Green Synthesis of Gold Nanoparticles Using Oil Palm Leaf Extract

by

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Dissertation submitted in partial fulfillment of
the requirements for the
Bachelor of Engineering (Hons)
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CERTIFICATION OF APPROVAL

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Approved by,

_____
(AP Dr. Sekhar Bhattacharjee)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
MAY 2014

CERTIFICATION OF ORIGINALITY

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

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(Nur Arina binti Rafiuddin Yeo)

ABSTRACT

Nanoparticles are known to have applications in various fields such as electronics, agriculture and medicine. These nanoparticles are usually produced using physical or chemical methods that are costly and produce toxic residue. In recent years, interest in synthesizing nanoparticles using eco-friendly methods has peaked and various research has been done to produce these nanoparticles using leaves, fruits and roots of plants. Therefore, it is imperative to study the possibility of producing gold nanoparticles from oil palm leaf extract, which may result potent biological applications. The nanoparticles are produced by the bioreduction of gold ions in chloroauric acid after mixing the acid solution with oil palm leaf extract. The preliminary Fourier Transfrom Infrared spectroscopy analysis carried out on the oil palm leaf extract indicates that functional groups present in the oil palm leaf extract are O—H from phenols and carboxylic acid, C=C aromatics, alkane, alkenes and ethers. The colour change observed and Ultraviolet-Visible spectroscopy analysis done on the reacting mixture of chloroauric acid and palm oil leaf extract resulting in an absorbance peak at around 530 to 550 nm indicated that the synthesis is feasible. The FESEM and EDX analysis further confirmed the presence of gold nanoparticles and their resulting shapes.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The science of nanotechnology is a burgeoning area of research due to the wide applications of nanoparticles in diverse fields that include catalysis, water purification, materials science and electronics, to name a few (Abdel-Raouf, Al-Enazi, & Ibraheem, 2013; Suman, Radhika Rajasree, Ramkumar, Rajthilak, & Perumal, 2014). Interest in the study of metal nanoparticles, specifically gold nanoparticles, has increased over the past years due to their possible uses in the medical field such as cancer therapy, drug delivery and antimicrobial activities in the human body (Geetha et al., 2013).

These nanoparticles are usually derived using physical or chemical methods, which are expensive and likely to produce residual toxic chemicals, hence unsuitable for bio-medication (Zayed & Eisa, 2014). The chemicals used for conventional syntheses of gold nanoparticles are also not environmentally friendly. Therefore, in modern nanotechnology, numerous amounts of research have been done to attempt to synthesize these gold nanoparticles using a more environmentally friendly alternative that does not use harsh chemicals by using plant extracts, which is now commonly known as the green synthesis of nanoparticles (Chakraborty, Das, Sinha, Dey, & Bhattacharjee, 2013).

This present project revolves around the study of green synthesis of gold nanoparticles. After extensive review of previous literature, it has been found that the synthesis of gold nanoparticles from oil palm (*elais guineensis jacq.)* leaf extract has not been attempted. However, the synthesis of gold nanoparticles from palm oil mill extract (POME) has been carried out and the study proved that oil palm constituents can produce these nanoparticles (Gan, Ng, Huang, & Li, 2012).

According to the Malaysian Palm Oil Council (MPOC), Malaysia is one of the largest exporters and producers of palm oil. Hence, this country is rich with oil palm crops and the leaves are easily obtainable for the execution of this project. Synthesizing gold nanoparticles from the oil palm leaves would be a low-cost and environmentally benign approach that could potentially be industrialized and practiced by nanoparticle manufacturers.

1.2 Problem Statement

As mentioned in the background of the study, current methods of synthesizing gold nanoparticles usually involve the use of hazardous chemicals as reducing and stabilizing agents. Noble metal nanoparticles are targeted for use in the medical industry. Hence, it is best that these nanoparticles do not contain any pathogenic, toxic or carcinogenic residues from chemical or physical syntheses. Moreover, these syntheses of nanoparticles require high temperature and pressure and are not cost-effective. This project is executed to address the issue of the unsuitable conventional methods of synthesizing gold nanoparticles for medical use.

In addition to that, the synthesis of gold nanoparticles using the oil palm leaf extract as a reducing agent has not been carried out before. Therefore, it is important to explore this possibility due to the abundance of oil palm trees in Malaysia.

1.3 Objectives of Study

These objectives were outlined to achieve at the end of the study:

- To synthesize gold nanoparticles from oil palm (*elais guineensis jacq.*) leaf extract.
- To investigate the characteristics of gold nanoparticles synthesized from oil palm (*elais guineensis jacq.*) leaf extract.

1.4 Scope of Study

The gold nanoparticles in this study are synthesized from a leaf extract specifically from the oil palm (*elais guineensis jacq.*) tree.

The synthesis of gold nanoparticles in this study is carried out by reducing chloroauric acid ($HAuCl_4$) with the leaf extract. The experiments will be done at the provided laboratories in Universiti Teknologi PETRONAS (UTP). Characterization is to be done on the gold nanoparticles using Field Emission Scanning Electron Microscopy (FESEM), Fourier Transform Infrared (FTIR), Transmission Electron Microscopy (TEM) and UV-Visible Spectrophotometer.

1.5 Relevancy of The Project

Nowadays, a lot of attention and research is directed towards creating alternatives of established methods to ultimately conserve the environment. This project is highly relevant because it addresses the issue that the conventional syntheses of gold nanoparticles are costly, toxic and non-environmentally friendly. Moreover, the project will help to bridge the gap between theory and practice of synthesizing the gold nanoparticles from oil palm leaves, which are abundantly available in Malaysia.

1.6 Feasibility of The Project

The project is done experimentally and consists of three basic stages: the preparation of leaf extract, the synthesis of gold nanoparticles and the characterization of gold nanoparticles. Prior to that, extensive research and study on relevant literature is done to have a thorough understanding of the subject matter. These three stages of the main experiment including the preliminary study will be done over the span of two semesters, equivalent to seven months. The tasks to be completed within the first semester include research and acquiring the relevant chemicals, which are easily attainable. The experiments and data analysis are covered in the second semester. Realistically, the project is feasible to be executed within the given timeframe.

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of Gold Nanoparticles

Nanoparticles generally have a large surface area for reaction compared to a bulk material due to their small size (Jayaseelan, Ramkumar, Rahuman, & Perumal, 2013). Gold nanoparticles, particularly, possess significant characteristics such as electrical, magnetic and thermal conductivity as well as chemical and bio-stability (Boruah, Boruah, Sarma, Medhi, & Medhi, 2012). Gold nanoparticles also have a unique and flexible surface Plasmon resonance (SPR) that can be tuned by altering the shape and size of the particles (Sujitha & Kannan, 2013). This results in yields of different and vibrant colours. The varying SPR values of gold nanoparticles allow them to be tailored specifically to meet various functions (Philip, 2010).

2.2 Synthesis of Gold Nanoparticle

Gold nanoparticles are produced from the reduction of Au^{3+} ions in chloroauric acid ($HAuCl_4$, $3H_2O$) by using a reducing agent via chemical reduction, physical reduction, photochemical reduction and heat evaporation (Dubey, Lahtinen, & Sillanpää, 2010). However, these methods will likely result in toxic residuals and by-products that causes the nanoparticles to be unusable in biomedical applications (Zayed & Eisa, 2014). Due to the fact that these nanoparticles are increasingly used in medicinal applications, gold nanoparticles synthesized from plant extracts have progressively gained attention (Arunachalam, Annamalai, & Hari, 2013).

Plants can be used in the synthesis of gold nanoparticles because they contain natural reducing agents such as ascorbic acid, citric acid, reductases and flavonoids (Pandey, Oza, Mewada, & Sharon, 2012). Undeniably, other organisms such as fungi, bacteria and viruses have also been used to synthesize gold nanoparticles (Dubey et al., 2010) however, plant extracts yield gold nanoparticles in various shapes and sizes thus increasing the possibilities of applications (Aswathy Aromal & Philip, 2012).

The table below shows some of the previous research done on the synthesis of gold nanoparticles using plant extracts and the resulting size of the particles produced:

TABLE 1: Plant species used in the synthesis of gold nanoparticles

No.	Plant species used	Size of gold nanoparticles (nm)	Reference
1	*Citrus limon, Citrus reticulate, Citrus sinensis*	15 – 60	Sujitha and Kannan (2013)
2	*Morinda citrifolia*	12 – 38	Suman et al. (2014)
3	*Pheonix dactylifera L.*	32 – 45	Zayed and Eisa (2014)
4	*Galaxaura elongata*	4 – 77	Abdel-Raouf et al. (2013)
5	*Terminalia arjuna*	20 – 50	Gopinath, Venkatesh, Ilangovan, Sankaranarayanan, and Arumugam (2013)
6	*Rosa rugosa*	11 (average)	Dubey et al. (2010)
7	*Moringa oleifera*	20 – 60	Chakraborty et al. (2013)
8	*Momordica charantia*	10 – 100	Pandey et al. (2012)
9	*Memecylon umbellatum*	15 – 20	Arunachalam et al. (2013)
10	*Couroupita guianensis*	7 – 48	Geetha et al. (2013)
11	*Camellia sinensis*	3 – 46	Boruah et al. (2012)
12	Grape seeds (Proanthocyanidin)	17 – 29	Vinodhini, Govindaraju, Singaravelu, Mohamed Sadiq, and Kumar (2014)(2014)
13	*Plantago ovata*	8 – 30	Amin et al. (2013)
14	*Trigonella foenum-graecum*	20 (average)	Aswathy Aromal and Philip (2012)
15	*Mimosa pudica*	10 – 50	Iram et al. (2014)
16	*Abelmoschus esculentus*	45 – 75	Jayaseelan et al. (2013)
17	*Zingiber officinale*	5 – 15	Kumar, Paul, and Sharma (2011)
18	*Cassia auriculata*	12 – 41	Venkatachalam et al. (2013)

2.3 Applications of Gold Nanoparticles

The applications of gold nanoparticles span through various fields, which include medicine, catalysis and electronics (Amin et al., 2013). Their biological applications such as imaging and effective drug delivery has resulted in numerous studies done in recent years (Suman et al., 2014). For instance, gold nanoparticles synthesized from *cassia auriculate* were found to have anti-diabetic properties whereby they inhibit the activity of protein tyrosine phosphate 1B (Venkatachalam et al., 2013). In addition to that, green synthesized gold nanoparticles were also found to have anti-cancerous properties (Geetha et al., 2013) and are highly effective against bacteria namely *E.coli* and *K.pneumoniae (Abdel-Raouf et al., 2013)*. Similarly, a study done on the synthesis of gold nanoparticles from grape seeds have resulted in the discovery of cardioprotective properties of these particles against myocardial injury (Vinodhini et al., 2014).

Gold nanoparticles can also be used in the agricultural sector. These particles synthesized from *abelmoschus esculentus* seeds proved to have antifungal properties against a fungus growing on rice paddy. Furthermore, gold nanoparticles can also be used to induce pollen germination and mitotic cell division in plants thus increasing crop yield (Gopinath et al., 2013).

2.4 The Oil Palm Tree

British colonists first introduced the oil palm tree in Malaysia as an ornamental plant in the 1870s. It was later on in 1917 that oil palm was first commercialized and cultivated in plantations. Currently, oil palm plantations cover a total area of 4.49 million hectares of land in Malaysia, making it one of the largest producers and exporters of palm oil in the world ("The Oil Palm Tree,").

The oil palm tree belongs to the genus *Elais* and is scientifically knows as *Elais gunineensis* (Sundram, Sambanthamurthi, & Tan, 2003). The tree originates from West Africa and is now produced commercially in tropical counties such as Indonesia, Malaysia and India (Sreekala, Kumaran, & Thomas, 1997). The average oil palm tree can grow up to 60 feet high ("The Oil Palm Tree,") and live up to 30 years (Uexkull). A mature oil palm tree consists of the roots, trunk, fronds, fruit

bunches and the leaves. The fruits, which are made up of the endocarp, kernel and mesocarp, are the vital elements in producing palm oil and palm kernel oil (Sundram et al., 2003).

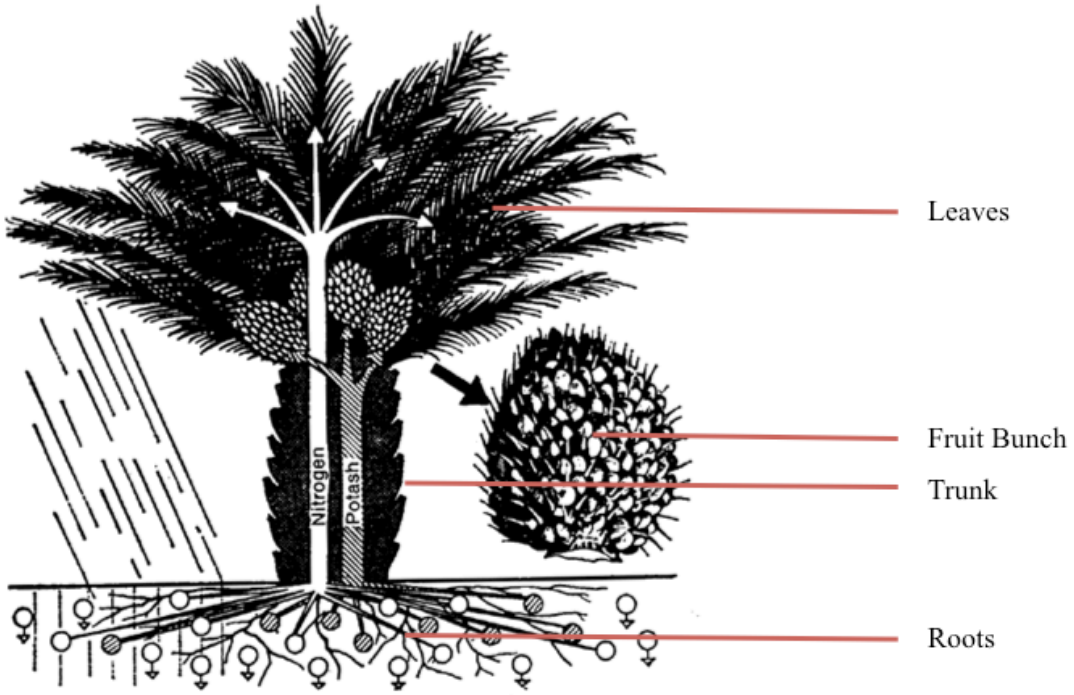

FIGURE 1: The Oil Palm Tree *(The Oil Palm, 1990)*

The palm oil itself holds various nutritional values such as presence of beta-carotene ("Palm Oil Facts and Figures," 2013), vitamin E, carotenoid and antioxidants (Sundram et al., 2003). Elements of the tree are also used in traditional medicine, especially in its native continent of Africa. The sap of the plant is known as a laxative, pulverized leaves are used to heal wounds and ash prepared from the fruit husk is used to treat skin infections (Sreenivasan Sasidharan, 2012). In a study done on the toxicity oil palm leaf extracts, it was concluded that the extract can be consumed as a health product or supplement to prevent injury (Zaid O. Ibraheem, 2012).

FIGURE 2: The oil palm fruit *("Palm Oil Facts and Figures," 2013)*

Furthermore, according to Zaid (2012), several phytochemicals are present in the oil palm leaf extract such as phenols, tannins, flavonoids, polyphenols, saponines and terpanoids. A study was also carried out to investigate anti-aging effects of the oil palm leaf extract on humans. It was found that the extract displays a high scavenging ability which allows free radicals in the human body to be located (Soundararajan & Sreenivasan, 2012). In the aforementioned study, the oil palm leaf extract was analyzed with a Fourier Transform Infrared Spectroscopy (FTIR) analysis and it can be concluded that the extract contains the presence of an OH group, carboxylic acid group, aromatic double bond group, alkena and other minor phenol groups. With the presence of hydroxyl groups in aromatic rings of the phenolic molecules, the reduction of gold could occur as follows (Gan et al., 2012):

$$AuCl_4^- + 3R{-}OH \rightarrow Au^0 + 3R{=}O + 3H^+ + 4Cl^-$$

CHAPTER 3

METHODOLOGY

3.1 Research methodology

Prior to deciding the experimental methodology for this project, extensive research was done to obtain a feasible and executable project plan. Journals and research papers were read and reviewed to get a general understanding of synthesizing gold nanoparticles from greener procedures as opposed to other chemical methods. Subsequent to reviewing related literature, a project plan was developed to achieve the project objectives a shown in the figure below:

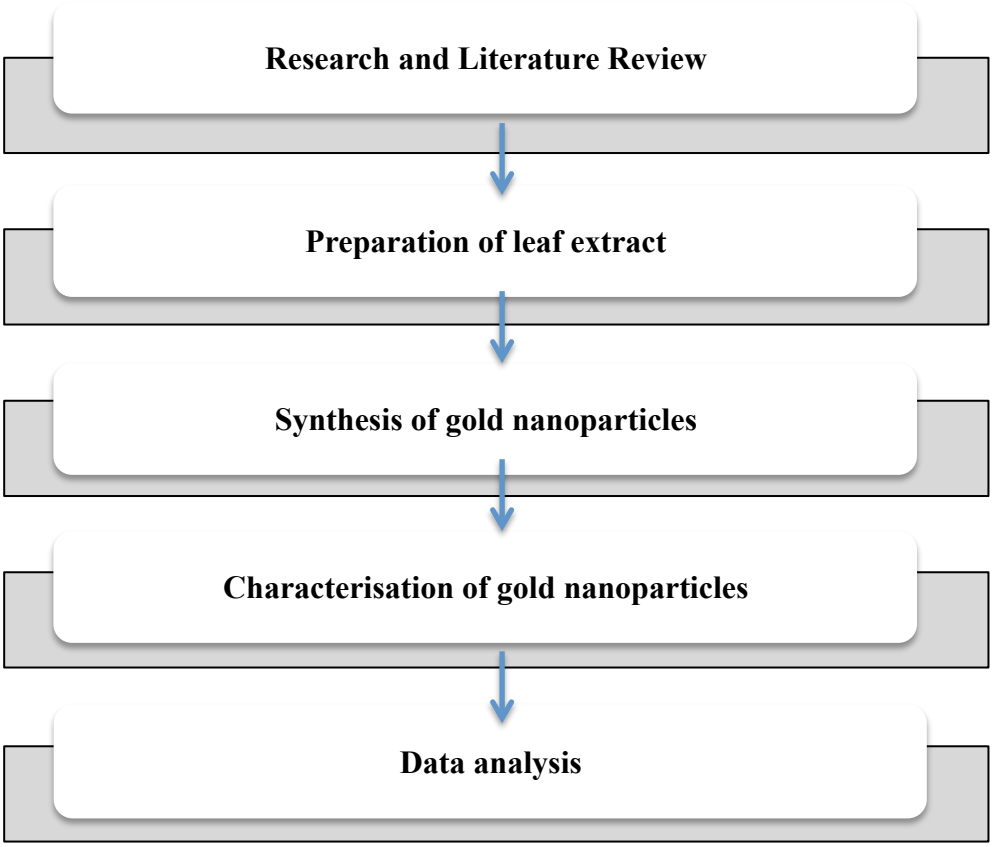

FIGURE 3: Project Flow Chart

3.2 Project Gantt Chart and Key Milestones

As mentioned in the introduction, the execution of the project is done over the span of two semesters. The Gantt charts for the January 2014 (FYP I) and May 2014 (FYP II) semesters are shown in FIGURE 4 and FIGURE 5:

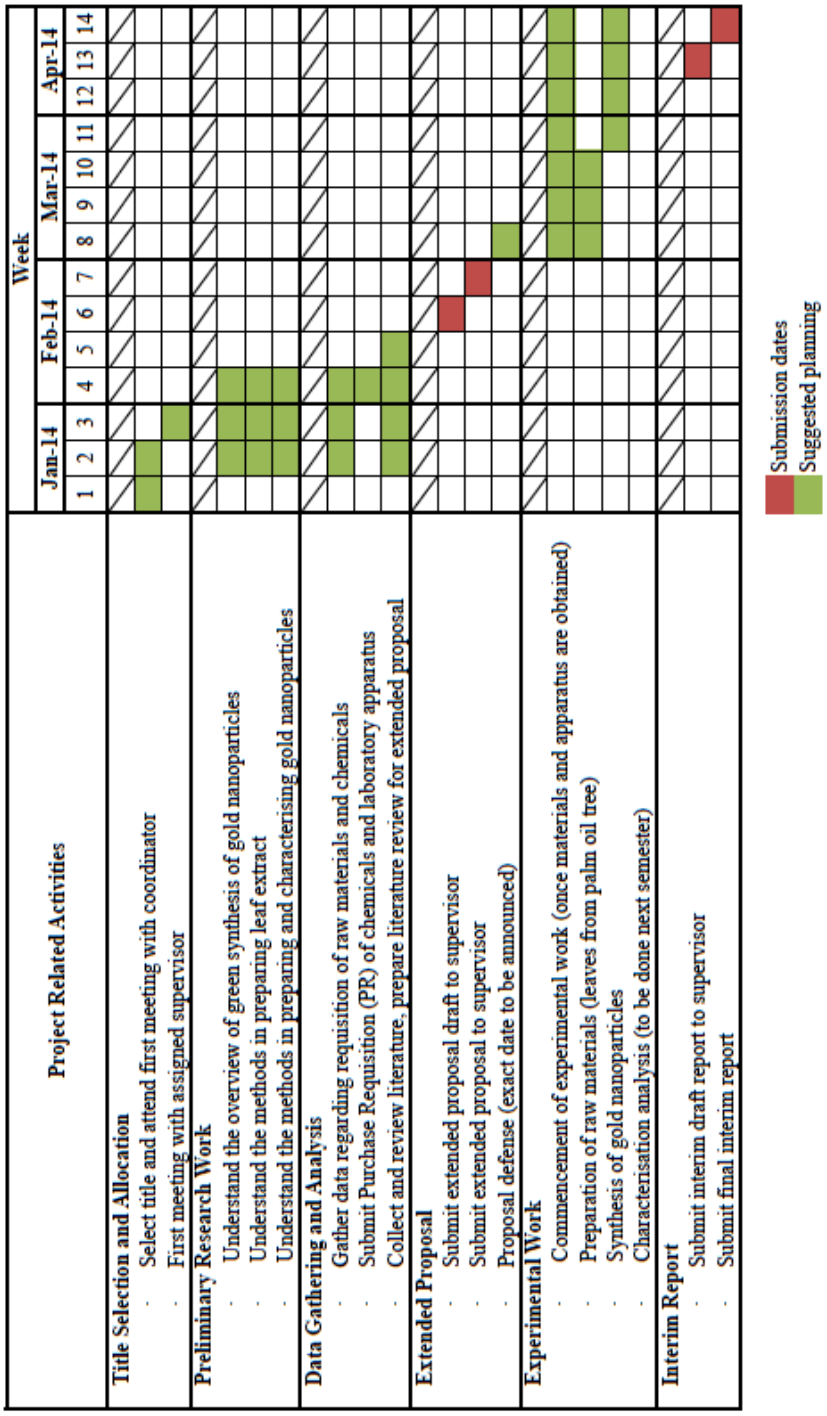

FIGURE 4: Project Gantt Chart for FYP 1

Project Related Activities	Week														
	May-14		Jun-14				Jul-14				Aug-14				Sep-14
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Continuation of Experimental Work															
· Synthesis of gold nanoparticles/ experimentation with variables															
· Characterization and Analysis															
Submission of Progress Report															
· Submit progress report to supervisor															
Pre-EDX															
· Present project to internal examiners															
Dissertation and Technical Paper															
· Submit draft report to supervisor															
· Submit dissertation to supervisor and internal examiner															
· Submit technical paper to supervisor															
· Prepare poster for oral presentation															
· Oral Presenation of project to supervisor and internal examiner															

Important dates
Process

FIGURE 5: Project Gantt Chart for FYP II

To measure the progress of the project, several key milestones are determined. The important milestones for the project are listed in the table below:

TABLE 2: Project Key Milestones

No.	Project Key Milestones	Estimated Date of Execution
1	Acquisition of raw materials	February 2014
2	Preparation of oil palm leaf extract	March 2014
3	Synthesis of gold nanoparticles	June 2014
4	Characterization and analysis of gold nanoparticles	Mid July 2014

3.3 Experiment methodology

3.3.1 Materials

The chemicals required for this project are chloroauric acid ($HAuCl_4$, $3H_2O$), absolute alcohol (C_2H_5OH) and triple distilled water. Absolute alcohol and distilled water was obtained from UTP's laboratory. On the other hand, raw materials required are leaves of the oil palm tree (*Elaeis guineensis jacq.)*. The leaves were obtained from an oil palm tree of the *dura* x *pisifera* progeny on 4th March, 2014 at Felcra Berhad Nasaruddin Oil Palm Plantation located in Bota, Perak. On the other hand, 500 mg of gold (III) chloride hydrate ($HAuCl_{4x}$, xH_2O) was purchased from Sigma-Aldrich through a local vendor i.e. Avantis.

3.3.2 Apparatus and Equipment

The apparatus needed for this experimental project include pipettes to obtain small amounts of the extract and chloroauric acid to be mixed. In addition to that, small vials to store the extract and colloidal solution containing gold nanoparticles are needed. Other typical laboratory apparatus such as beakers, Erlenmeyer flasks, volumetric flasks, measuring cylinders as well as a magnetic stirrer for mixing are also necessary for the execution of this experiment.

Equipment for characterization analysis of gold nanoparticles will be explained further in section 3.3.5.

3.3.3 Procedure for preparation of Oil Palm Leaf Extract

The oil palm leaf extract was prepared in two methods; using absolute alcohol (ethanol) and distilled water for extraction. The basic procedure for the extraction is as follows:

1. The leaves were sun-dried for two weeks and washed with distilled water to remove dust particles
2. The leaves were oven dried at 50 °C just for 30 minutes to remove excess water.
3. The leaves were grinded with an IKA® grinder and sifted though a 0.25 mm sieve. The powder was stored in an airtight container for further use.

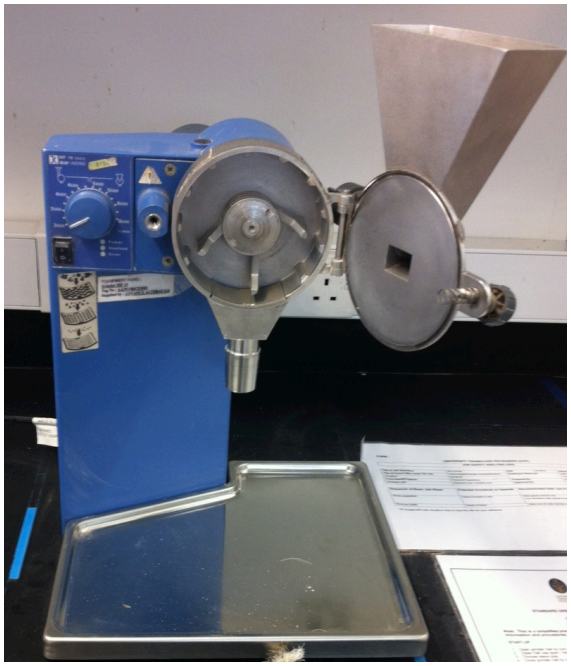

FIGURE 6: IKA Grinder

4. The extracts were prepared using different solvent to powder ratios based on the solvent used:
    a. 50 ml of ethanol was added to 5 g of oil palm leaf powder and the mixture was stirred with a magnetic stirrer for 3 hours.
    b. 100 ml of distilled water was added to 10 g of oil palm leaf powder and the mixture was stirred and heated at 100 °C with a magnetic stirrer for five minutes

FIGURE 7: Mixing with Magnetic Stirrer

5. The liquid from both extractions were filtered out via gravity filtration using a Whatman No. 1 filter paper. The supernatant liquid was measured and then stored at 8 °C until further use.

FIGURE 8: Gravity Filtration

3.3.4 Procedure for Synthesis of Gold Nanoparticles

The stock solution of chloroauric acid was first prepared by adding 500 mg of gold (III) chloride hydrate to 50 ml of distilled water in a volumetric flask. This stock solution of 1wt % (2.943 mM) is used to prepare dilutions to study the effect of concentration of $HAuCl_4$ on the synthesis of gold nanoparticles.

The working solutions used for initial experimentations are prepared using the following equation:

$$M_1V_1 = M_2V_2$$

where M_1 = molarity of the stock solution (mM)

V_1 = volume of stock solution (ml)

M_2 = molarity of the working solution (mM)

V_2 = volume of working solution (ml)

A sample calculation for the dilution of the stock solution can be found in APPENDIX A. Once the working solutions are obtained, the synthesis of the gold nanoparticles are hence carried out using the following procedure, which is based off of the procedure in the studies done by Chakraborty et al. (2013) and (Boruah et al., 2012):

1. 2 ml of oil palm leaf extract is added dropwise to 20 ml of distilled water in an Erlenmeyer flask.
2. 2 ml of 0.02943 mM chloroauric acid is added to the mixture, which is stirred vigorously using a magnetic stirrer at room temperature.
3. A pH reading of the solution is taken before and after the synthesis of gold nanoparticles.

The experiment was carried out with variations in three main parameters i.e. ratio of volumes between chloroauric acid and plant extract, concentration of chloroauric acid, type of plant extract (ethanolic or aqueous) and temperature of reaction. The following table represents the experiment matrix that was developed for the experimentations:

TABLE 3: Experiment Matrix

Variable	: *Type of leaf extract*		
Fixed Variables	• Concentration of chloroauric acid : • Volume ratio (acid:extract) • Temperature of reaction		
	Start (time)	**End (time)**	**Observations**
Ethanolic			
Aqueous			
Variable	: *Temperature of reaction*		
Fixed Variables	• Concentration of chloroauric acid : • Volume ratio (acid:extract) • Type of extract		
	Start (time)	**End (time)**	**Observations**
Room (27 °C)			
40 °C			
60 °C			

Variable	: *Concentration of chloroauric acid*		
Fixed Variables	: • Type of leaf extract • Volume ratio (acid:extract) • Temperature of reaction		
	Start (time)	**End (time)**	**Observations**
0.14715 mM			
0.2943 mM			
0.44145 mM			
Variable	: *Volume ratio (acid:extract)*		
Fixed Variables	: • Concentration of chloroauric acid • Type of leaf extract • Temperature of reaction		
	Start (time)	**End (time)**	**Observations**
2:1			
1:1			
1:2			

The results of these variations will be further discussed in the results and discussion section of this report.

3.3.5 Procedure for Characterization of Gold Nanoparticles

Characterization of the gold nanoparticles is imperative to determine the size and morphology of the substances. The characterization will also be carried out on the leaf extract to determine the chemical composition of the extracts obtained from the different methods. Once the gold nanoparticles are obtained, they will be characterized using the following methods:

TABLE 4: Types of Characterization Analysis

Characterization Analysis / Model	**Description**
Ultraviolet (UV) Visible spectrophotometer/ Perkin Elmer Lambda 25	This analysis is used to indicate the actual presence of gold nanoparticles in the colloidal solution. The output of this analysis is a graph of absorbance vs.

	wavelength whereby a presence of gold nanoparticles will result in a peak in the absorption curve due to Surface Plasmon Resonance (SPR).
Field Emission Scanning Electron Microscopy (FESEM)- Electron Dispersive X-ray (EDX)/ Zeiss Supra 55VP	The main function of FESEM is to provide a high-resolution electron microscopic image of the gold nanoparticles. The FESEM is fully integrated with an EDX analyzer, which will provide the presence of elemental gold in the samples.
Fourier Transform Infrared (FTIR)/ Perkin Elmer	FTIR is used to determine the possible molecules in the leaf extract which will interact with the nanoparticles to produce gold nanoparticles. The analysis is carried out with potassium bromide (KBr) pellets as carriers for the samples and then analyzing the samples by using electromagnetic radiation.
Transmission Electron Microscopy (TEM)/ Zeiss Libra 200 FE	TEM analysis will provide the size distribution of the gold nanoparticles through a micro image produced under different magnifications. This analysis can also be used to determine the growth pattern and distribution of shapes of the synthesized nanoparticles.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preparation of Leaf Extract

The leaves obtained from the oil palm trees were initially cut into small pieces after washing and drying. Then, they were ground with a grinder, in which they were filtered through a 0.25 mm sieve. During the grinding process, a lot of powder was lost to the surroundings due to the air movement in the laboratory and the pushing force of the powder coming out from the grinder. Moreover, fibers from the veins of the leaves were too big to pass through the sieve. Hence, only around half of the powder in weight could be produced from the same weight of leaves.

The leaf extract was prepared through two methods of extraction; by using ethanol and by using distilled water. Based on literature analysis, the common ratio for leaf powder to solvent was used as indicated in TABLE 5. The table also indicates the resulting amount of extract obtained.

TABLE 5: Results of Extraction

Solvent	**Volume of Solvent (ml)**	**Weight of Leaf Powder (g)**	**Mixing time (minutes)**	**Temperature**	**Resulting extract (ml)**
Ethanol	50	5	180	Room temperature	35
Distilled Water	100	10	5	100 °C	50

From the experiments, it can be observed that ethanol yields a larger amount of extract from the original solvent volume as compared to distilled water. Moreover, the extract obtained through ethanol extraction has a darker green appearance compared to the extract obtained with distilled water, which has a pale brown colour. This result is similar to the study done by Zaid O. Ibraheem (2012), in which he stated that extraction with ethanol is ideal because alcohol has a strong tendency to build intra-molecular bonds with the polyphenols present in the oil palm leaf.

The dark green appearance from the ethanolic extract would most possibly be from the chlorophyll present in the leaves. The appearance of the extract obtained with distilled water is a dull brown because the leaf powder did not bind to the distilled water as well as it did with ethanol**.** Moreover, during mixing, it can be clearly seen that the distilled water is initially immiscible with the leaf powder. Increasing the temperature as suggested by numerous studies resulted in a faster extraction time and better miscibility.

4.1.1 FTIR Analysis

FTIR spectroscopy analysis was carried out on the oil palm ethanolic and aqueous leaf extracts as well as the oil palm leaf powder obtained through grinding. The resulting FTIR spectra are shown in the following figures:

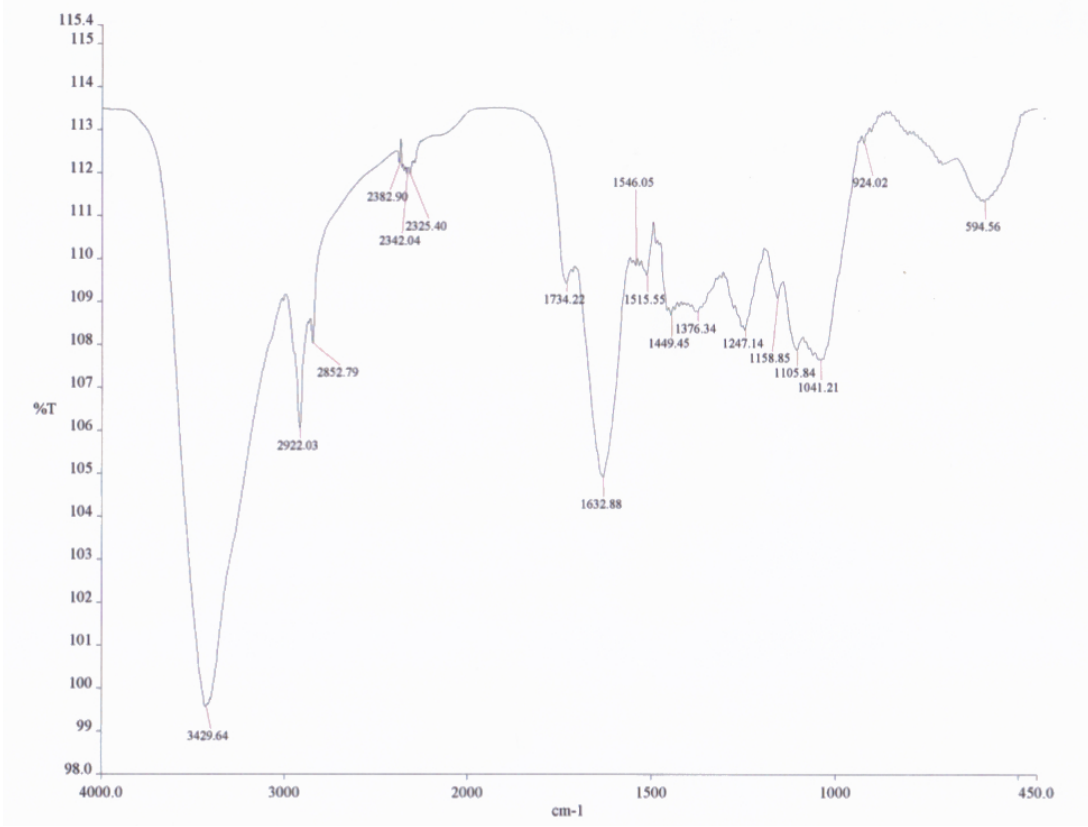

FIGURE 9: FTIR Spectrum of Oil Palm Leaf Powder

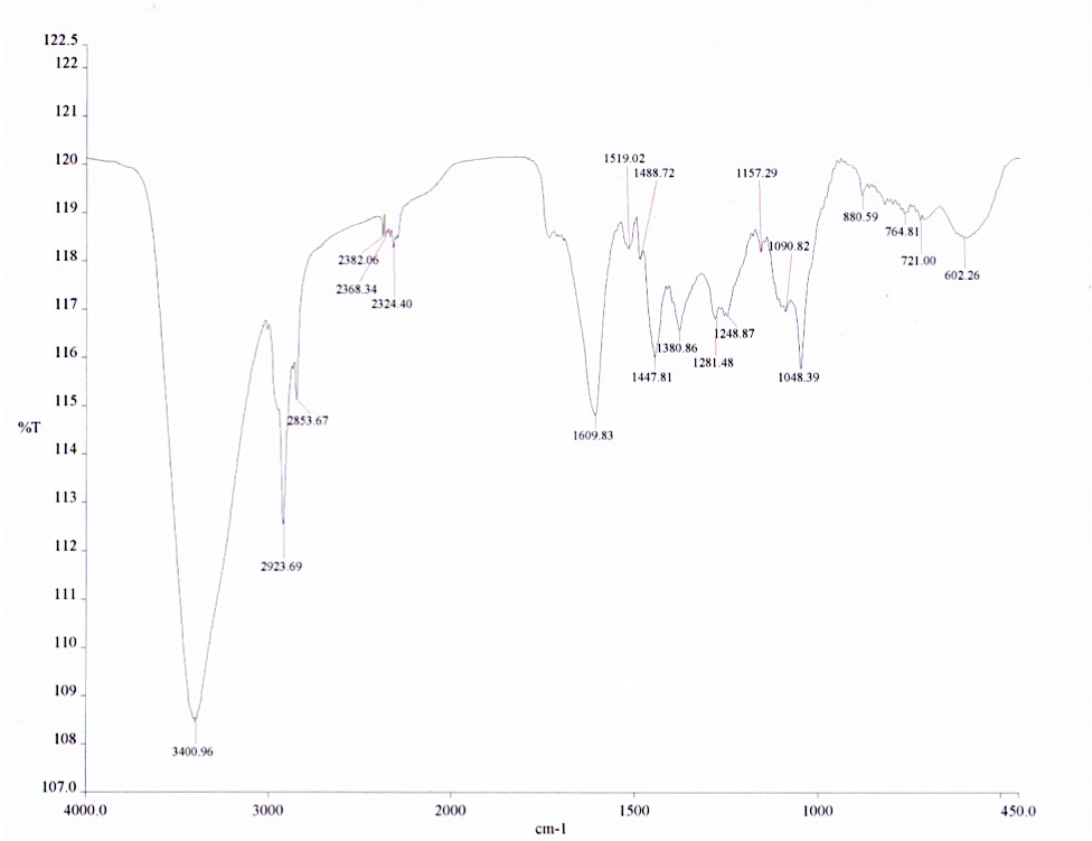

FIGURE 10: FTIR Spectrum of Oil Palm Leaf Extract (Ethanol Extraction)

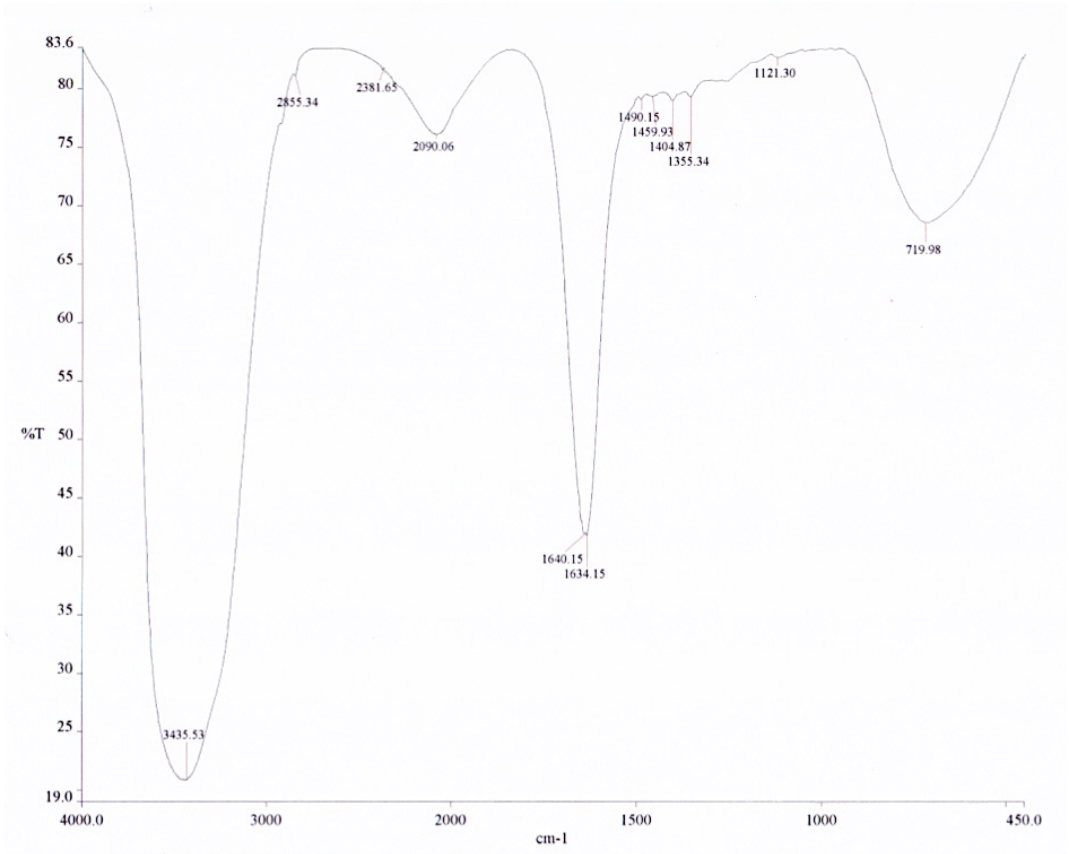

FIGURE 11: FTIR Spectrum of Oil Palm Leaf Extract (Water Extraction)

As it can be observed in the FTIR spectra, the most apparent peak for all three spectra is in the range of 3000 to 3500 cm^{-1}, which indicates the presence of O—H stretching bonds in phenol. The peaks around 2823 to 2852 cm^{-1} implies the presence of C—H asymmetric bonds in CH_3 and CH_2 groups. This peak is not apparent in the spectrum for the oil palm leaf extract using water extraction. Next, the peak at 1734 cm^{-1} in the spectrum of the oil palm leaf powder is an indication of a C=O stretching bond of carboxylic acids. Also observed in the same spectrum is a peak at around 1632 cm^{-1} which shows the presence of C=C in aromatics or alkenes. The peaks at 1105 and 1041 cm^{-1} could indicate the existence of C—O and C—O—C functional groups from cellulose and lignin. The functional groups present in the oil palm leaf extract can be concluded to be O—H from phenols and carboxylic acid, C=C aromatics, alkanes, alkenes and ethers.

This result is parallel to a study done to verify the feasibility of *Elaeis guineensis* methanolic leaf extract as an antioxidant. In the study done by Soundararajan and Sreenivasan (2012), it was found, through the FTIR spectrum of the methanolic extract, that the oil palm leaves have a moderately high phenolic content and traces of carboxylic acid, C=C aromatics, alkena, alcohol primer and other minor phenolic groups.

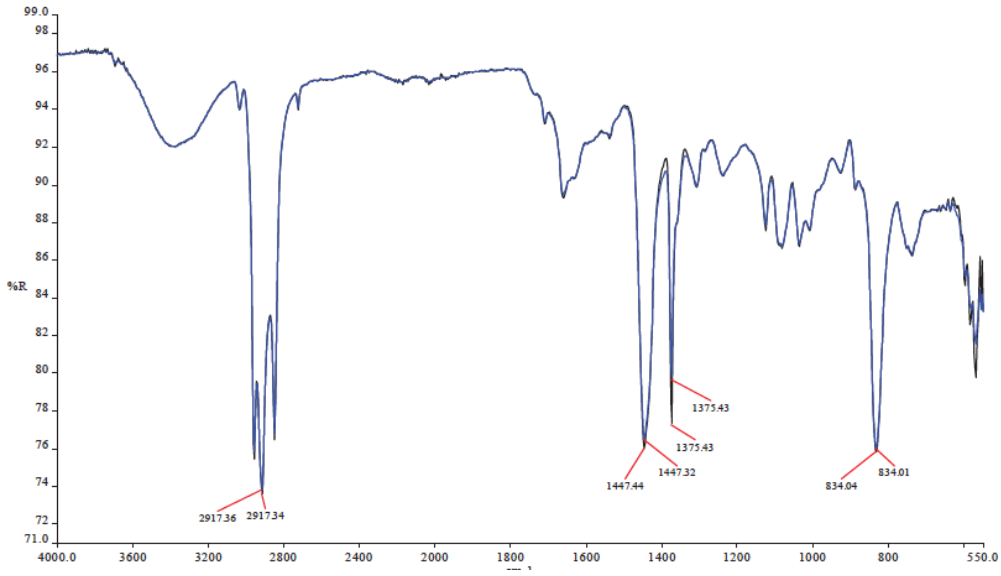

FIGURE 12: FTIR spectrum of methanolic extract from *E. guineensis* leaves *(Soundararajan & Sreenivasan, 2012)*

Similarly, in a study done by Sidik et al. (2012), oil palm leaf powder that was analyzed using FTIR resulted in absorption peaks in the 3000 to 3500 cm^{-1} range, indicating the presence of O—H from phenol. In addition to that, peaks at 2823 to 2852 cm^{-1} and 1734 cm^{-1} signifies the existence of C—H asymmetric bonds and C=O bonds, respectively. The FTIR spectrum of the oil palm leaf powder also showed transmittance in the range 1103 to 1060 cm^{-1}. Sidik et. al. states that this is due to the presence of C—O and C—O—C stretching vibrations in cellulose and lignin, respectively.

The main difference between the spectra of the ethanolic extract and the leaf powder is the reduction of transmittance of functional groups in between the range of 1700 to 1500 cm^{-1}. On the other hand, the spectrum of the aqueous leaf extract shows an overall reduction in all peaks. However, all the spectra obtained indicates the presence of the O—H bond in phenols and C=O stretching bond. These two bonds are the main elements required in the reduction of gold in chloroauric acid to produce gold nanoparticles and to cap the gold nanoparticles, preventing agglomeration (Abdel-Raouf et al., 2013; Gan et al., 2012).

4.2 Synthesis of Gold Nanoparticles

4.2.1 Visual Observations

The preliminary assessment of the presence of gold nanoparticles was observed from the colour change occurring in the colloidal solution. Since the ethanolic plant extract was a rich dark green colour, the pale yellow of the diluted chloroauric solution was not clearly visible. However, colour changes to the reaction mixture could still be observed. On the other hand, the initial colour for the aqueous plant extract was light brown. According to Sujitha and Kannan (2013), colloidal solutions exhibit intense colours due to their SPR. Gold nanoparticle solutions will appear to be wine red in colour with no sedimentation.

The first set of experiments was carried out to test the feasibility of the oil palm leaf extract in reducing gold ions in chloroauric acid. The initial experiments used ethanolic leaf extract with varying concentrations of chloroauric acid using trial and error method. The experiments were repeated with aqueous extract. From these

experimentations, it is deduced that the concentration of 0.02943 mM of the chloroauric acid in these mixtures is too low for the reduction of gold ions to occur. The minimum concentration that exhibited a colour change in the mixture is 0.14715 (20 times dilution of the stock solution). This is consistent with the study conducted by Gan et al. (2012), in which it was concluded that chloroauric acid with concentrations of 0.1 and 0.25 mM did not yield any gold nanoparticles.

The trial experiments that displayed a significant change in colour, did so within 10 minutes of the reaction. This result is supported by the studies by Zayed and Eisa (2014), Gopinath et al. (2013) and Dubey et al. (2010) in which their leaf extract and chloroauric acid mixture displayed a colour change in the first 10 to 15 minutes of the reaction.

A major difference between the reactions using aqueous and ethanolic extract is that the colloidal solution that was reduced using ethanolic extract will turn out purple in colour and particles tend to agglomerate after just 10 minutes from the time of reaction. The particles will then settle down to the bottom of the Erlenmeyer flask. As stated before, gold nanoparticle colloidal should be a rich wine red colour. This is the case for the experiments carried out with the aqueous extract.

FIGURE 13: Colour Change Observed During the Experiments

Increasing the ratio between the chloroauric acid and leaf extract resulted in faster reactions. Undoubtedly, increasing the volume of the leaf extract from 1 ml to 2 ml resulted in a shorter reaction time but once the volume of the leaf extract surpassed the volume of the acid, the reaction became slower.

Varying the temperature of the reaction also had an effect on the synthesis of gold nanoparticles. The reaction rate increased with increasing temperature hence colour change was clearly faster at 60 °C compared to at lower temperatures. However, according to Sujitha and Kannan (2013), a high rate of reaction hinders the capping process this causing the aggregation of gold nanoparticles. This can be observed in the colloidal solution reduced using the ethanolic extract, in which the agglomerated particles were much bigger at 60 °C than at 40 °C.

FIGURE 14: Colour Change Between Aqueous Extract and Colloidal Solution

FIGURE 14 shows the colour change from the light brown colour of the aqueous leaf extract to the red colour of the colloidal solution. After a week, the wine red colour of the colloidal solution was still the same indicating stable gold nanoparticles.

4.2.2 UV-Vis Analysis

As a preliminary assurance of the presence of gold nanoparticles, the samples obtained were characterized using a Perkin Elmer Lambda 25 spectrophotometer, using distilled water as a blank. The resolution used was 1 nm between 400 and 700 nm. When exposed to visible light, gold nanoparticles will form a plasmon band that has an absorption peak in between 510 and 550 nm. From the UV-Vis obtained from three samples as shown in FIGURE 15, there is a clear peak in the said range, confirming the presence of gold nanoparticles.

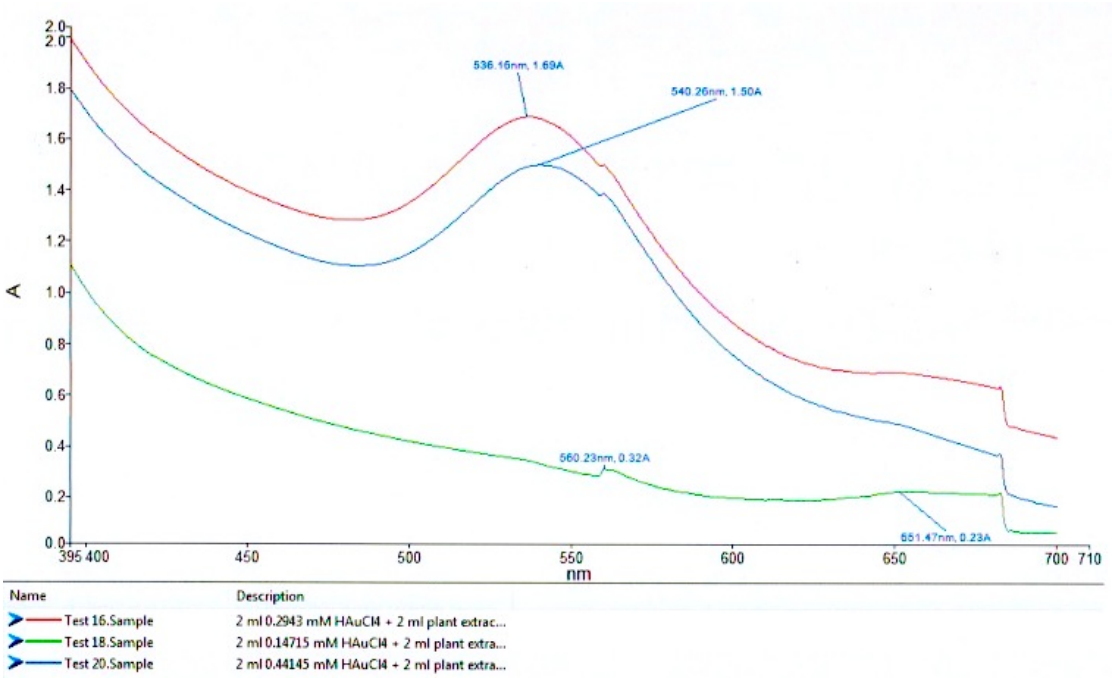

FIGURE 15: UV-Vis Spectra of The Gold Nanoparticles as a Function Of Chloroauric Acid Concentration

These samples were reduced using aqueous plant extract. As mentioned in the visual observations, the colloidal solutions reduced using ethanolic plant extract produced sedimentations. Hence, it was impossible to obtain a satisfactory spectrum from the samples. Another point to note in FIGURE 15 is that the sample using 0.14715 mM chloroauric acid did not create an absorbance peak in the 510 to 550 nm range, albeit exhibiting a colour change.

From the spectra, it can also be deduced that the most efficient concentration is 0.2943 mM, due to a higher absorbance. A red shift in SPR bands was observed for the higher concentration of chloroauric acid (0.44145 mM). As stated by Pandey et al. (2012), this red shift is due to the increase in the particle size and decrease in distance between the gold nanoparticles. In addition to that, all spectra show an increasing absorption tail in the near IR regions. According to Zayed and Eisa (2014), an apparent absorption tail extended to the near IR regions may be due to the size distributions of the gold nanoparticles or the formation of non-spherical gold nanoparticles. The apparent absorption peak seen before 400 nm may also be attributed to the plant extract.

4.2.3 FTIR Analysis

As mentioned in section 4.1.1, the preliminary FTIR analysis was carried out on the oil palm leaf extract to determine the functional groups present in the oil palm leaves. A reduction of absorbance peaks in the FTIR spectrum of the colloidal solution will help identify which functional groups contribute to the biosynthesis of gold nanoparticles.

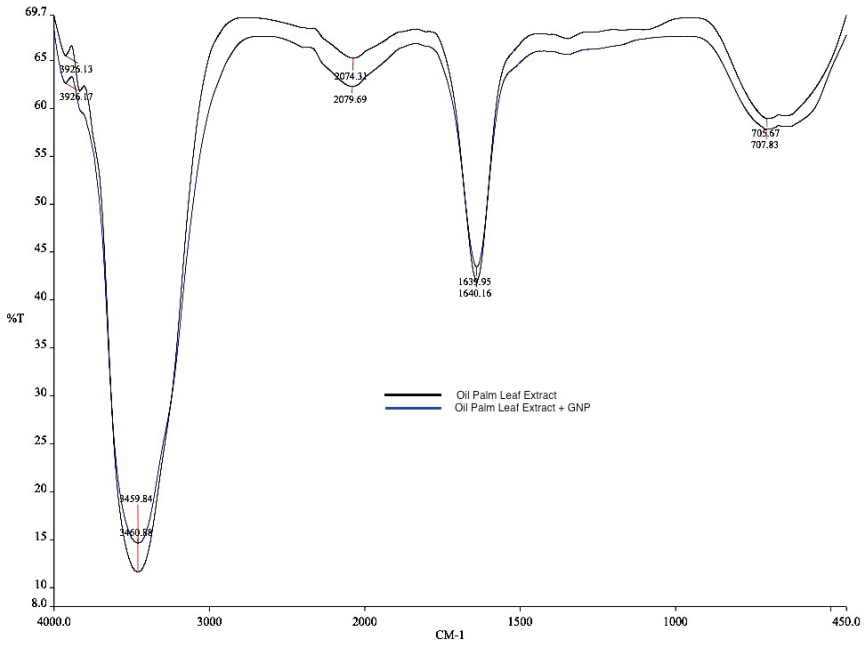

FIGURE 16: FTIR Spectra of Oil Palm Leaf Extract and Colloidal Gold Solution

Referring to FIGURE 16, there is a reduction in the transmittance peak at approximately 3400 cm^{-1} and 1640 cm^{-1} in the spectrum of the colloidal gold solution. This indicates that O—H and C=O groups present in palm oil leaves play a crucial role in the bioreduction of gold ions in choloroauric acid to gold nanoparticles. As suggested by Gan et al. (2012), the capping and stabilization of gold nanoparticles may be due to the presence of polyphenols and carboxyl groups, which are present in the oil palm leaves.

4.2.4 FESEM and EDX Analysis

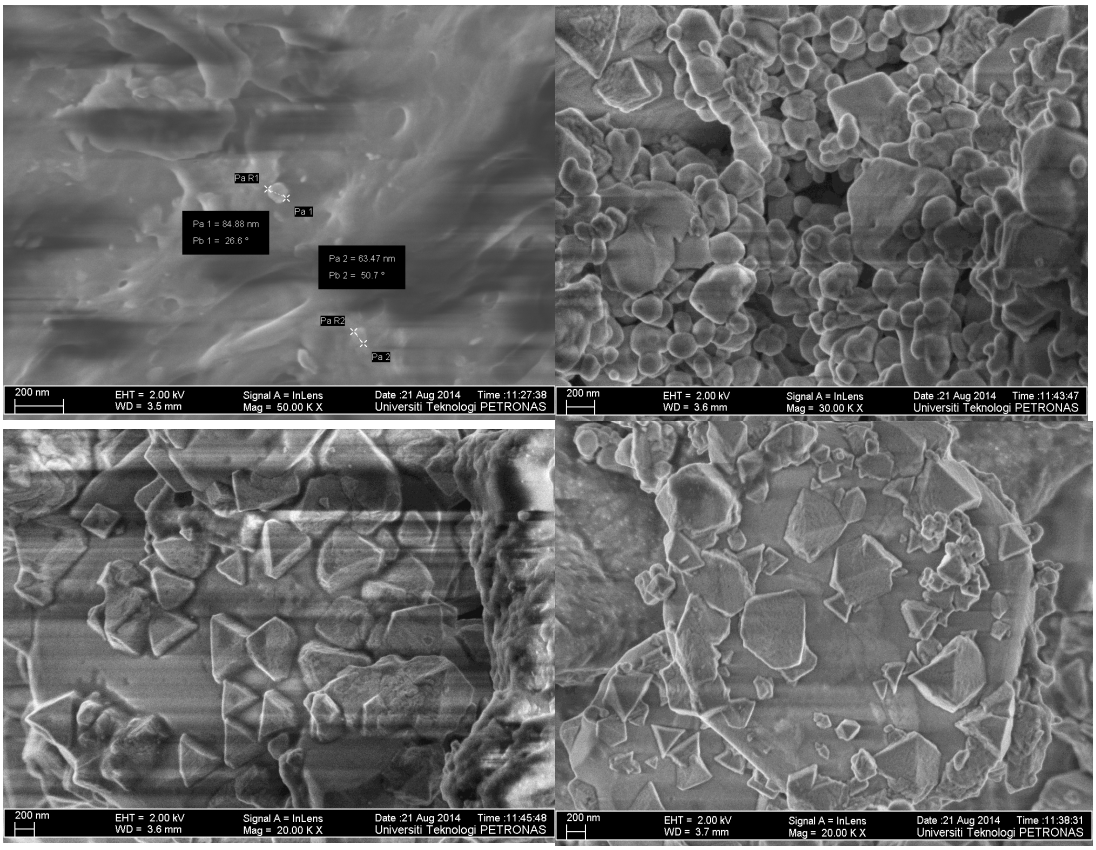

FIGURE 17: SEM Photographs of the Synthesized Gold Nanoparticles

FIGURE 17 shows the images obtained using the Zeiss Supra 55VP FESEM. From the images, it can be confirmed that the shapes of the gold nanoparticles obtained are irregular, with some being triangular, spherical and tetrahedral in shape. The EDX spectrum shown in FIGURE18 confirms the presence of elemental gold in the sample. The high amount of carbon and oxygen can be attributed to the biomolecules of the oil palm leaves.

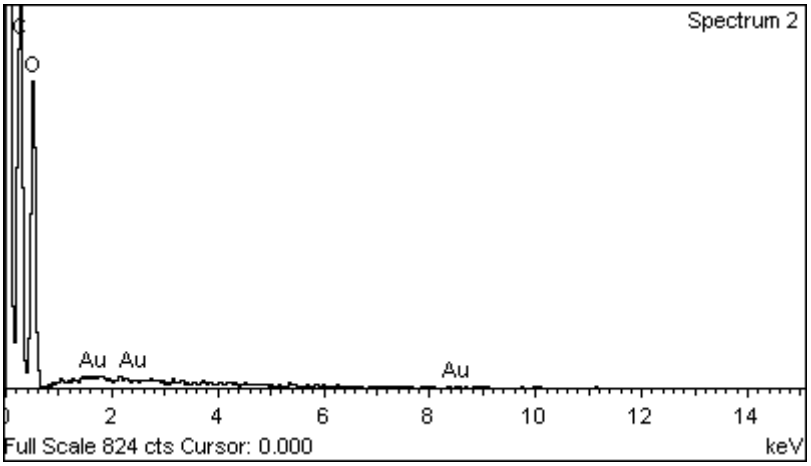

FIGURE18: EDX Spectrum Recorded from the Synthesized Gold Nanoparticles

Due to equipment unavailability in UTP, other characterization analysis to determine the shape and morphology of the gold nanoparticles using TEM were unable to be carried out within the given time frame. However, the results from the UV-Vis analysis is sufficient to indicate that oil palm leaf extract can act as a precursor to reduce gold ions in chloroauric acid.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

In conclusion, this study is significant because the results can contribute to the advancement in nanotechnology, particularly biosynthesis of nanoparticles for the use of medicine. As mentioned in this report, biosynthesis of gold nanoparticles is a growing area of interest. Since the oil palm itself has various medicinal properties, it is imperative that the study is attempted to confirm the feasibility of the oil palm leaf extract as a precursor for the bioreduction of Au^{+} ions. From the preliminary FTIR analysis of the plant extracts, it was found that oil palm leaf extract contains the relevant phytochemicals that can reduce the gold ions into gold nanoparticles. The biosynthesis is confirmed through the preliminary experiments carried out using oil palm leaf extract and chloroauric acid in which a colour change in the colloidal solution and an absorption peak in the 510 to 550 nm range of the UV-Vis spectra of the aforementioned solution was observed. Moreover, the presence of elemental gold can be observed from the EDX spectrum. FESEM images of the gold nanoparticles show that the particle shapes are irregular. Lastly, the FTIR analysis of the oil palm leaf extract and the colloidal solutions shows the crucial functional groups present in the oil palm leaves that may be involved in the bioreduction of gold nanoparticles are hydroxyl groups.

Recommendations and future works for this project include experimenting with different variations in the reaction parameters such as pH value of the reaction mixture and characterizing the gold nanoparticles using advanced characterization equipment i.e. X-ray Diffraction (XRD). Moreover, once stable GNPs are formed using the oil palm leaves, it is recommended to test the effectiveness of the synthesized nanoparticles in the biomedical field.

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APPENDIX A

Sample calculations for dilution of 2.943 mM $HAuCl_4$ stock solution

If the desired volume and concentration of the working solution is 100 ml of 0.02943 MM respectively, the volume of the stock solution required is as follows:

$$M_1V_1 = M_2V_2$$

$$2.943V_1 = (0.02943)(100)$$

$$2.943V_1 = 2.943$$

$$V_1 = 1\ ml$$

Thus, to prepare the working solution, 1 ml of the stock solution is added to a 100 ml volumetric flask. Then, 99 ml of distilled water is added to fill up the volumetric flask and the solution is shaken with the flask's stopper in place.