Characterization and Antibacterial Activity of Synthesized Copper Nanoparticles from *Melastoma malabathricum* Leaves Extract

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A report submitted in fulfilment of the requirements for the degree of Bachelor of Applied Science (Natural Resources Science) with Honours

Faculty of Earth Science

UNIVERSITI MALAYSIA KELANTAN

2017

DECLARATION

I declare that this thesis entitle Characterization and Antibacterial activity of Synthesized Copper Nanoparticles from *Melastoma malabathricum* leaves extract is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any degree.

Signature: _____

Name: Muhamad Najib Bin Abdul Rahman

Date: _____

ACKNOWLEDGEMENT

First and foremost, I would like to express my thankful to Allah S.W.T for provide me with passion and good health so that I can finish my Final Years Project (FYP). Secondly, I would like to express my gratitude to my supervisor, Dr. Irshad Ul Haq Bhat for his continuous guidance and support that allowed me to successfully complete my final year project. His understanding and knowledge have given me to settle down all the problem that I faced during finishing my final year project.

Secondly, I would like to thank to all las assistants that help me in monitoring, guiding and teach me on conducting the experiment for this study. I also would like to express my gratitude to Miss Kyariatul Syafinie Abdul Majid as my academic advisor that always advised me and keep me motivated during finishing my final year project.

To all my friends and course mates, thank you for your helping me throughout my final year project. Throughout the experience that I gained from completing this final year project, I will use it in facing the future challenges.

Last but not least, I owe my sincere gratitude to my family members, especially my parents and all my sibling that continuously encourage and support me in my study in Universiti Malaysia Kelantan (UMK) and finishing my final year project. Without support from them, it would be impossible for me to finish this thesis.

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ABSTRACT

In the 21th century, the wide use of nanoparticles in different field had led to variety of methods of synthesis nanoparticles. Some method used had cause diverse impact in the environment. Thus, research on other method that more eco-friendly was required. In this study, the green synthesis approach was adopted to synthesize Copper nanoparticle (CuNPs) by using *Melastoma malabathricum* leaves extract. The prepared Copper nanoparticles were characterize by UV-visible spectroscopy (UV-vis), X-ray Powder Diffractometer (XRD), X-ray florescence (XRF) and Fourier Transform Infrared Spectroscopy (FT-IR). The UV-visible spectra of CuNPs revealed abroad absorption band between 370-380 nm contributing to the surface Plasmon vibration of metal nanoparticles. The XRF analysis confirmed the presence of silver in nanoparticles and XRD result reviled the monoclinique shape with 13-29 nm size of CuNPs. The presence of alcohol (O-H), alkene (C=C), organohalide (C-F) and oxygen ether (C-O) was confirmed by FT-IR analysis. Antibacterial activity of CuNPs against *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) was carried out by disc diffusion method with increasing concentration (0.003, 0.006, 0.009, 0.012, 0.015 and 0.02μg/ml) of nanoparticle enhance inhibition zone was observed.

Pencirian dan Aktiviti Antibacteria bagi Kuprum Nanopartikel yang disintesis menggunakan extrak daun *Melastoma malabathricum*

ABSTRAK

Dalam abad 21 kini, nanopartikel digunakan secara meluas dalam pelbagai bidang dan terdapat pelbagai cara untuk nanopartikel di sintesis. Sesetengah daripada cara tersebut boleh merosakkan alam sekitar. Dalam kajian ini, pendekatan sintesis hijau digunakan untuk sintesis nanopartikel Kuprum (CuNPs). Nanopartikel Kuprum yang telah di sintesis dicirikan menggunakan *UV-visible spectroscopy* (UV-vis), *X-ray Floresence* (XRF), *X-ray Powder Diffractor* (XRD) dan *Fourier Transform Infrared Spectroscopy* (FT-IR). UV-vis spektra bagi CuNPs menunjukkan penyerapan lengkungan luas antara 370-380 nm menyumbang kepada getaran permukaan Plasmon bagi nanopartikel logam. XRF analisis meyakinkan kehadiran Kuprum dalam nanopartikel dan hasil analisis XRD mendedahkan bentuk CuNPs adalah monoclinic dan saiznya ialah 13-29nm. Kehadiran alcohol (O-H), Alkena (C=C), Organohalida (C-F) dan Oksigen eter (C-O) ditentukan menggunakan FT-IR analisis. Aktiviti anti-bacteria bagi CuNPs menentang *Escherichia coli* (*E. coli*) dan *Staphylococcus aureus* (*S. aureus*) dijalankan menggunakan kaedah resapan cakera dengan kepekatan nanopartikel ditingkatkan iaitu (0.003, 0.006, 0.009, 0.012, 0.015 dan 0.020 μg/ml) bagi menggalakkan zon perencatan dapat diperhatikan.

LIST OF FIGURES

LIST OF ABBREVIATION

ANOVA	Analysis of variance
a.u	Absorbance unit
$CuCl_2$	Copper Dichloride
CuNPs	Copper nanoparticles
DNA	Deoxyribonucleic Acid
DLS	Dynamic Light Scattering
FT-IR	Fourier Transform Infrared Spectrometry
MDR	Multi Drug Resistance
MIC	Minimum Inhibition Concentration
NA	Nutrient agar
nm	Nanometer
XRD	X-ray Powder Diffraction
XRF	X-ray Florescence
µg/ml	Microgram per millilitre
µg/L	Microgram per liter
µm	Micrometer

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Nanoparticles are metallic or polymeric particles that size within range of 1 – 100 nm and potentially applied in pharmaceutical, biotechnology, information technology, aerospace, defence, agriculture, energy, textile, automotive, telecommunication (Nimesh, 2013). Metal nanoparticles have been comprehensively studied attributed to their versatile antimicrobial properties and catalytic activity (Das *et al*., 2013). Nanoparticles can be synthesized by sonochemical, supercritical fluid, solvothermal and hydrothermal, microwave, spray pyrolysis, templet synthesis and green chemistry (Bensebaa, 2013). Green chemistry or green synthesis method is a method that simple, easy, efficient, and eco-friendly (Mubayi *et al*., 2012). Due to this factor, scientist nowadays made a dipper exploration on this method toward the effectiveness in synthesis metal nanoparticles.

In order to synthesis nanoparticles by using green method, plant material such as leaf, root, stem, flower and fruit or plant extract is required. In this research, the leaf of *Melastoma malabathricum* will be use as the plant material. *Melastoma melabathricum* is a part of pantropical family that comprises of 22 family (Noormawati *et al*., 2015). In traditional, *M. malabathricum* was used as medicine for treat stomach ache, white disease, minor wound, pox sports and women after childbirth (Ismail *et al*., 2003)

In terms of antibacterial activity, synthesize copper nanoparticles was tested on the multi-drug resistant (MDR) bacteria which is in this research *Escherichia coli*

(E. coli) and *Staphylococcus aureus* (*S. aureus*). The *E. coli* was used in this research to represent gram-negative bacteria (Gupta & Sivakumaran, 2016) while *S. aureus* represent gram-positive bacteria (Okafor *et al*., 2013). Base on (Sharma *et al*., 2015) antibacterial or antibiotic can be classified into several class which is sulphonamides, macrolides, β-lactams, penicillin, arsenicals and aminoglycosides. As the technology on antibiotic increase, some mutation was occur within the genotype of bacteria to confront the resistance again the antibiotic (Lenski, 1998).

1.2 Problem Statement

Bacteria usually exhibit resistance to different treatment due to ability of it to evolve and adapt to the environment. Thus new method based on nanoparticles and green approach are needed in order to against and control bacteria growth for future wellbeing.

1.3 Objective

- To characterize the synthesized copper nanoparticle by using *Melastoma malabathricum.*
- To evaluate antibacterial activity of *E. coli* and *S. aureus* by using synthesis Copper nanoparticles.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanoparticles

Nanoparticle is particle that has size between one to hundreds nanometres. Synthesize of nanoparticle is under the study of nanotechnology which concern to variety sizes, shape, chemical composition and control dispersity and their potential use for human benefits (Kathiravan *et al*., 2015). Metal nanoparticle have high specific surface area and high fraction of surface atom (Marquis *et al*., 2016). Even though nanoparticle can give a lot of benefit to human life, but it also have bad impact to the environment include human as the process in evaluate the nanoparticle it quite critical and difficult in quantify the hazard level (Bensebaa, 2013). Nanoparticle can be divided to 4 groups which are: (i) inorganic metals, (ii) inorganic semiconductors, (iii) inorganic insulator including oxides and sulphide and finally (iv) organic and polymers (Bensebaa, 2013).

In common, there is two way of producing or synthesize the nanoparticle which is wet method and dry method. In order to synthesize nanoparticle using wet method, there is two way it can be use which is bottom-up synthesis that use chemical base process and the second way is top-down method that using physical process (Bensebaa, 2013). Same as wet method of synthesis of nanoparticle, dry method also has two way of synthesis which is chemically and physically (Bensebaa, 2013).

2.2 Copper nanoparticles

Copper with the chemical element symbol (Cu) is a metal that reddish-orange in colour for the pure one. It is well known in the medical and industry field as inhibitor of bacteria and other microorganism (Patel *et al*., 2016). Lately, Copper nanoparticles (CuNPs) have been extensively study due to some characteristics, some example of study on CuNPs is the toxicity of it toward *Daphnia magna* under different exposure condition (Xiao *et al*., 2016), importance of extracellular speciation and corrosion of CuNPs on lung cell membrain integrity (Hedberg *et al*., 2016) and other. Due to special cherectiristic of CuNPs, it was widely applied as catalysis, optical limiting, photovoltaics and biosensors (Xu *et al*., 2016). Copper nanoparticles have absorbance rate at 560 to 600a.u and make it suitable for dye removal activity from the water source (Mokhtari *et al*., 2016).

In recent years also, the green synthesis of CuNPs was done using *Ginco biloba Linn* leaves (Figure 2.1). As the result of using Transition Electron Microscopy (TEM) analysis, the size of synthesize Copper nanoparticles using *Ginco biloba* leaves as the reduction agen is between 15 to 20 nm (NasrillahZadeh & Sajadi, 20015). By using TEM and Dynamic Light Scattering (DLS), the shape of CuNPs also can be determine which is spherical and monodispered (Prasad *et al*., 2016).

Figure 2.1: *Ginko biloba* leaves

(Source: Carole Anne Tomlinson, 2015)

2.3 Green synthesis

Green synthesis is a process to synthesize product by using green method that involve plant material as reduction agent. Plant material have been extensively use in synthesis activitity as it will reduce and elaminate the use of hezourdous substance or chemical (Wang & Liu, 2016). The other specialty of plant material that make resercher interest to make research on it is because of the contain of exceptional of chemical stabilisation that help in formation of nanoparticles (Gardea-Torresdey *et al*., 2008). In recent years, variety type of plant species have been use as plant material to synthesis nanoparticle such as *Euphorbia tirucalli* (Malleshappa *et al*., 2014), *Vigna unguiculata* (Mohammadi, *et al*., 2016), *Emblica officinalis* (Ramesh *et al*., 2015) and many other plant species.

2.4 *Melastoma malabathricum*

Melastoma malabathricum or called as Singapore Rhododendron or Senduduk belong to the family of Melastomataceae (Balamurugan *et al*., 2014). In this recent years, much research on this tree was widely practiced. For example *In-vitro* antiproliferative and antioxidant activities and total phenolic contents of the Extracts of *Melastoma malabathricum* leaves (Zakaria *et al*., 2011), Performance of fruit extract of *Melastoma malabathricum* as sensitizer in DSSCs (Dye-sensitized solar cells) (Singh *et al.*, 2014) and the latest one is the effects of this plant on erosion rate of slope soil at different slope orientations (Halim & Normaniza, 2015). However, the research about the synthesis of nanoparticle and the antibacterial activity is not much done yet on this plant.

In Southeast Asia include Malaysia, the family of Melastomataceae comprise of 22 species, two subspecies and three varieties which are classified by the colour of the flower, petals-light-pink magenta, dark-purple magenta and white (Rajenderan, 2010). In tropical Asian country, the most comment type of Melastoma species found is *M. afiine* (Figure 2.1), *M. candidum* (Figure 2.2), *M. malabathricum* (Figure 2.3), *M. polyanthum* (Figure 2.4) and *M. sanguineum* (Figure 2.5). In common, people is hard to differentiate between *M. candidum* and *M. sanguinium*. *M. candidum* and *M. sanguineum can be* differ markedly in indumenturn of leaf and hypanthmm (a cup-shape structure which bears the sepals, petals and staments (Lui *et al*., 2014). But, in this research, we was focused on the use of *Melastoma melabathricum* extract for synthesis CuNPs and test on the antibacterial properties of Copper nanoparticles.

Figure 2.2: *Melastoma affine*

(Source: Robert Whyte, 1993)

Figure 2.3: *Melastoma candidum*

(Source: Hungda, 2012)

Figure 2.4: *Melastoma malabathricum*

(Source: Daniel Mosquin, 2013)

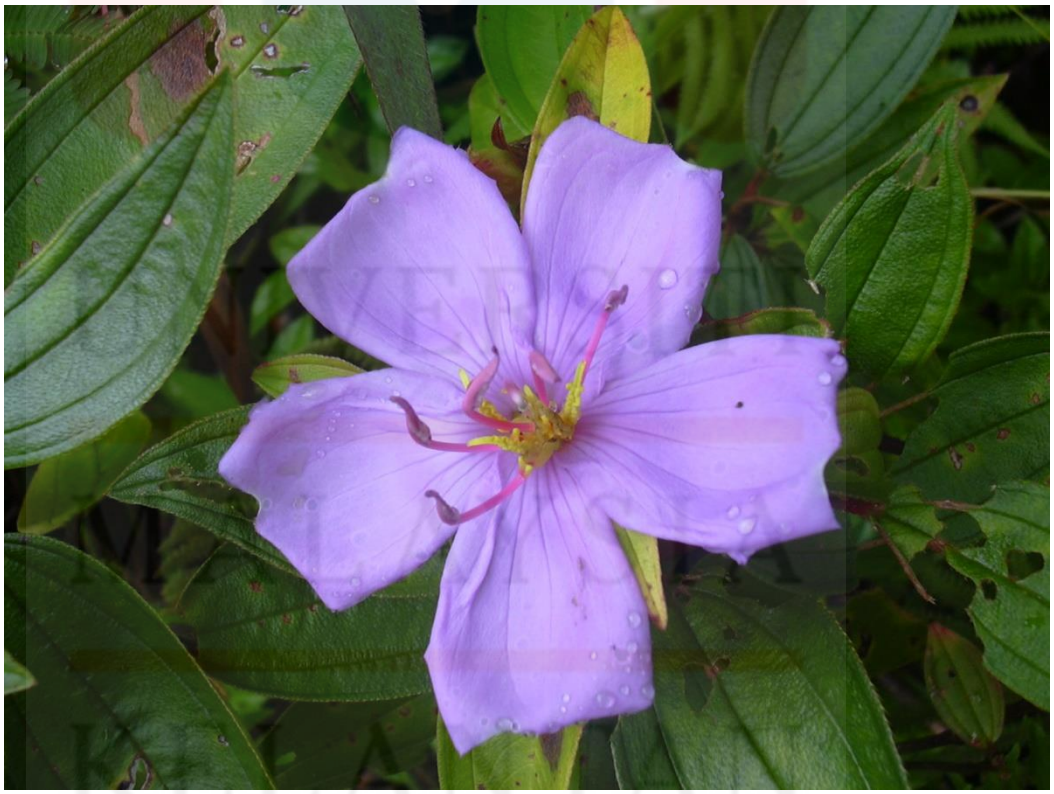


Figure 2.5: *Melastoma polyanthum*

(Source: Andreas Lambrianides, 2013)

Figure 2.6: *Melastoma sanguinium*

(Source: Kaiyan Wong, 2012)

2.5 Antibacterial activity

Antibacterial activity is any activity that inhibits the increase in bacteria population or growth either chemically, physically or biologically. Due to bacteria can evolve to against or adapt to the anti-bacteria agent, a continuous research is required to against it. Due that, extensive research are required to identify and find the effective anti-bacteria agent that capable to destroy the bacteria instead of control the population by focus on break the bacteria DNA. In classic life cycle of bacteria, four stages of it was determine which is lagging, log, stationary and death phase as in (Figure 2.6) but in the modern and more scientifically research, the log phase was divided into tree part which is early log, mid-log and late log (Cooper, 1991). In general, there are a few factors that contribute to antibacterial activity such as temperature, pressure, humidity, nutrient available and pH (Held *et al*., 2005).

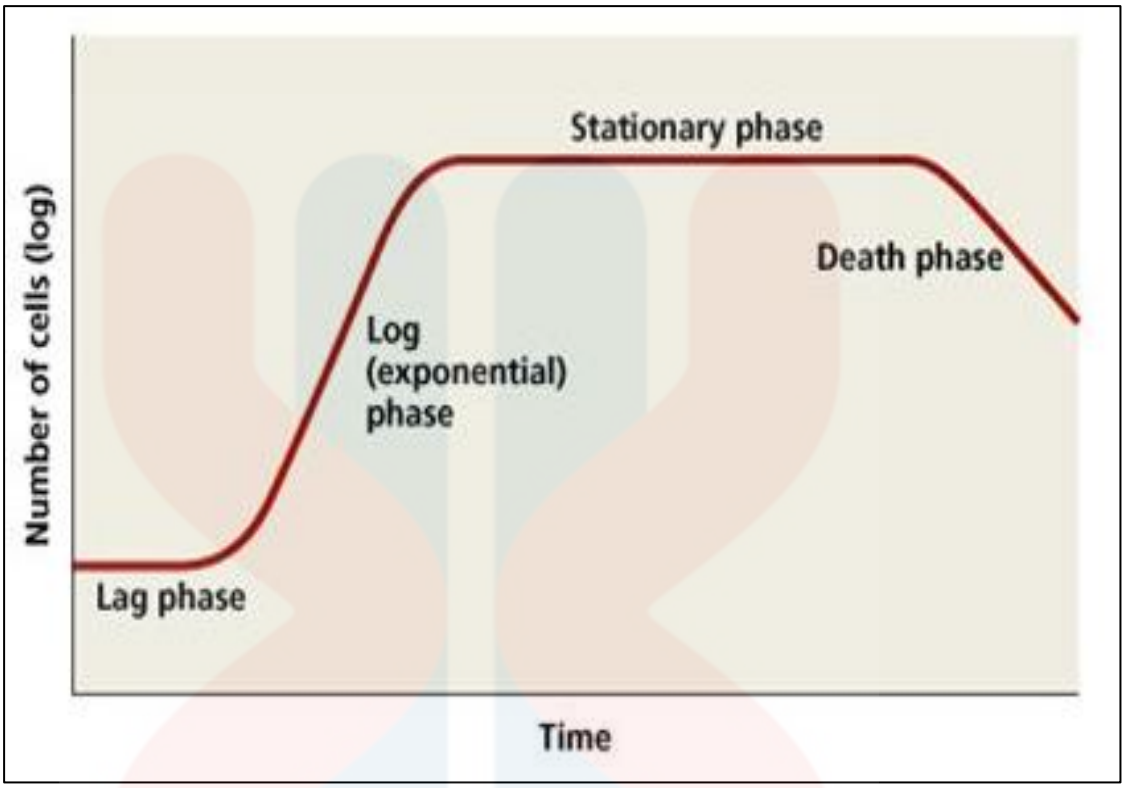


Figure 2.6: Graph of number of bacteria against time

(Source: Benjamin Cumming, 2006)

E. coli or *Escherichia coli* was firstly discovered in 1885 by Dr Theodore Escherich (Bell & Kyriakides, 1998). The presence of E.coli mean the source is contaminated with the faeces and can cause severe disease (Shaibani *et al*., 2016). *E. coli* also play an epidemiology role in the spread of resistance as it will substitute as antimicrobial resistance gene when it enter the gastrointestinal track of animal or human (Alonso *et al*., 2016).

Staphylococcus aureus or S. aureus was one of the pathogens that capable to provide tremendous infection (Radhakrishna & Aishwarya, 2015) and also cause contagious and chronic mastitis in dairy cattle (Cremonesi *et al*., 2015). This pathogen required iron from haemoglobin to growth like other bacteria pathogens (Ratner, 2015)

and able to adapt to the host to continue the survival event in the critical condition (Alonzo & Torres, 2013).

In order to against bacteria growth, it is importance to identify the Minimum Inhibition Concentration (MIC) so that synthesize copper nanoparticle can be used effectively. The MIC is the minimum concentration that required to inhibit the bacteria growth and the common unit use is μg/ml (Papich, 2013)

CHAPTER 3

MATERIALS AND METHODS

3.1 Chemical

Copper (II) chloride 2-hydrate ($CuCl_2.2H_2O$)) from Merck (Darmstadt, Germany), Nutrient Agar (NA) from Oxoid (Hampshire, England).

3.2 Instrument

UV-Visible spectroscopy (from Thermo Fisher Scientific model 4001/4), X-ray Powder Diffractometer (XRD) from (Bruker Karlsruhe, Germany with serial number 208493), X-ray Florescence (XRF) from (Bruker Karlsruhe, Germany with serial number 208543), Fourier Transform Infrared Spectrometry (FT-IR) from (Thermo scientific with model number iZ10) and Centrifuge from (Eppendorf German, model 5810R)

3.3 Plant leaves collection and powdering

Fresh leaves of *Melastoma malabathricum* were collected in the area around Agropark of Universiti Malaysia Kelantan, Jeli campus. The leaves were washed using running tap water and washed again using distilled water. The leaf was dried in shade and powder of dried *Melastoma malabathricum* leaves were made using blender

3.4 Preparation of *Melastoma malabathricum* extract

M. malabathricum leaves extract was prepared by mixing 10 g of the dry leaf powder with 100 ml of double distilled water and kept in 60 °C water bath for 10 minute. The extracts was then filtered using cotton gauze and followed by using filter paper. The filtered extract was stored in 4 °C incubator for further studies.

3.5 Synthesis of Copper nanoparticles

Synthesized Copper nanoparticles was prepared by followed the method that had been use by (Das *et al*., 2013) with abit of change. The synthesized copper nanoparticle was prepared by adding 10 ml of aqueous extract of *Melastoma malabathricum* into conical flask and followed by adding 90 ml of 1 mM of $CuCl_2$ solution. The mixture was then incubated in incubator at 37 °C.

3.6 Characterization of synthesized copper nanoparticles

The characterization of Copper nanoparticles was made using UV-Visible spectroscopy to determine the different in analysis of transition metal ions in synthesized copper nanoparticles. The wavelength used was from 200nm to 1100nm. Characterization of Synthesized CuNPs was continued using X-ray Powder Diffractometer (XRD to analyse the crystalline nature of synthesized Copper nanoparticles.

The characterization of copper nanoparticles was continued using X-ray Florescence (XRF). XRF was used to identify the elemental composition in the synthesized CuNPs sample. Lastly, Fourier Transform Infrared Spectrometry (FT-IR)

was used to determine the functional group that have in the sample that respond for the reduction of CuNPs.

3.7 Bacterial pathogens

Multidrug resistance strain (MDR) that was *Escherichia coli* and *Staphylococcus aureus* was obtained from Sigma-Aldrich. The bacteria was then cultured by using streak plate method in the petri dish that fill with nutrient agar (NA) and incubate at 37 °C temperature.

3.8 Antibacterial activity of copper nanoparticle.

A different concentration of solution contain synthesized copper nanoparticle was prepared on 0.020µg, 0.015µg, 0.012µg, 0.009µg, 0.006µg and 0.003µg/ml to test on MIC and 5µl of each different concentration of synthesized copper nanoparticle was dropped on petri dish that contain cultured *E. coli* and *S. aureus*. In the meantime, 5µl of 0.003µg/ml of amoxicillin was dropped into the both plate contain the cultured bacteria as control (C). At the same, 5µl of plant extract (PE) was also dropped into the plate contain the cultured bacteria. All the solution that was dropped into the plate that contain culture bacteria with the sterile disk and labelled with 0.020µg/ml, 0.015µg/ml, 0.012µg/ml, 0.009µg/ml, 0.006µg/ml, 0.003µg/ml, PE and C. All the plate was then incubate at 37°C for 24 hour. After incubation period, the zone of inhibition of bacteria for different concentration will be determine using ruler.

3.9 Statistical analysis

The inhibition zone of antibacterial activity are express as means ± standard deviation. Analysis of variance (ANOVA) and Tukey-Kramer multiple-comparison was used to analyse the difference in mean and the inhibition zone of bacteria. The software of IBM SPSS statistics 23 was used to run the statistical analysis. The *P* value that <0.05 considered as significant.

CHAPTER 4

RESULT AND DISCUSSION

4.1 UV-Visible spectroscopy of synthesized copper nanoparticle

Refer to the Figure 4.(a) below, incubation of 10 ml of plant extract with 90 ml of 1.0mM of $CuCl_2$ solution cause change in colour. The colour change from very light blue ($CuCl_2$) solution with yellowish (*M. malabathricum*) extract to brown. The change in colour indicate the reduction of copper chloride solution to copper nanoparticle after incubation period due to surface plasmon vibration metal nanoparticle (Chan *et al*., 2007). The change of colour also prove that the plant extract not just act as reduction egent but also the stabillizing agent (Marquis *et al*., 2016).

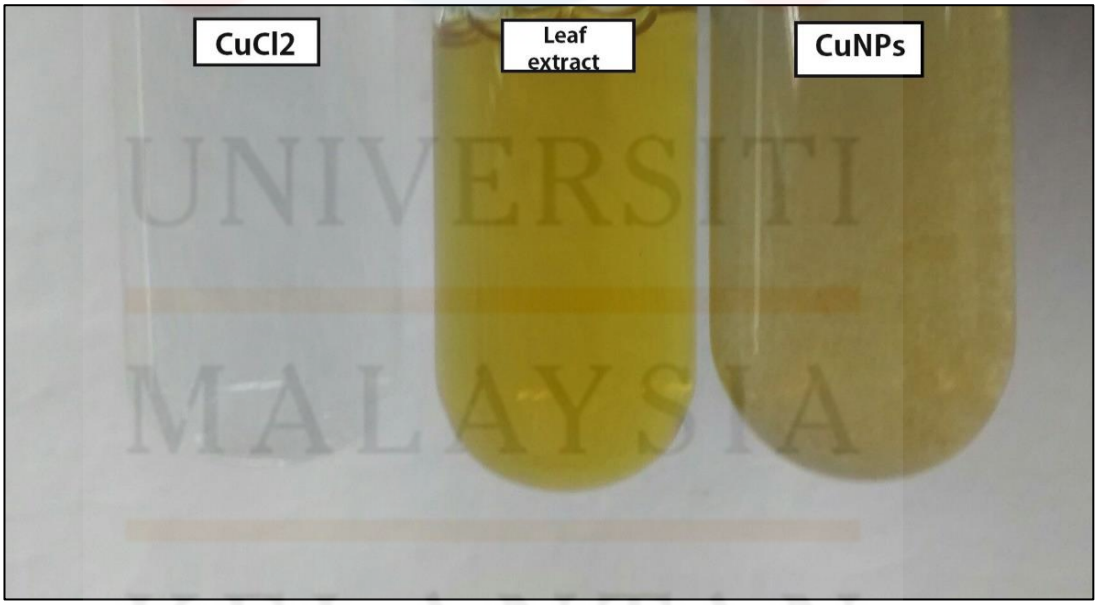


Figure 4.1(a): Colour change of synthesize CuNPs

The excitation of Copper nanoparticle was then characterize using Uv-Vis spactroscopy. In this method, the synthesize nanoparticle was run using wavelength in range of 200nm to 1100 nanometre for 2,3,4 and 5 hour of incubation period. The result get was then interparate as in Figure 4.1(b). From the graph, it show that the band occur within the range of 370 to 380 nanometers with a shifted of band at range of 650 to 750 nanometre.

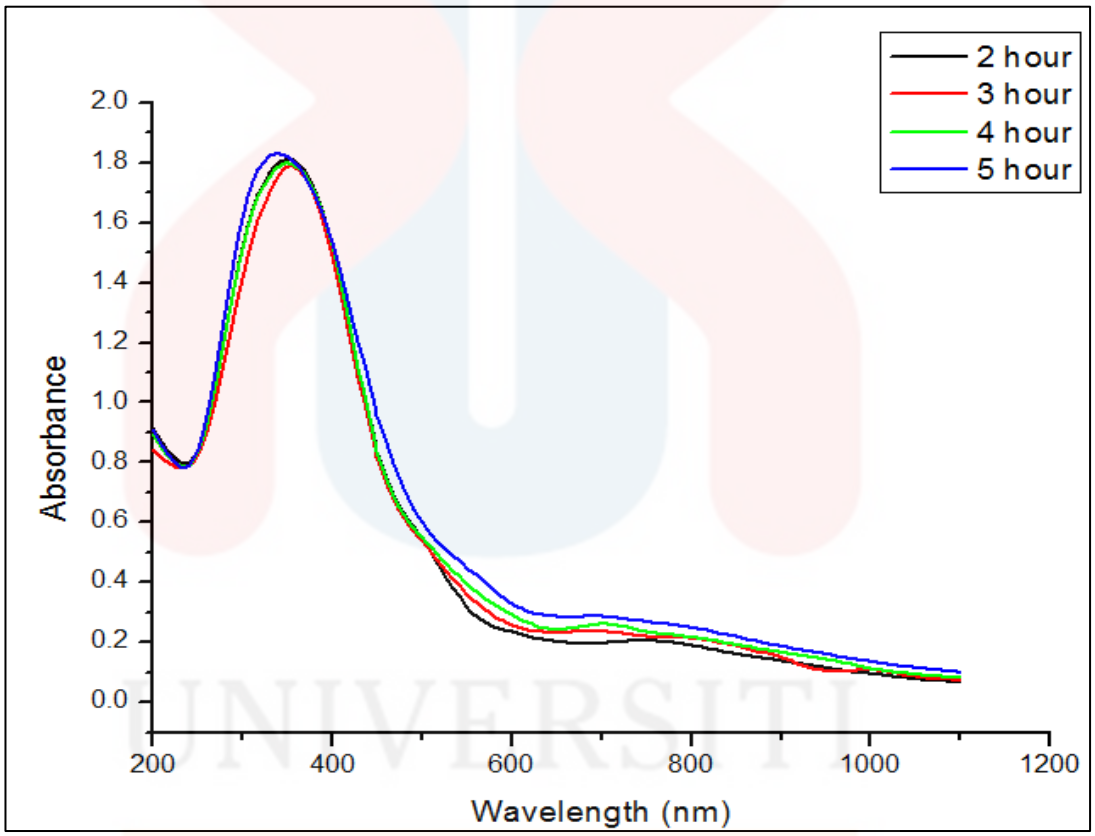


Figure 4.1(b): Uv-Vis spectrum of CuNPs for different time interval of incubation

Same as at the wavelength of 200nm, the absorption of CuNPs was at range of 0.8 to 0.9 with shift. From Figure 4.1(b) also, it indicate that the formation of CuNPs only occur at the early period of incubation which was below than 2 hour because the absorbance within the different incubation period was not show much change.

4.2 XRF analysis

Based on Figure 4.2, the high percentage of copper element which is 30.58% from the sample of synthesize nanoparticle indicate that the effectiveness of plant extract act as the reduction agent for the formation of Copper nanoparticle. In the meantime, the second highest percentage of element that had in the synthesize nanoparticle was Sodium with 26.16%. This element might came from the plant extract itself and followed by chlorine, phosphorus, sulphur and other.

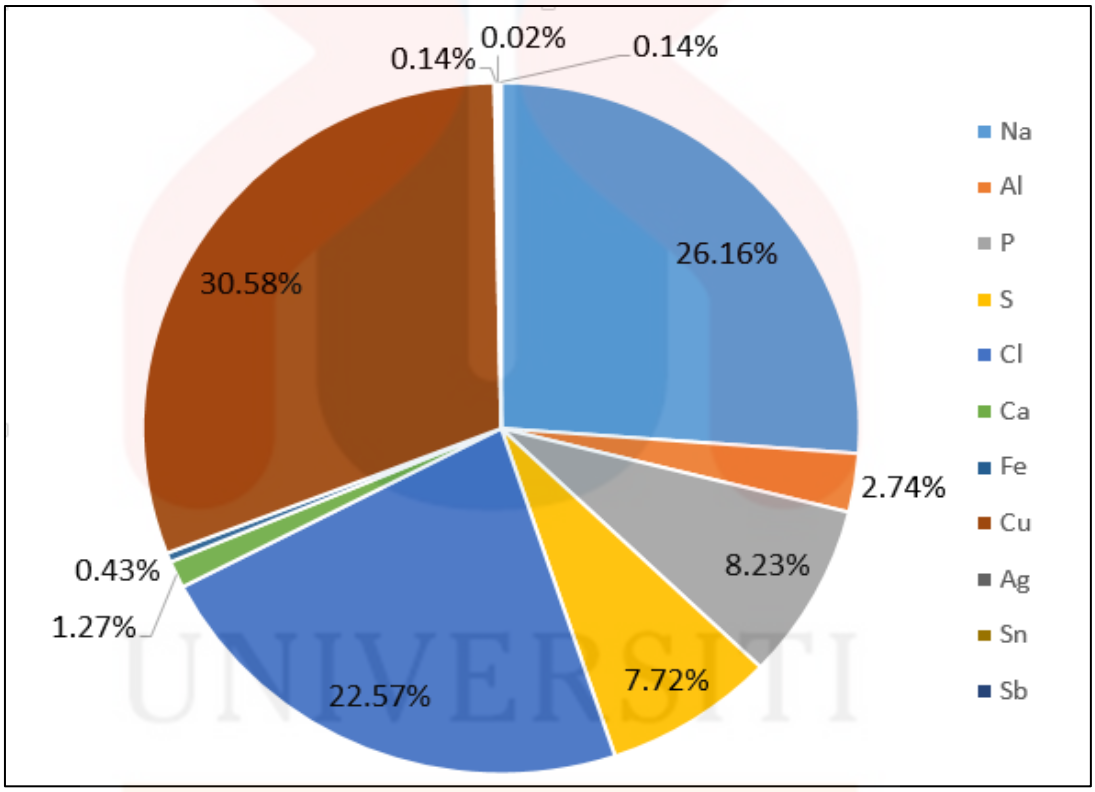


Figure 4.2: Percentage of element in synthesized CuNPs

The entrance of chloride ions into the Cu(II) coordination sphere during the incubation was indicated by the colour change of the starting solution from very light blue to black-green copper after the centrifuse process and this is due to complexes compound from plant extract (Cedzynska *et al*., 1981).

4.3 XRD analysis

The crystalline nature of synthesize CuNPs was identified through XRD analysis graph as on Figure 4.3. The XRD spectrum show 4 discrete peak at 26.10°, 31.62°, 46.55° and 47.08° respected to 2θ value with d value was 3.9033, 3.0234, 2.1377 and 1.9516 respectively and corresponding lattice plane was (-1 -1 -1), (-3 -1 0), (-4 -2 0)and (-4 -2-2) with lattice parameter of a= 9.5611Å.

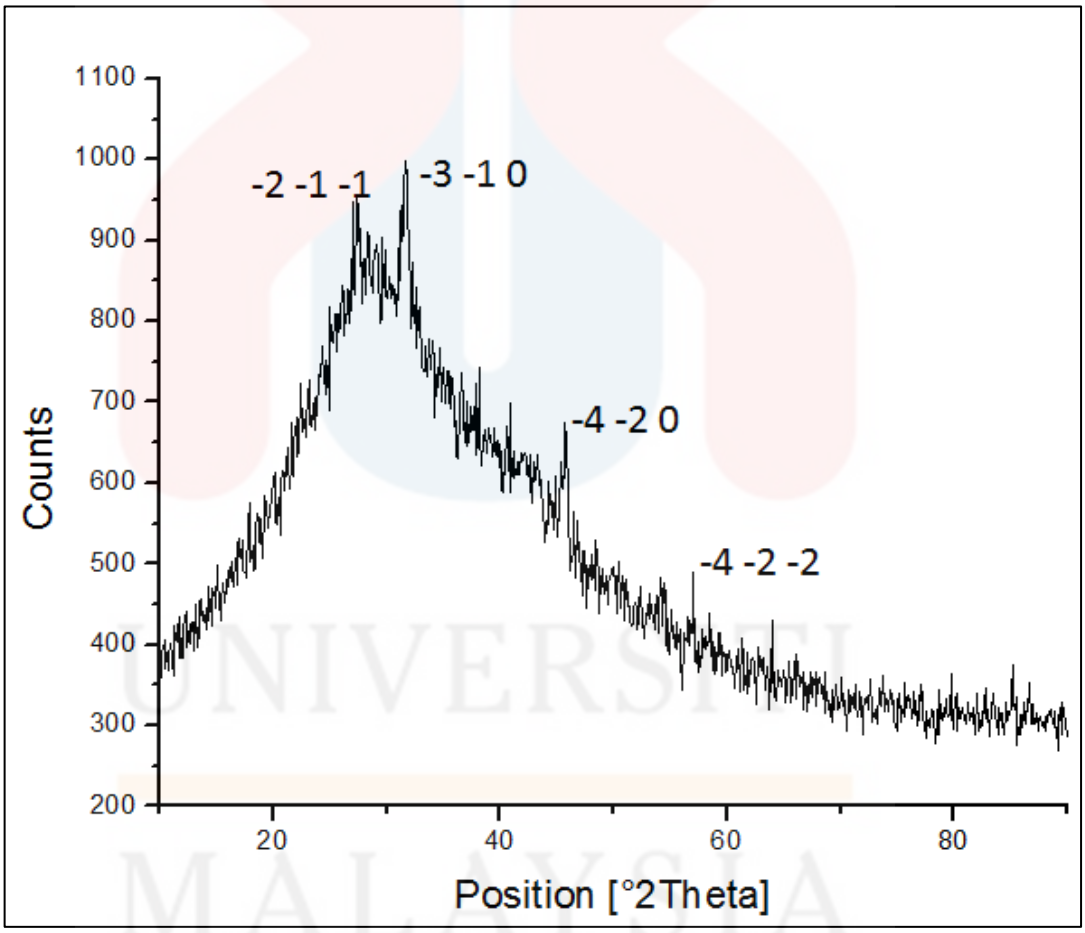


Figure 4.3(a): XRD patterns of synthesize CuNPs from aqueous extracts of *M. malabathricum*

The crystallinity of this spectrum was observe from 10° to 90°. The crystalline nature of the synthesized CuNPs was identify by using Diffraceva software from Bruker, and the crystalline nature identified was monoclinique. But from other

research, the crystalline nature of synthesized CuNPs was monoclinic structure (Awwad *et al*., 2015) and face centered cubic (Manikandan & Sathiyabama, 2015). After the peak was identified, the size of synthesized CuNPs was calculate using Scherrer equation as follow:

$$D_p = \frac{0.94\lambda}{\beta_{1/2}\cos\theta}$$

Where: D_p= Average crystallinity size

λ= X-ray wavelength

β = Line broadening in radians

θ = Bragg angle

Figure 4.3(b): Scherrer equation

(Source: Mahendra Kumar Koppolu, 2013)

After calculation, the size of synthesized CuNPs was 13.55 nm, 17.71 nm, 26.23 nm and 28.79 nm and the lattice strain was at 0.0735, 0.0466, 0.0217 and 0.0196. The different in crystalline nature of synthesized copper nanoparticle may occur due to the different method of synthesized and the type of plant use that lead to different functional group that cause the different in reduction of CuNPs.

4.4 FT-IR analysis of CuNPs

FTIR spectroscopy was used to identify the functional group in plant extract (PE) that cause biologically reduction $CuCl_2$ solution to copper nanoparticles (CuNPs). In figure 4.4, it showed the spectrum of plant extract and the synthesized copper nanoparticles. The shift occur from 3281.56 from plant extract to 3231.31 of CuNPs indicate the reduction of copper ions due to O-H bond (alcohol) that stretch and free and as the result of reduction was the formation of O-H bond that stretch and H-bonded. At the

1621.52 of PE was shift to 1582.51 and the functional group that respond to it was C=C (alkene) group and the functional group that form after the reduction still C=C (alkene). This reduction occur for the formation of CuNPs but it still maintained the functional group which is alkene.

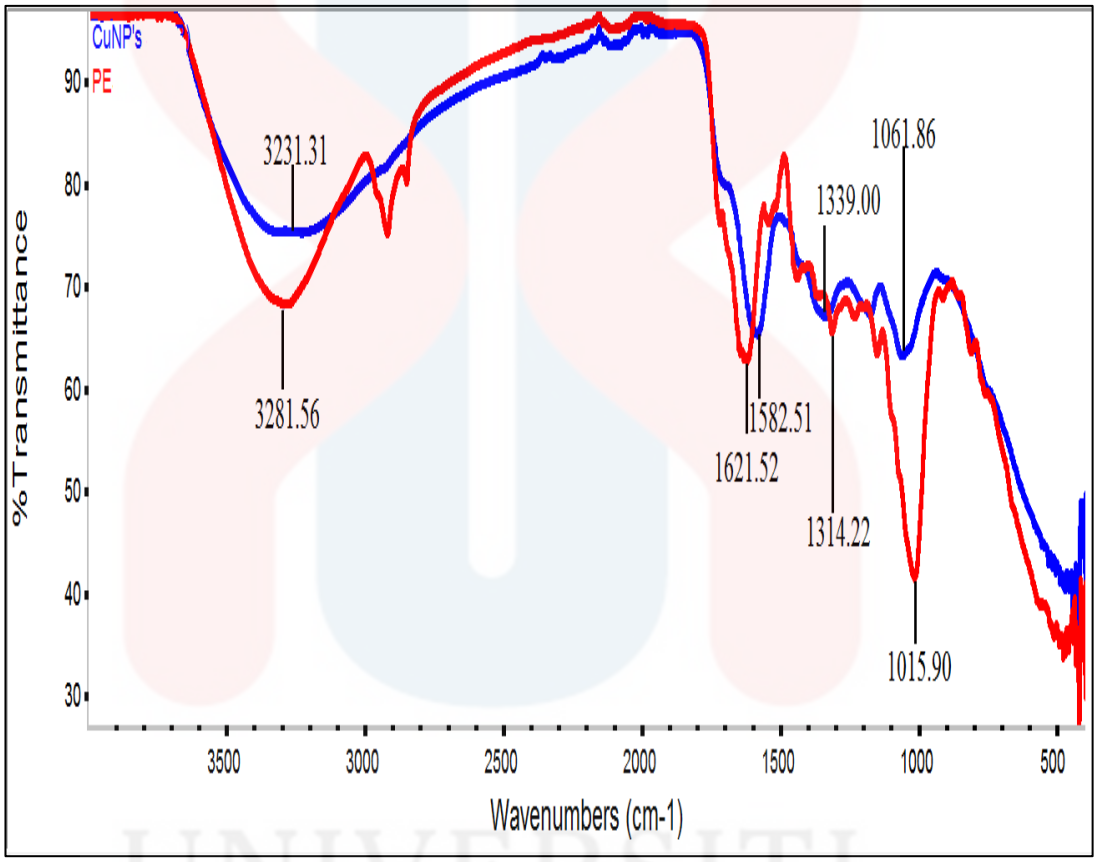


Figure 4.4: FTIR spectrum of leaves extract and synthesized CuNPs

The shift that occur from 1314.22 of PE to 1339.00 of CuNPs was due to the present of C-F (organohalide) group and as the result, the C-N (primary amine) was form. Lastly, the shift that occur at 1015.90 of PE to 1061.86 of CuNPs is due to C-O (oxygen ether) group and the functional group that form after the reduction was (C-O) alcohol ether. From the FTIR spectrum of plant extract and synthesize copper nanoparticle using *M.malabathricum* extract, minor change in position of reduction was occurred. Based on FTIR spectrum, it can indicate that the synthesized CuNPs

was capped by proteins that had functional group of alcohols, alkenes, organohalide and oxygen ether.

4.5 Antibacterial activity of Copper nanoparticle

Antibacterial activity against gram positive bacteria (*S.aureus*) and gram negative bacteria (*E.coli*) was investigate using disc diffusion method. Disc diffusion method was a method that easy and flexible to test on antimicrobial activity (Jiang, 2011). From Figure 4.5, inhibition zone for the Synthesize CuNPs against *E.coli* was highest at the concentration of 0.015µg/ml which is 1.5 cm while, for S.aureus was highest at 3 different concentration which is 0.009, 0.012 and 0.020 µg/ml with 1.0 cm of inhibition zone. From the result, it indicate that synthesize copper nanoparticle was effective against gram positive and gram negative bacteria.

Minimum inhibition concentration is the lowest concentration of anti-microbial agent that can be identify through naked eyes after overnight incubation (Andrews, 2001). MIC for the *E.coli* and *S.aureus* was same, which was 0.003µg/ml. In previous, the MIC of CuNPs against *E.coli* was 10µg/ml (Figueroa *et al*., 2014), 0.076µg/ml (Zain *et al*., 2014) and 1.37×10^{-3}mg/ml (Dugal & Mascarinhas, 2015). For *S. aureus*, the previous reserch has identify the different MIC which was 2.5-20µg/ml (Behiry, 2014), 0.078µg/ml (Kanchana & Santhanalakshmi, 2016) and 0.06µg/ml (Hundakova et al., 2013). The different in MIC of Copper nanoparticles in against *E. coli* and *S. aureus* in this study with respact to previous reserch may be due to the method of synthesize nanoparticle that will effect the structure of nanoparticle, size and number of copper cations (Cu^{2+}) from CuNPs. As the solution of nanoparticle was droped into the double stralized paper disc, the dispersion of nanoparticle on the agar that already spread with MDR strains (*E. coli* and *S. aureus*) was evently spread. As

the result, the inhibition zone around the disc can be identify after 24 hours of incubation.

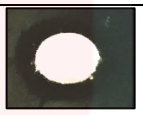
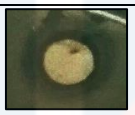
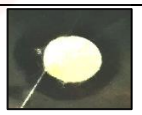
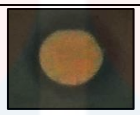
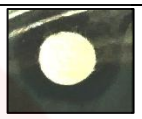
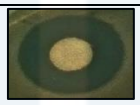
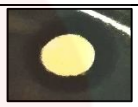
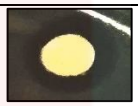
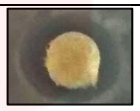
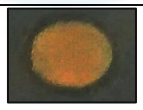
Chemical	Concentration (µg/ml)	Against *E.coli*	Inhibition Diameter (cm)	Against *S.aureus*	Inhibition Diameter (cm)
CuNPs	0.003		0.8 ± 0.2		0.8 ± 0.2
	0.006		1.2 ± 0.6		0.9 ± 0.2
	0.009		1.4 ± 0.6		1.0 ± 0.3
	0.012		1.2 ± 0.2		1.0 ± 0.3
	0.015		1.5 ± 0.3		0.9 ± 0.1
	0.020		1.1 ± 0.2		1.0 ± 0.2
Amoxicillin	0.003		1.0 ± 0.0		0.7 ± 0.1
Plant extract	1×10^5		0.6 ± 0.1		0.7 ± 0.1

Figure 4.5: Antibacterial activity of synthesize CuNPs against *E. coli* and *S. aureus*

This inhibition area occur due to Cu^{2+} from synthesized copper nanoparticles that attach on the negatively charge on the cell wall of the bacteria cell. This increased the permeability of mambrain cell of bacteria cause denatured on the cell and lastly cause the cell death (Lin *et al*, 1998). From the statistical analysis, the 2 concentration that did not exceed the significant value was 0.015 µg/ml and control. This may occur due to some mistake during conducting the experiment and some parallex arror.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this research, we had demonstrate the use of *M.malathricum* leaf extract as the reduction agent to synthesize copper nanoparticles. Then, the synthesized copper nanoparticle was used against the multi-drug resistance pathogens to identify the minimum concentration inhibition (MIC). The MDR pathogens use in this research was *Escherichia coli* to represent gram negative bacteria and *Staphylococcus aureus* to represent gram negative bacteria and it was greatly prove that the synthesize copper nanoparticle can inhibit the bacteria growth and can replace the use of drug (amoxicillin). Through the XRD analysis, the shape of nanoparticle was identified and through the FTIR analysis, the functional group that cause the reduction in the formation of copper nanoparticle was identified. In conclusion, the green method of synthesize copper nanoparticle using *Melastoma malabathricum* as the reduction agent is one of the method that did eco-friendly, cheap and easy compared to other method such as chemically or physically.

5.2 Recommendation

In this research, we was focus on synthesize of copper nanoparticle using the *M.malabathricum* as the reduction agent and the minimum inhibition zone of synthesized copper nanoparticles toward gram positive (*S.aureus*) and gram negative (*E.coli*) strain. Even though, more research are required to identify other plant that

may act as the reduction agent to synthesize nanoparticles and the DNA cleavage of the bacteria strain due to nanoparticle.

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APPENDIXES

Figure A (1): Fresh leaves of *M. malabathricum*

Figure A (1): Dry leaves of *M. malabathricum*

Wavelength (nm)	2 hour	3 hour	4 hour	5 hour
200	0.915	0.844	0.893	0.912
250	0.844	0.827	0.837	0.845
300	1.517	1.413	1.512	1.617
350	1.812	1.788	1.798	1.819
400	1.526	1.490	1.526	1.536
450	0.84	0.818	0.837	0.958
500	0.547	0.538	0.550	0.600
550	0.318	0.362	0.394	0.450
600	0.235	0.259	0.292	0.330
650	0.203	0.233	0.243	0.286
700	0.198	0.237	0.261	0.286
750	0.206	0.220	0.237	0.269
800	0.190	0.214	0.217	0.249
850	0.161	0.188	0.193	0.219
900	0.138	0.149	0.166	0.187
950	0.115	0.105	0.144	0.161
1000	0.095	0.107	0.114	0.136
1050	0.078	0.086	0.093	0.115
1100	0.067	0.074	0.082	0.100

Figure A (2): Rough data of UV-visible spectroscopy

		N	Correlation	Sig.
Pair 1	Cont.0.003 & Treatment	6	.124	.815
Pair 2	Cont.0.006 & Treatment	6	-.321	.536
Pair 3	Cont.0.009 & Treatment	6	-.490	.324
Pair 4	Cont.0.012 & Treatment	6	-.365	.477
Pair 5	Cont.0.050 & Treatment	6	-.834	.039
Pair 6	Cont.0.020 & Treatment	6	-.243	.643
Pair 7	Control & Treatment	6	-.933	.007
Pair 8	PE & Treatment	6	.408	.422

Figure A (3): Paired sample correlation

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	Cont.0.003 – Treatment	-.68333	.54924	.22423	-1.25973	-.10694	-3.048	5	.029
Pair 2	Cont.0.006 – Treatment	-.45000	.77395	.31596	-1.26221	.36221	-1.424	5	.214
Pair 3	Cont.0.009 – Treatment	-.30000	.86023	.35119	-1.20276	.60276	-.854	5	.432
Pair 4	Cont.0.012 – Treatment	-.40000	.64807	.26458	-1.08011	.28011	-1.512	5	.191
Pair 5	Cont.0.050 – Treatment	-.26667	.86178	.35182	-1.17105	.63772	-.758	5	.483
Pair 6	Cont.0.020 – Treatment	-.43333	.60222	.24585	-1.06532	.19866	-1.763	5	.138
Pair 7	Control – Treatment	-.65000	.71484	.29183	-1.40018	.10018	-2.227	5	.076
Pair 8	PE – Treatment	-.80000	.51769	.21134	-1.34328	-.25672	-3.785	5	.013

Figure A (4): Paired sample test