Ionic Liquid Mediated Green Synthesis of Gold Nanoparticles using Elaeis Guineensis (Oil Palm) Leaf Extract

by

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Dissertation submitted for partial fulfilment of
the requirements for the
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Universiti Teknologi PETRONAS
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Perak

CERTIFICATION OF APPROVAL

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Approved by,

____
(Assoc. Prof. Dr Sekhar Bhattacharjee)

UNIVERSITI TEKNOLOGI PETRONAS
BANDAR SERI ISKANDAR, PERAK
September 2015

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgment, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

NORHAMIZAH HAZIRAH BINTI AHMAD JUNAIDI

ABSTRACT

In recent years, ionic liquids (ILs), as green and designer solvents, have accelerated research in analytical chemistry. This green synthesis of gold nanoparticles (AuNPs) has emerged as an important area in nanotechnology primarily due to its eco-friendly approach. In this project, AuNPs were synthesized using palm oil leaf extract. Palm oil leaf, which is considered as the major waste in palm oil industry are chosen since it possess anti-oxidant properties. It serves as a reducing agent and stabilizing agent during the synthesis process, palm oil leaf extract simultaneously, hence, no additional reagent such as surfactant, template, and capping agent are needed. Green synthesizing method is rapid, cost effective, nontoxic and eco-friendly and these features are making it more compatible for biomedical application. The characteristics of the synthesized AuNPs were analyzed using UV-Visible Spectrophotometer that confirms the presence of AuNPs.

ACKNOWLEDGEMENT

First and foremost, I praise to God, the almighty for granting me with good health and strength to complete this Final Year Project (FYP). This thesis was successfully done with the assistance and guidance from several parties. Therefore, I would like to take this opportunity to acknowledge and extent my heartfelt gratitude to the persons who made the completion of this Final Year Project possible. Firstly, I would like to extent my gratitude to my beloved parents, for their countless support.

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CHAPTER 1
INTRODUCTION

1.1 Background

Ionic liquids (ILs) are organic salts that melt below 100 °C. Interest in ILs stems from their potential application as ‘green solvents’. Specifically, their non-volatile character and thermal stability make them attractive alternatives for volatile organic solvents.

According to Itoh, Naka, & Chujo (2003) room-temperature ionic liquids are attracting much interest in many fields of chemistry and industry, due to their potential as a “green” recyclable alternative to the traditional organic solvents. Ionic liquid are non-volatile and provide an ultimately polar environment for chemical synthesis. Some ionic liquids are immiscible with water and organic solvents, giving biphasic ionic liquid systems, which enables easy extraction of products from the ILs.

Ionic liquids are used in leave extraction process because it is reported to have higher efficiency for the gold synthesizing. Therefore, a study of the effect of solvent concentration in the solution is necessary. According to Tuutijarvi (2009), synthesis of metal nanoparticles such as gold nanoparticles (AuNPs) have been of great interest due to their distinctive features such as optical, catalytic, electrical and magnetical properties. Physical and chemical methods are the example of methods that have been developed to synthesize well-defined metal nanoparticles. However, in medical application, the energy intensive chemical and physical methods of synthesizing AuNPs are not favoured when nanoparticles are to be used since it involves toxic and aggressive chemicals as reducing and capping agents (Milaneze, 2013).

Green synthesis of AuNPs using plants extracts has been carried out by many scientist and researchers. Most of developed green synthesis approaches are using plant species which are difficult to be found in Malaysisa such as *Morinda citrifolia L*, *Terminalia arjuna* and *Rosa rugosa*. However, there are less researchers are using ionic liquid as the solvent for the green synthesis.

In the past, ionic liquids have been used in aqueous solvent systems for the extraction of alkaloids. For example, Li et al (2005), described the extraction of the opium alkaloids codeine, papaverine and morphine from biological samples, using ionic liquids in two phase solvent systems. The ionic liquid used was BMIM (1-butyl- 3-methylimidazolium) chloride, and an additional salt such as K_2HPO_4 was used to ensure the correct extraction environment, in particular the correct pH.

Therefore, this study will explore the effect of ionic liquid as the solvent for plant extraction in synthesizing AuNPs using oil palm leaf extract as an alternative to the available methods. According to Yin et. al. (2013), oil palm leaf are chosen over the other parts of the oil palm tree because it possesses anti-oxidant properties under-utilised and also considered as a major waste in the oil palm industry.

1.2 Problem Statement

Synthesizing of gold particles (AuNPs) through physical and chemical method are not safe to be used in biomedical industry since the process involves hazardous chemicals as reducing and stabilizing agents. Currently, scientists and researchers are working on green synthesis as an alternative approach by using various plant extract. However, most of the established green approaches are using plant species which are difficult to find or having other significant role. Therefore, it is vital to develop an approach of synthesizing AuNPs using plant species or biomass which are widely available in Malaysia. Since oil palm leave is considered as a major waste in the oil palm industry, and due to its anti-oxidant properties, oil palm leave extract can be used as the reducing agent in the AuNPs synthesizing process. In order to get better solvent efficiency, ionic liquid is introduced as the solvent for the leave extraction process. Ionic liquid shows wide range of unique properties such as extremely low vapour pressure, good microwave-absorbing ability and excellent dissolving and extracting ability. Hence, there is a need for developing an approach of synthesizing AuNPs using plant species or biomass using ionic liquid as the solvent for extraction process of the leaves.

1.3 Objectives

The main objectives of this project are:

a) To synthesize gold nanoparticles (AuNPs) using oil palm leaf extract.

b) To determine the effect of ionic liquid in synthesizing AuNPs.

c) To study morphological characteristics of the synthesized gold nanoparticles.

1.4 Scope of Study

In this project, ionic liquids are used as the solvent to get the leave extract from Elaeis Guineensis (oil palm) leave. The conditions involves in the synthesis process includes the concentration of the solvent, temperature and the extraction time. Gold nanoparticles (AuNPs) are to be synthesized by using this leave extract as the reducing agent to reduce chloroauric acid ($HAuCl_4$). The optimum conditions for the formation of AuNPs will be determined by manipulating reaction parameters which includes the concentration of acid, volume of leaf extract, temperature and reaction time. Characterization on the synthesized AuNPs were done using UV-Visible Spectrophotometer. From the UV-Vis spectrophotometer, the presence of AuNPs in the colloidal solution will be confirmed.

CHAPTER 2
LITERATURE REVIEW

2.1 Properties of Ionic Liquid

In simple word, ionic liquids (ILs) can be easily defined as salt in liquid form consisting of ion and ion pairs. ILs have received escalating attention recently in basic research because of its vast potential as a green replacement for volatile organic solvents. It can be defined that Room Temperature Ionic liquids (RTILs) are salts that normally melt at low temperature which are most likely ambient temperature.

Since ionic liquids are composed of only ions, therefore they show very high ionic conductivity, non-volatility, and non-flammability. The use of ionic liquids has been considered as the key element in designing more environmentally benign methods for separation processes. ILs display several properties as solvents that make them feasible as a potential basis for a green separation processes, such as negligible vapour pressure, good thermal stability and a wide liquid range.

In recent years, most of the attention in ionic liquids are focused on the design of new solvents. Not only that, the development of the new type of solvents has led to more possible applications of ILs. Besides, there is also more potential for development in electrochemical applications as well. Figure 2.1 below shows the potential applications of ionic liquid various industries as according to Robin (2005).

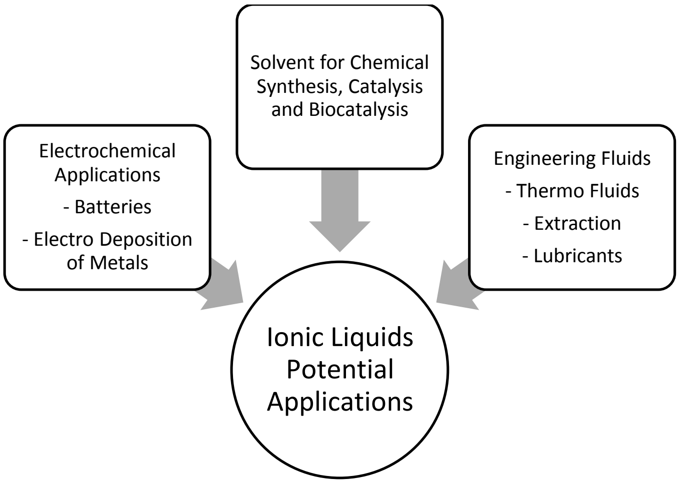

Figure 2.1 Application of Ionic Liquids

Basically, Room temperature ionic liquids (RTILs) are organic salts that exist in liquid form at ambient temperature. Normally, it is composed of relatively large asymmetric organic cations and inorganic or organic anions. According to Mizuuch (2007), this RTIL have great interest in the industry as a green solvents, due to the impressive characteristics of RTILs such as insignificant vapour pressure and solubility of a wide range of organic and inorganic compounds. One of the examples of RTIL is 1-alkyl-3- methylimidazolium.

Imidazole is defined as organic compounds of the heterocyclic series which consists of three carbon atoms and two nitrogen atoms at nonadjacent positions. The formula of imidazole is $C_3H_4N_2$. Figure 2.2 below show the molecular structure of imidazole.

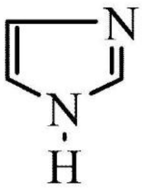

Figure 2.2 Molecular Structure of Imadazole

Imidazolium salts are salts that have cation of imidazole, such as imidazolium chloride. This type of salts have been used widely in ionic liquids application because of their ability to infinitely be recycled and remain as solvent or liquid at room temperature, indirectly making them excellent green solvents. There are salts where imidazole exists as an anion. These salts are known as imidazolide salts. The example of this type of salt are sodium imidazolide.

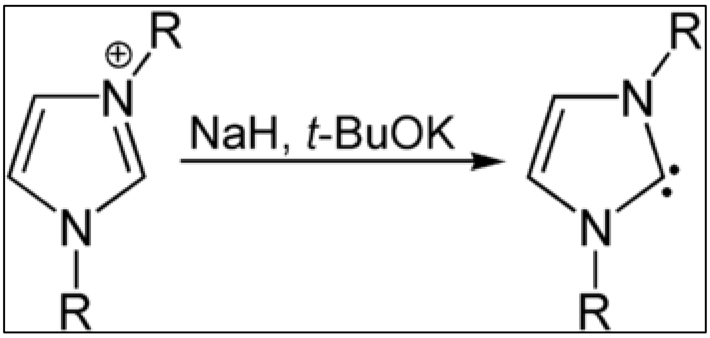

Figure 2.3 Molecular Structure of Imadazole Salt

According to Chu (2009), Imidazolium salt also has shown good potential as desulphurization agent presumably due to its similarity in structure to the rings of sulphur compounds.

2.2 Extraction of Plant Leaves using Ionic Liquids

In the past few years, the utility of ionic liquids as green and alternative solvents has significantly expanded. The high polarity of ionic liquids combined with their low vapor pressure, large liquid range and high thermal stability make them a promising class of solvents for various of chemical and biochemical applications. To be surprise, there are many research has shown that ILs are good solvents for making biofuels. Some recent studies have shown that hydrophilic ILs such as 1-butyl-3-methylimidazolium chloride ([Bmim][Cl]), 1-methyl-3-methylimidazolium dimethyl phosphate ([Mmim][Me2PO4]), 1-allyl-3-methylimidazolium chloride ([Amim][Cl]) and 1-ethyl-3-methyl-imidazolium acetate ([Emim]- [Ac]) show promise for dissolving agricultural products. However, for the enzymatic hydrolysis of cellulose, the preferred choice is ([Emim][Ac]), because it has a low melting point.

According to Wei et. al. (2012), extraction is an important key to quantitative analysis of active components in plant material. Nowadays, environment friendly techniques catch more attraction due to the development of the Green Chemistry. ILs which are composed of relatively large asymmetric organic cations with smaller inorganic or organic anions, are in liquid state near room temperature or below 100 °C. This ILs display a wide range of unique properties, including excellent microwave-absorbing ability, extremely low vapor pressure, good dissolving and extracting ability and designable structures. Thus, they have been applied as green solvent to replace the volatile organic solvents in sample preparation such as liquid–liquid extraction, aqueous biphasic systems, as well as solid-phase and liquid phase microextraction.

Researches indicated that the structures of ILs have significant effect on its physicochemical properties, and affect the extraction efficiency of target compounds (Wei, 2012). 1-Alkyl-3-methylimidazoliumtype ILs are the most popular type for sample preparation. This type of ionic liquid has been successfully used to extract alkaloids, resveratrol and phenolics from plant materials.

2.3 Properties of Gold Nanoparticles

Nanoparticles can exhibit excellent catalytic activity due to their high surface area to volume ratio and their interface-dominated properties, unlike metals in a bulk form. Besides, nanoparticles have a unique optical and electrical property which relies on the size and shape of nanoparticles as an effect of quantum confinement of electrons (Sujitha et al., 2012). According to Philip (2009), AuNPs specifically have a unique and tuneable surface Plasmon resonance. The phenomenon of Surface Plasmon Resonance (SPR) occurs within visible frequencies and result in strong optical absorbance and scattering properties of AuNPs (Jain et al., 2007).

In addition, AuNPs are well known as excellent scatterers and absorbers of visible light due to their strongly enhanced SPR at optical frequencies. The optical properties of the AuNPs can be tuned simply by manipulating the size and shape of the AuNPs (Jain et al., 2006).

According to Amin et. Al. (2013), AuNPs are versatile materials with a very broad range of applications and uses in a variety of fields which include medicine, catalysis and electronics. The example of the uses of AuNPs is in the detection and treatment of cancer cells (Soppimath and Betageri, 2008). This method of cancer diagnosis and treatment are different with previous methods which are harmful and costly. Instead, AuNPs provide an inexpensive and safer route for targeting only cancerous cells without harming the other healthy cells (Pellequer and Lamprecht, 2009).

This AuNPs has wide application in this industry that includes electronics, photodynamic therapy, and also it has been used in diagnostics. Table 2.1 shows the range of application of AuNPs.

Table 2.1 Application of AuNPs (according to Sigma-Aldrich)

Industry	Application of Gold Nanoparticles
Electronics	Gold nanoparticles are designed for use as conductors from printable inks to electronic chips. As the world of electronics become smaller, nanoparticles are important components in the chip design. Nanoscale gold nanoparticles are being used to connect resistors, conductors, and other elements of an electronic chip.
Photodynamic Therapy	Near-IR absorbing gold nanoparticles (including gold nanoshells and nanorods) produce heat when excited by light at wavelengths from 700 to 800 nm. This enables these nanoparticles to eradicate targeted tumors. When light is applied to a tumor containing gold nanoparticles, the particles rapidly heat up, killing tumor cells in a treatment also known as hyperthermia therapy.
Therapeutic Agent Delivery	Therapeutic agents can also be coated onto the surface of gold nanoparticles. The large surface area-to-volume ratio of gold nanoparticles enables their surface to be coated with hundreds of molecules (including therapeutics, targeting agents, and anti-fouling polymers).
Sensors	Gold nanoparticles are used in a variety of sensors. For example, a colorimetric sensor based on gold nanoparticles can identify if foods are suitable for consumption. Other methods, such as surface enhanced Raman spectroscopy, exploit gold nanoparticles as substrates to enable the measurement of vibrational energies of chemical bonds. This strategy could also be used for the detection of proteins, pollutants, and other molecules label-free.
Probes	Gold nanoparticles also scatter light and can produce an array of interesting colors under dark-field microscopy. The scattered colors of gold nanoparticles are currently used for biological imaging applications. Also, gold nanoparticles are relatively dense, making them useful as probes for transmission electron microscopy.
Diagnostics	Gold nanoparticles are also used to detect biomarkers in the diagnosis of heart diseases, cancers, and infectious agents. They are also common in lateral flow immunoassays, a common household example being the home pregnancy test.
Catalysis	Gold nanoparticles are used as catalysts in a number of chemical reactions. The surface of a gold nanoparticle can be used for selective oxidation or in certain cases the surface can reduce a reaction (nitrogen oxides). Gold nanoparticles are being developed for fuel cell applications. These technologies would be useful in the automotive and display industry.

2.4 Green Synthesis of Gold nanoparticles

AuNPs can be produced by the reduction of Au^{3+} in chloroauric acid ($HAuCl_4$) in a reaction with reducing agent by chemical, physical or photochemical methods (Dubey et al., 2010). However, chemical methods usually involve the use of hazardous solvents and harmful reducing agents such as sodium borohydride (Van Hyning and Zukoski, 1998) and hydrazine (Guzman et al., 2009). In addition, most of AuNPs produced through these methods are unstable, thus require a stabilizing agent in the process of synthesis. This causes some toxic chemical species to be absorbed on the surface of AuNPs and may cause adverse effect if it is being used in medical applications (Gan et al., 2012).

Hence, the use of microorganism or plant for the biosynthesis of AuNPs could be advantageous because biomass is capable of secreting biomolecules which can act as both reducing and capping agents during the reaction. In addition, by using this biomass, it can capable to produce AuNPs which are more biocompatible (Lukman et al., 2011). According to Narayanan and Sakthivel (2010), microbial synthesis is not preferred as compared to plant because it is time consuming, tedious, and the size distribution, shape and crystallinity of synthesized nanoparticles are hard to control. In addition, Philips et. al. (2012) also reported that nanoparticles produced by plants are more stable and the rate of synthesis is much faster than the one using microorganisms.

Table below shows some of previous research done on the synthesis of AuNPs using various plant extracts and the size of resulting AuNPs:

Table 2.2 Green synthesis using plant species

No	Plant Species	Size of AuNPs (nm)	Reference
1	Cinnamomum zeylanicum	25	Smitha et al. (2009)
2	Mangifera indica	20	Philip (2010)
3	Rosa rugosa	11	Dubey et al. (2010)
4	Momordica charantia	10 -100	Pandey et al. (2012)
5	Plantago ovato	8 – 30	Amin et al.(2013)
6	Citrus limon, Citrus reticulate, Citrus sinensis	15 – 60	Sujitha and Kannan (2012)
7	Moringa oleifera	20 -60	Chakraborty et al. (2013)
8	Mimosa pudica	40	Iram et al. (2014)
9	Pheonix dactylifera L.	32 – 45	Zayed and Eisa (2013)

2.5 Oil Palm (Elaeis Guineensis) Leaf

Scientifically, oil palm tree are known as Elaeis Guineensis and it is classified as tropical tree which normally grown and harvested for industrial production of vegetable oil. The leaf is considered as the major waste in oil palm industry. However, some researchers take an initiative to transform the waste into valuable products. Sasidharan et. al (2012) reported that juice squeezed from oil palm leaves can enhance wound healing process. Besides, this leaf is also useful for treatment of rheumatism, cancer, headaches as well as an aphrodisiac liniment and diuretic (Irvin, 1985). Yin et al (2013) stated that the interests in investigating the constituent of the oil palm leaf have grown over the past few years because it possesses some medical values.

Besides, according to Ibraheem et. al. (2012), the oil palm leaf is reported to contain some amount of bioactive compounds that are higher in concentration compared to oil palm fruit and more diverse. Phytochemical screening have been done by some researchers on the oil palm leaf extract confirms the presence of tannins, phenolic, terpenoids, flavonoids, alkaloids, saponins, and steroids and coumarins as phytochemical constituents in oil palm leaf which could be responsible for the biological activities. Fortunately, the high content of phenolic, tannin and flavonoid compounds in the oil palm leaves extract may contribute to its antioxidant activity (Yin et al., 2013).

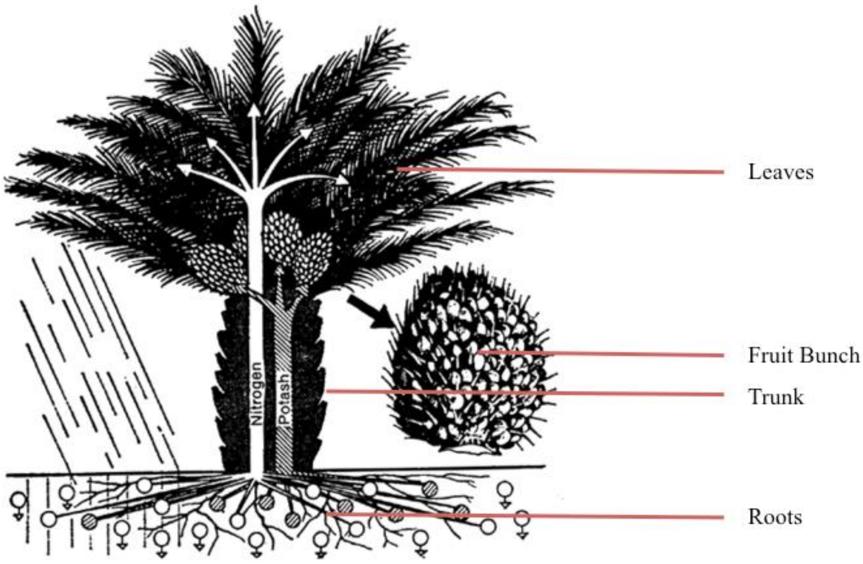

Figure 2.4 The Oil Palm Tree (The Oil Palm, 1990)

CHAPTER 3
METHODOLOGY

3.1 Research Methodology

An extensive research study was done on several journals and research papers in order to obtain a feasible and executable project plan. Figure 3.1 shows the project plan developed in order to achieve the objectives of the project.

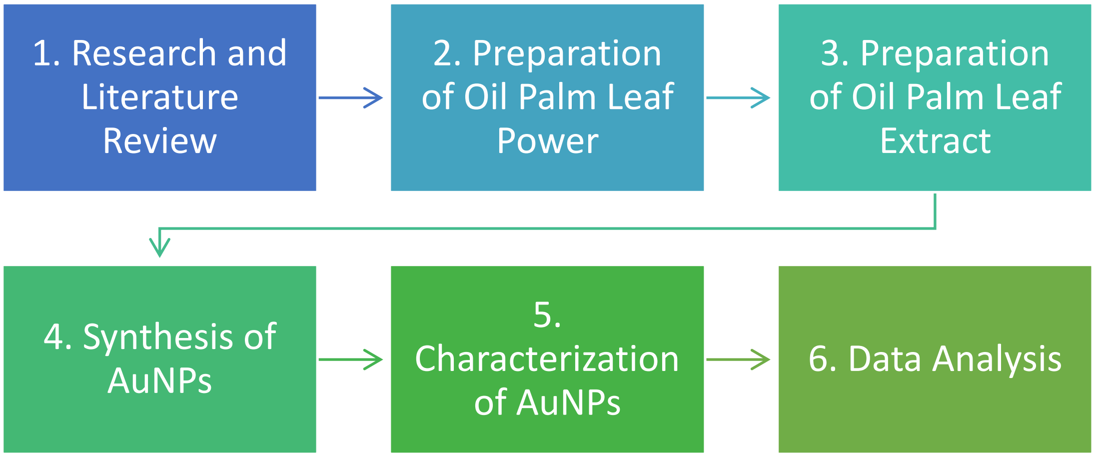

Figure 3.1 Project Flow

3.2 Experimental Methodology

3.2.1 Materials

There are severals materials are important in this experiment. Chloroauric acid ($HAuCl_4.H_2O$) is the main chemical used to synthesize the AuNPs was purchased from Sigma-Aldrich. 1-ethyl-3-methylimidazolium (Emim) Br_2 are the ionic liquid used. The oil palm leaves are obtained from Felcra Nasaruddin Oil Palm Mill that are located in Bota, Perak and triple distilled water was obtained from Chemical engineering Laboratory in Universiti Teknologi PETRONAS.

3.2.2 Apparatus and Equipment

Some laboratory apparatus are used to conduct this experiment. This includes the conical flask, beaker, pipette, filter funnel and filter paper. Hot plate and magnetic stirrer are used to heat the sample and stir the solution. In order to grind the dried oil palm leaf into powder, IKA grinder are used with sieve of 0.25 mm. For storage purpose, vials were used to keep the colloidal solutions of AuNPs. For characterization of AuNPs, the equipment used are UV-Vis spectrophotometer.

3.2.3 Experimental Procedure

Preparation of Oil Palm Leaf Powder

Oil palm leaves are taken from oil palm tree. The leaves are then been washed to remove dust particles and they are left sun dried for 2 weeks. The leaves are then been oven-dried for about 6 hours. The dried leaves are then grinded into powder using IKA grinder with 0.25 mm sieve. The leaf powder is stored in an airtight container for further use.

Figure 3.2 Dried Oil Palm Leave

Figure 3.3 Oil Palm Leaf Powder

Preparation of Oil Palm Leaf Extract

1. 2 wt% of ionic liquid in leaf extract are prepared by adding 0.4g of ionic liquid into 20g of distilled water in a flask.
2. 1g of oil plam leaf powder was added to the mixture and was stirred at 1000 rpm, heated up to 70ºC for 15 minutes.
3. The solutions were filtered by gravity filtration using Whatman No. 1 filter paper.
4. The supernatant liquids were collected and stored in a freezer at 4ºC in order to retain the activity of enzymes until further use.

Synthesis of Gold Nanoparticles

1. Add oil palm leaf extract vary from 1 – 4 ml in a drop-wise manner to an Erlenmeyer flask containing 20 ml of distilled water at room temperature.
2. Add 2 ml of chloroauric acid with concentration vary from 2% to 10% into the mixture and stir the solution using a magnetic stirrer at 360 rpm for a reaction time ranging from 5 to 45 minutes.
3. Record the time required for the solution to change colour into wine-red colour.
4. Store the gold colloidal in a vial and kept in cold water at 4 ºC in order to freeze the reaction.
5. Measure the maximum absorbance peak of the gold colloidal using UV-Vis spectrophotometer.

The parameters such as chloroauric acid concentrations, reaction temperature, reaction time as well as volume ratio between oil palm leaf extract and chloroauric are to be varies in order to determine the optimum conditions for synthesizing AuNPs using oil palm leaf extract. The experiment matrix developed are shown in Table 3.1 below.

Table 3.1 Experiment Matrix

<table>
<tr><td>Variable</td><td colspan="3">Volume of leaf extract</td></tr>
<tr><td>Fixed Variables</td><td colspan="3">Volume of acid
Concentration of acid
Heating method</td></tr>
<tr><td></td><td>Start Time</td><td>End Time</td><td>Observations</td></tr>
<tr><td>0.06 ml</td><td></td><td></td><td></td></tr>
<tr><td>0.12 ml</td><td></td><td></td><td></td></tr>
<tr><td>0.25 ml</td><td></td><td></td><td></td></tr>
<tr><td>0.50 ml</td><td></td><td></td><td></td></tr>
<tr><td>0.75 ml</td><td></td><td></td><td></td></tr>
<tr><td>Variable</td><td colspan="3">Concentration of acid</td></tr>
<tr><td>Fixed Variables</td><td colspan="3">Volume of acid
Volume of extract
Heating method</td></tr>
<tr><td></td><td>Start Time</td><td>End Time</td><td>Observations</td></tr>
<tr><td>1.27 mM</td><td></td><td></td><td></td></tr>
<tr><td>1.77 mM</td><td></td><td></td><td></td></tr>
<tr><td>2.28 mM</td><td></td><td></td><td></td></tr>
<tr><td>2.79 mM</td><td></td><td></td><td></td></tr>
</table>

Characterization of Gold Nanoparticles

The synthesized AuNPs are characterized using a Perkin Elmer Lambda 25 UV-Visible (UV-Vis) Spectrophotometer operated at a resolution of 1 nm at a scan speed of 480 nm/min. High quality quartz cuvette (Perkin Elmer optics) is used as a vessel to record the spectra. The output of this analysis is an absorbance vs. wavelength graph. It is important because this analysis is used to determine the presence of AuNPs in the colloidal solution. For the presence of AuNPs, it will result in a peak in the absorbance curve due to Surface Plasmon Resonance (SPR) of the colloidal gold. Assessment on the stability of synthesized AuNPs toward aggregation can also be done periodically by monitoring the evolution of their UV–Vis spectra in time.

3.3 Project Key Milestones

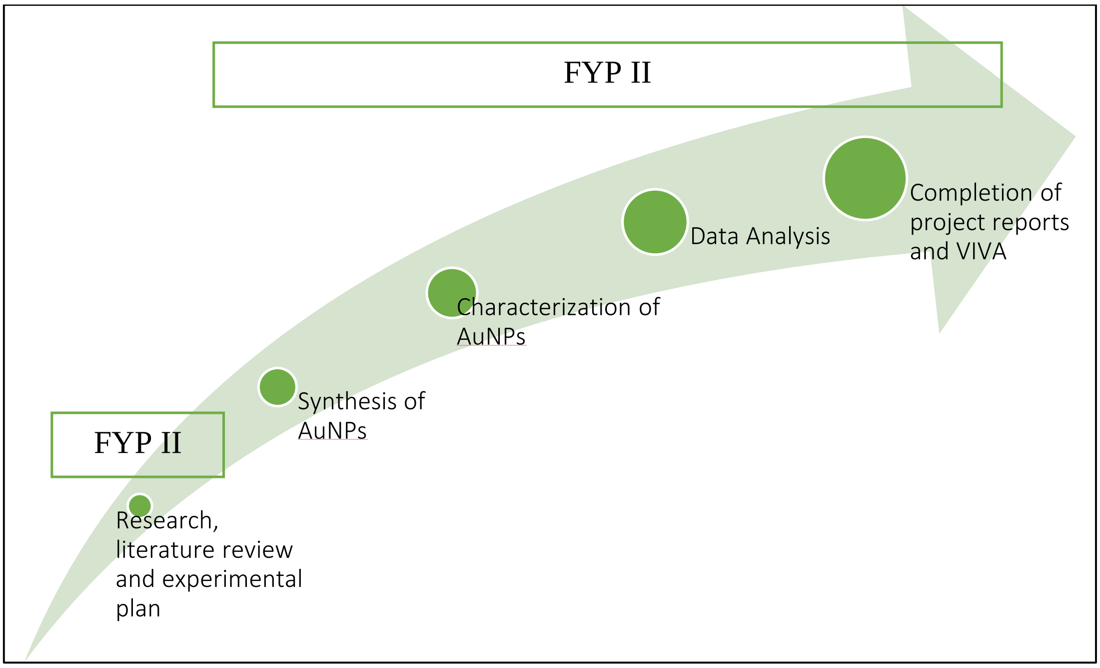

Figure 3.4 Project Key Milestones

3.4 Project Gantt Chart

Table 3.2 Project Gantt Chart

ACTIVITY	PERIODS (Week)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Preparation of leaf extract	■	■													
Synthesis of AuNPs		■	■	■	■	■									
Study of optimum conditions for synthesizing AuNPs		■	■	■	■	■									
Characterization of synthesized AuNPs						■	■								
Data analysis						■	■	■	■	■					
Submission of Progress Report								■							
Pre-SEDEX											■				
Submission of Draft Final Report											■				
Submission of Dissertation (soft bound)												■			
Submission of Technical Paper												■			
Viva oral presentation													■		
Submission of Project Dissertation (hard bound)															■

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Oil Palm Leaf Extract

Oil palm leaf extract are one of the important element in order to synthesis the AuNPs in this project. This is because it will determine the ability of the extract to acts as the reducing agents as part of the synthesis process. According to Fatin (2015), the best extract that can reduce gold ion in chloroauric acid into AuNPs are the extract that are using leaves which has been sun-dried for 2 weeks and further dried using oven at 50°C for 8 hours. This is because the phenolic content of plant are directly proportional to the drying temperature (Chen, 2011). Therefore, the drying process of the oil palm leaves are not to be performed in high temperature as it will degrade the biomolecule or change its structure of the biomolecule itself that present in the leaves.

The extract was prepared by adding 1 g of leaf powder into the mixture of 20 g of distilled water and 0.5 g of ionic liquid to produce a 2.0 wt% leaf extract. The mixture are then been heated in a hot plate at 70ºC for 15 minutes. During the mixing, it can be seen that the leaf powder was initially immiscible with the mixture of distilled water and ionic liquid. Thus, the stirring speed was increased up to 1000 rpm in order to have a well-mixed extract. The mixture are then been filtered to get the aqueous oil palm leaf extract. It was found that the colour of the extract was dark brown. The extract was then been collected and was preserved in small vial inside a freezer for further use.

Table 4.1 Extract Preparation

IL used as solvent	Amount of IL (g)	Amount of distilled water (g)	Amount of leaf powder (g)
Emim Br_2	0.5	20	1
Emim Ac	0.5	20	1

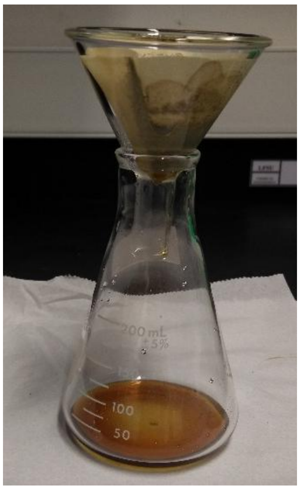

Figure 4.1 Gravity Filtration

Both solvent are tested to synthesis the AuNPs to find the best solvent. However, it was found that solvent containing Emim Br_2 are much better than Emim Ac to be the solvent for the leaf extract. This is because the synthesis of AuNPs with leaf extract solved in Emim Br_2 gives higher absorbance at a lower wavelength. Besides, the reaction time are faster using Emim Br_2 than Emim Ac when using conventional heating. The colour of the synthesized gold solution are also shows a very good colour, indicating the presence of AuNPs.

4.2 Synthesis of Gold Nanoparticles

The synthesis process of the AuNPs was carried out by adding 1 ml of 2.28 mM gold solutions into 5 ml of distilled water. The lead extract was then added with different volume in order to test for the optimum volume that will results in good gold nanoparticles presence. Two sets of samples are prepared to synthesize the gold nanoparticles in two different method, conventional heating and microwave heating. Several parameters such as chloroauric acid concentration, volume ratio and temperature were varied in order to determine the optimum conditions in synthesizing AuNPs.

Figure 4.2 Synthesis of AuNP using Conventional Heating Method

4.2.1 General Observation

Physically, the visual observation on the colour change of the solution in the sample can act as a preliminary assessment on the presence of AuNPs. However, according to Zayed et al. (2014), UV-Vis spectroscopy is a technique that is widely used in order to confirm the presence of GNPs. From the UV-Vis Sepctrophotometer, AuNPs will exhibit surface plasmon resonance in the range of 510 nm to 550 nm (Chakrabortry et al., 2013). This is because, the position of SPR band in UV-Visible spectra is sensitive to various parameters such as particle size, shape, local refractive index and its interaction with medium (Aromal et al., 2012).

Table 4.2 Observation on the Colour Changes

Heating Method	**Acid Concentration (mM)**	**Volume of acid (ml)**	**Volume of extract (ml)**	**Reaction Temperature (°C)**	**Time required for colour change, t (min)**	**Colour of solutions**
Conventional heating	2.28	1	0.06	70	60	Pale pink
	2.28	1	0.12	70	40	Light maroon
	2.28	1	0.25	70	30	Wine red
	2.28	1	0.50	70	30	Dark wine red
Microwave heating	2.28	1	0.06	Mode 3	2	Wine red
	2.28	1	0.12	Mode 3	2	Dark wine red
	2.28	1	0.25	Mode 3	2	Dark wine red
	2.28	1	0.50	Mode 3	2	Dark wine red
	1.27	1	0.12	Mode 3	2	Pale pink
	1.77	1	0.12	Mode 3	2	Red wine
	2.28	1	0.12	Mode 3	2	Red wine
	2.79	1	0.12	Mode 3	2	Dark wine red

4.3 Characterization of Gold Nanoparticles

4.3.1 Effect of Volume Ratio on the synthesis of AuNP

The effect of volume ratio of leaf extract on the synthesis of AuNPs are tested by varying from low to high. At first, 0.5 ml, 1 ml and 1.5 ml of leaf extract was used with 1 ml of 2.28 mM of gold solution and 5 ml of distilled water. Physically, the solutions shows that 0.5 ml of the oil palm leaf extract gives a good colour change. The test was then been furthered by reducing the range of the volume to be tested to 0.06 ml, 0.12 ml, 0.25 ml and 0.50 ml while keeping other parameters constant. The samples been tested by using both conventional method and microwave heating method each. For the conventional heating method, the solutions were heated at 70ºC and stirred with 500 rpm meanwhile for microwave heating, the solutions were heated with a very minimum power.

Table 4.3 Effect of Volume Extract on the synthesis of AuNPs

Heating Method	**Volume of extract (ml)**	**Time required for colour change, t (min)**	**Colour of solutions**	**Maximum wavelength, λ-max (nm)**	**Absorbance, A**
Conventional heating	0.06	60	Pale pink	560	0.455
	0.12	40	Light maroon	570	0.550
	0.25	30	Wine red	565	0.540
	0.50	30	Dark wine red	560	0.480
Microwave heating	0.06	2	Wine red	565	0.405
	0.12	2	Dark wine red	540	1.500
	0.25	2	Dark wine red	540	1.355
	0.50	2	Dark wine red	549	1.420

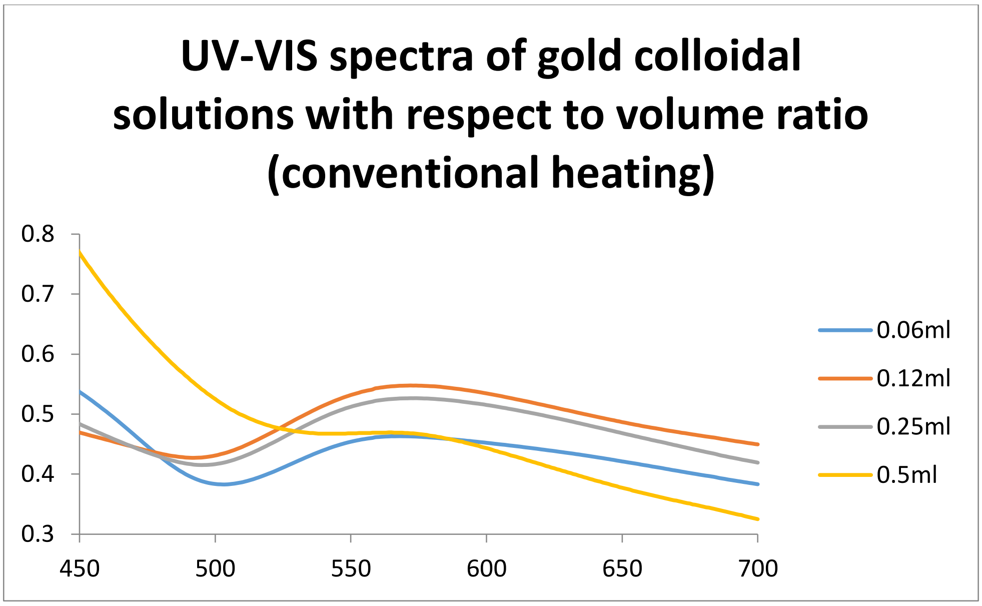

Figure 4.3 UV-VIS spectra with respect to volume ratio (conventional heating)

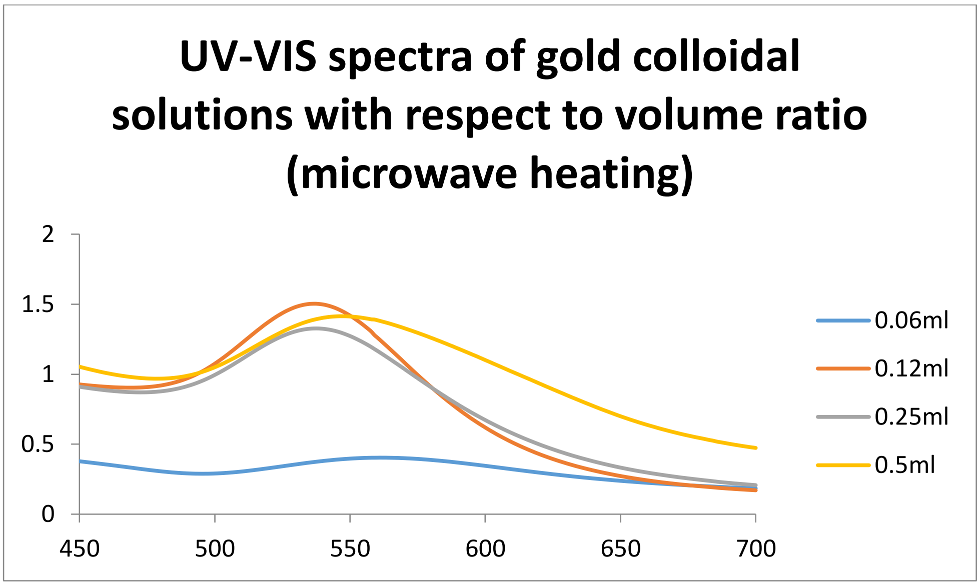

Figure 4.4 UV-VIS spectra with respect to volume ratio (Microwave heating)

From this study, it can be seen that by using conventional method, the absorbance are increasing as the volume of extract used is increased. This is because increasing the plant extract volume leads to more reduction occur in the gold ion inside the gold solution. However, it is observed that at some point, the absorbance and the wavelength of the gold solution decrease as the volume of extract increase. This is because the concentration of the gold inside the solution are decrease with the increase of extract which acts as the reducing agents.

Synthesis of AuNPs using microwave heating method are showing the same pattern of result. The different is that, the absorbance are higher and the maximum peak occurs at lower wavelength value. This is because ionic liquid works best with microwave heating (Tang, 2012).

4.3.2 Effect of Acid Concentration on the synthesis of AuNP

The concentration of chloroauric acid was studied in order to find the best gold concentration. The study was varied, starts with low concentration of gold solution to high concentration of gold while keeping other parameter at constant value. The synthesis is carried out using microwave heating method by adding 5 ml of distilled water and 0.12 ml of leaf extract into a flask. 0.12 ml of leaf extract are chosen to be added because from the study of volume extract, it shows that 0.12 ml of leaf extract gives the best peak and absorbance to the UV-Vis Spectrometer. After that, 1 ml of gold solution were added into the flasks with by changing the concentration of gold in each flask.

Table 4.4 Effect of acid concentration on the synthesis of AuNP

Concentration of gold solution	**Time required for colour change, t (min)**	**Colour of solutions**	**Maximum wavelength, λ-max (nm)**	**Absorbance, A**
1.27 mM	2	Pale pink	550	1.205
1.77 mM	2	Red wine	540	1.255
2.28 mM	2	Red wine	540	1.605
2.79 mM	2	Dark wine red	545	2.4555

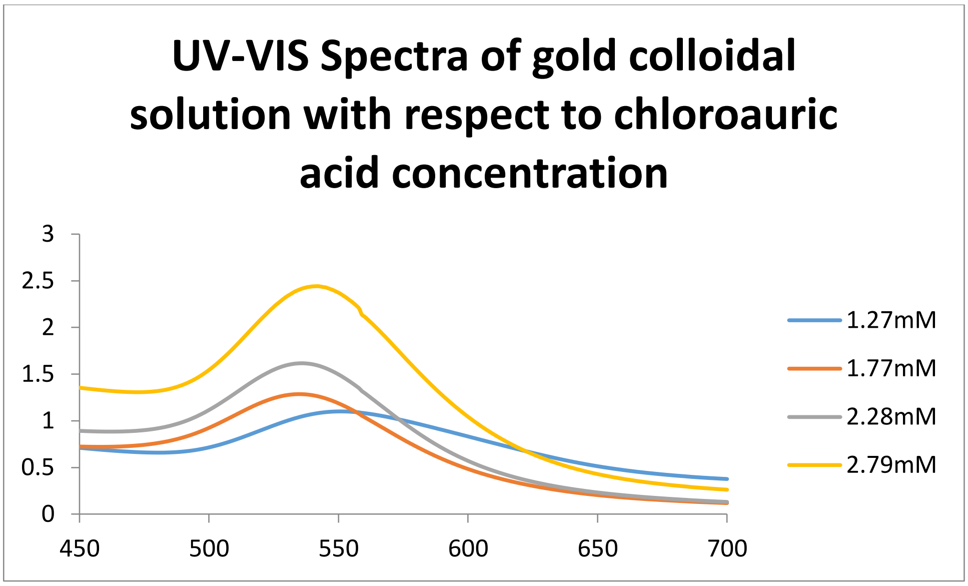

Figure 4.5 UV-VIS spectra with respect to chloroauric acid concentration

From the study, it is observed that different concentration of chloroauric acid produces different colour after the gold synthesis. According to Itoh (2003), amount of gold in the gold solution gives different result in the synthesis. In this study, maximum peak was only available once 2.79 mM of chloroauric acid was used and it results in a maximum peak at 545 nm. As the concentration of chloroauric acid increased, the maximum peak shifted to the right with increasing absorbance and took longer time to change the colour of the solution to wine red. Shifting of absorption peak to the higher wavelength indicates the increase in size of GNPs. This is due to the fact that, the amount of reducing agent available to reduce the gold ions inside the reaction mixture was relatively low as high concentration of chloroauric acid was used. Therefore, the small particles will aggregate to bigger ones until the total surface areas of particles small enough to be covered by the reducing agent.

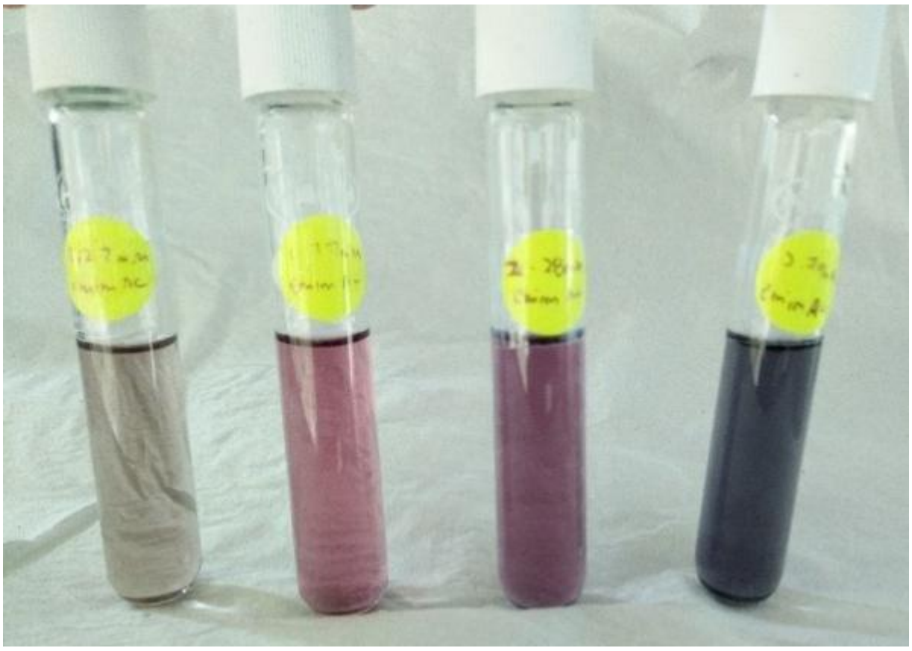

Figure 4.6 Observation on the colour change with respect to acid concentration

4.3.3 Effect of Microwave power

The effect of microwave power is studied by changing the microwave power from high to low. Since the exact power of the microwave in term of Watt cannot be obtained, thus, the power of microwave is first been tested by boiling plain water in bread mode with different power, 1, 2 and 3. The time taken for the water to boil is recorded.

Table 4.5 Time to boil water using microwave

Heating Mode	Time for water to boil (s)
Instant cooking	45
Mode 1	110
Mode 2	130
Mode 3	140

From the test, it can be concluded that Mode 1 is the highest power compared to Mode 2 and Mode 3 heating. Therefore, the synthesis of AuNP using this microwave mode is done by adding 5 ml of distilled water in three different flasks, and 1 ml of 2.28 mM of gold solution and 0.12 ml of leaf extract are added with the same amount at each flask.

Table 4.6 Effect of microwave power on the time for synthesis of AuNP

Microwave Heating Mode	Time required for colour change, t (min)	Colour of solutions
Mode 1	1.8	Dark wine red
Mode 2	2.5	Dark wine red
Mode 3	2.4	Dark wine red

CHAPTER 5
CONCLUSION AND RECOMMENDATION

This project research are carried out to determine the optimum conditions for synthesizing AuNPs using palm oil extract. Ionic liquid is used as the solvent in order to study the effect of solvent in synthesizing AuNPs since different solvents have different resolving strength towards plant constituents. As a conclusion, this study is to explore the possibilities of synthesizing AuNPs using palm oil (elais guineensis) leaf extract as an alternative way to available physical and chemical methods. There are preliminary result obtained from other researchers using UV-Vis stated that aqueous leaf extract is capable of reducing gold ion in chloroauric acid into AuNPs with an absorption peak produced at 535.77 nm. The aqueous solution change into wine-red colour after 20 minutes of reaction and the resulting colloidal solution is found to be stable without any agglomeration.

For betterment, it is highly recommended to produce palm oil leaf extract using different type of ionic liquid such as , IL-based silica and polymers that can enhance the extraction/separation of targets compounds have been synthesized by a large number of research groups. Although only a few studies have examined the extraction and separation of bioactive compounds, IL and IL-based materials have been applied successfully in analytical chemistry (Tang, 2012).

Besides, it is recommended to do the Transmission Electron Microscopy (TEM) analysis on the synthesized AuNPs to find the particle size distribution. Although UV-Vis analysis can indicates the presence of AuNPs in the colloidal solution, TEM analysis can confirm the formation of AuNPs. We can also get the shape of the AuNPs from the analysis.

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