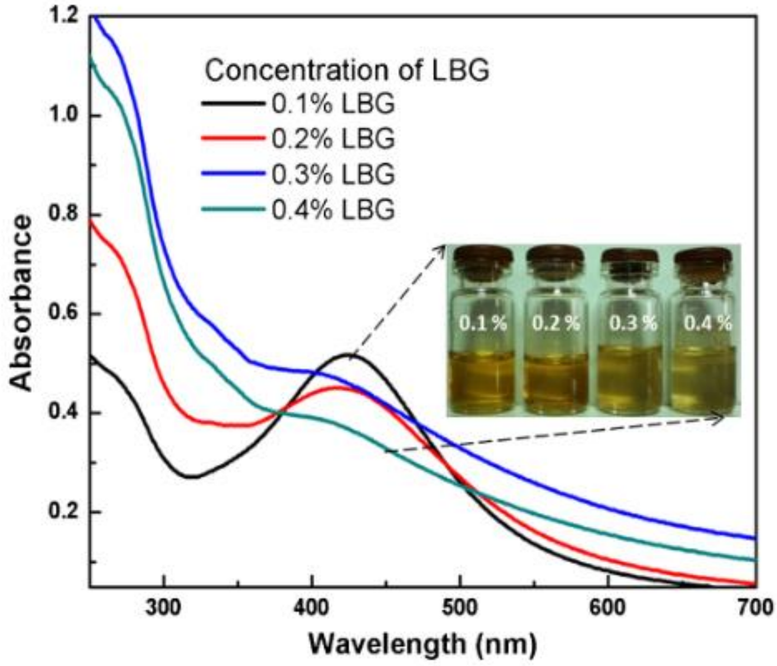


Figure 2.2 UV-vis spectra of AgNPs synthesised using different concentration of LBG (Tagad *et al.*, 2013).

2.3.2 Effect of the silver nitrate concentration on the green synthesis

Silver nitrate concentration is one of the parameters that affects the quality of green synthesised AgNPs. It acts as the silver ions source to be capped and reduced to produce AgNPs. However, it is not necessary that the higher $AgNO_3$ concentration would yield better quality. In this section, the optimal concentration of $AgNO_3$ from several reported studies is discussed. The summary of the effect of silver nitrate concentration from different studies is tabulated in **Table 2.3**.

Table 2.3 Overview of the green synthesis of AgNPs using different concentration of silver nitrate.

Plant extract	Concentration of $AgNO_3$ (mM)	Important findings	References
Locus bean gum polysaccharide	1 to 5	• Absorption intensity increase with an increase in $AgNO_3$ concentration • Similar surface plasmon bands are formed regardless the concentration of $AgNO_3$ • Increase number of AgNPs was observed with an increase in the concentration of $AgNO_3$	(Tagad *et al.*, 2013)
Banana peel extract	0.25 to 2.0	• At the range of 0.25 to 0.5 mM $AgNO_3$, yellow brown and light reddish-brown colors were observed • Darker brown color were observed at the concentration range between 1.0 to 2.0 mM • 1.75 mM $AgNO_3$ has the maximum peak intensity	(Ibrahim, 2015)
Acalypha wilkesiana extract	1, 2, 4, 6, 10	• Particle size increase at higher concentration • Optimum concentration of 1 mM gave the best SPR peak at 450 nm wavelength	(Dada *et al.*, 2019)

Table 2.3 (Continued)

Plant extract	Concentration of $AgNO_3$ (mM)	Important findings	References
C. microcarpa peel extract	2, 4, 6, 8	• Dark brown color was observed at the concentration of $AgNO_3$ at 8 mM • Intensity of the color increased significantly with the increased in $AgNO_3$ concentration • Maximum absorbance was recorded at 8 mM at a wavelength around 450 nm	(Jie *et al.*, 2020)
Tithonia diversifolia extract	1, 2, 4, 6, 8, 10	• The intensity increases as the concentration of $AgNO_3$ increase • A distinctive SPR peak at 430 nm was obtained at 1 mM concentration • The change in intensity was due to bathochromic shift, leading to a broader band, lower size, and higher aggregation • Best SPR peak was obtained at 1 mM concentration which results in good shape and size control	(Dada *et al.*, 2018)

Tagad *et al*., (2013) studied the effect of $AgNO_3$ concentration on the synthesis of AgNPs using LBG extract. The AgNPs was prepared using different concentration of $AgNO_3$ ranging from 1 to 5 mM. It was observed that the absorbance intensity increased with the increased of $AgNO_3$ concentration. This showed it has higher reduction efficiency of silver ions to AgNPs at higher $AgNO_3$ concentration. The amount of synthesised AgNPs increase with the increase in the concentration of $AgNO_3$. This is because more silver source is available to be capped and reduced to AgNPs.

The study conducted by Ibrahim, (2015) using banana peel extract varied the $AgNO_3$ concentration from 0.25 to 2.0 mM. At the $AgNO_3$ concentration of 0.25 to 0.5 mM, yellow brown and light reddish-brown colors were observed, respectively. Darker shades of brown color were observed at the concentration range between 1.0 to 2.0 mM. The SPR peak become distinct as the concentration of $AgNO_3$ increase. A maximum peak intensity was observed at 1.75 mM $AgNO_3$.

Dada *et al*., (2018) had conducted an experiment to evaluate the role of $AgNO_3$ concentration on the green synthesis of AgNPs using *Tithonia diversifolia* extract. The concentration carried out in the research is 1, 2, 4, 6, 8, and 10 mM. It was observed that the intensity increased as the concentration of silver ion increase. A distinctive SPR peak at 430 nm was obtained at 1 mM. Through the study, higher concentration (4, 6, 8, and 10 mM) would lead to a broader band, smaller size, and greater aggregation of AgNPs; lower concentration (1 and 2 mM) yields higher intensity, better absorbance, and narrower bands. The concentration of $AgNO_3$ would greatly affect the particle size. The best SPR recorded in this study was at $AgNO_3$ concentration of 1 mM, which gives a well dispersed size range and spherical shape.