



UNIVERSITI PUTRA MALAYSIA

***CHARACTERIZATION AND CYTOTOXICITY
ASSESSMENT OF BIOGENIC SYNTHESIS OF ZINC
OXIDE NANOPARTICLES (ZnO NPs)***

NURUL ATHIKAH ADILA BINTI ZAINAL

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BIOGENIC SYNTHESIS OF ZINC OXIDE NANOPARTICLES (ZnO NPs)**

By

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**Thesis Submitted to the Department of Physics, Faculty of Science,
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Bachelor of Science in Materials Science with Honours**

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DEDICATION

This thesis is dedicated to:

My project supervisor, Assoc. Prof. Dr. Che Azurahanim Che Abdullah,

My co project supervisor, Dr. Norazalina binti Saad,

My parents, Zainal Bin Sidek and Rohani Binti Yusoh,

My project coordinator, Muhammad Naziff Bin Ahamad Said,

My lecturers,

My siblings,

My friends,

and others who help me through the completion of this thesis.

Thank you for the help, support, love and encouragement.

ABSTRACT

CHARACTERIZATION AND CYTOTOXICITY ASSESSMENT OF BIOGENIC SYNTHESIS OF ZINC OXIDE NANOPARTICLES (ZnO NPs).

By

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FEBRUARY 2022

Supervisor: Assoc. Prof. Dr. Che Azurahanim Che Abdullah

Faculty : Faculty of Science

Nanotechnology is focusing with the production and application of nanoscale materials. Nanoscale dimensions result in a large surface-to-volume ratio for nanoparticles and, as a result, incredibly distinctive properties. Zinc oxide nanoparticles (ZnO NPs) have recently been studied because of their broad bandgap and high exciton binding energies. Aside from that, potential applications include biosensors, sunscreen production, antibacterial, antioxidant, and anti-cancer. Green methods or green synthesis have been established employing plants, fungus, bacteria, and algae due to the large number of hazardous substances and the toxic environment in the physical and chemical synthesis of these NPs. This project provided insight into the green synthesis, method, and characterization techniques used in the green synthesis of ZnO NPs from roselle and Dayak onion extract as reducing and capping agents. The synthesis of ZnO NPs was validated using X-Ray Diffraction (XRD), Fourier Transform Infrared Spectroscopy (FTIR), Ultraviolet-Visible Spectroscopy (UV-Vis), Photoluminescence (PL), Thermogravimetric Analysis (TGA) and Gas

chromatography–mass spectrometry (GC-MS) in order to get morphological properties. The result XRD shown the crystalline size of ZnO NPs was between 21 nm to 68 nm with wurtzite crystal structure. For all samples that using roselle and Dayak onions extract, FTIR analysis indicated the Zn-O stretching bond. The UV-Vis spectra of ZnO NPs containing roselle extract and Dayak onions extract revealed a distinct optical energy band gap ranging from 3.12 to 3.89 eV. The sample is stable in nitrogen gas combustion at a temperature of 200 °C for TGA analysis. Additionally, GC-MS analysis revealed the presence of phytochemical compounds acting as reducing and capping agents in roselle and Dayak onion extracts. In addition, the toxicity of ZnO NPs *in vitro* was evaluate using normal and breast cancer cell lines. MTT assays was conducted for both MCF-10A and MCF-7 cell lines following 24h of exposure to synthesised ZnO NPs. The cytotoxicity test demonstrated that IC₅₀ for MCF-7 for ZnO NPs with roselle extract and ZnO NPs with Dayak onions was 2.503 µg/ml and 5.540 µg/ml, respectively. While for MCF-10A, the IC₅₀ for ZnO NPs containing roselle extract was 16.47 µg/ml and 15.77 µg/ml for ZnO NPs containing Dayak onions extract.

ABSTRAK

PERINCIAN DAN PENILAIAN SITOTOSIKSITI SINTESIS BIOGENIK

NANOPARTIKEL ZINK OKSIDA (ZnO NPs).

Oleh

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Nanoteknologi menfokuskan penghasilan and aplikasi untuk bahan yang berskala nano. Dalam dimensi berskala nano, disebabkan oleh nisbah antara luas permukaan dengan isipadu, partikel nano mempunyai sifat-sifat yang unik. Oleh itu, nanopartikel zink oksida (ZnO NPs) dikaji dan di analisa disebabkan oleh jurang jalur tenaga optikalnya yang besar. Selain itu, kemampuannya dalam pelbagai bidang juga diambil kira seperti di dalam pengesan bio, dalam pembuatan product pelindung dari sinar UV, anti- bakteria, anti- oksida dan juga anti- kanser. Justeru itu, pendekatan sintesis hijau digunakan untuk mencegah peningkatan jumlah bahan yang berbahaya and bertosik untuk alam sekitar di dalam proses sintesis fizikal dan sintesis kimia di dalam nanopartikel. Oleh itu, projek ini dijalankan bagi bertujuan untuk melakukan sintesis,

proses dan kaedah perincian bagi sintesis hijau ZnO NPs menggunakan ekstrak rosel dan bawang Dayak. Kaedah perincian dijalankan dengan menggunakan Belauan Sinar-X (XRD), Spektroskopi Transformasi Fourier Inframerah (FTIR), Spektroskopi Ultraungu-Kelihatan (UV-Vis), Photoluminescence (PL), Thermogravimetrik Analisis (TGA) and Gas kromatografi- spektrometri jisim (GC-MS) untuk mengetahui ciri- ciri ZnO NPs. Daripada analisis XRD, saiz untuk ZnO NPs menunjukkan diantara 21 nm hingga 68 nm. Analisis FTIR menunjukkan ikatan peregangan Zn-O untuk semua jenis sampel. Spektrum UV-Vis menunjukkan ciri jurang jalur tenaga optikal pada nilai 3.12 eV ke 3.89 eV. Untuk TGA analisis, sampel stabil pada suhu 200 °C di dalam pembakaran gas nitrogen. Bagi GC-MS analisis menunjukkan kehadiran bahan kimia yang berperanan sebagai agen pengurang dan agen pengikat bagi kedua-dua ekstrak. Tambahan pula, toksisiti bagi ZnO NPs dalam *vitro* diuji dengan menggunakan sel payudara dan sel kanser payudara. Selepas itu, ujian MTT dijalankan untuk kedua-dua sel dengan mendedahkan kepada ZnO NPs yang telah disintesis. Seterusnya, sitotosikiti menunjukkan nilai IC₅₀ sel MCF-7 untuk ZnO NPs dengan rosel ekstrak dan ZnO NPs dengan bawang dayak ialah 2.503 µg/ml and 5.540 µg/ml. Manakala untuk MCF-10A, nilai IC₅₀ untuk ZnO NPs yang mengandungi rosel ekstrak ialah 16.47 µg/ml dan 15.77 µg/ml untuk ZnO NPs yang mengandungi bawang Dayak ekstrak.

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LIST OF ABBREVIATION

α	Optical Absorption Coefficient
β	Full Width at Half-Maximum Peak Intensity
$^{\circ}\text{C}$	Degree Celsius
λ	X-ray Wavelength
θ	Bragg Angle
τ	Average Size of The Crystals
ν	Frequency Of The Incident Photon
A	Constant For A Direct Transition
a.u	Arbitrary Unit
AgNPs	Silver Nanoparticles
CdO	Cadmium Oxide
cm^{-1}	Reciprocal Centimetre
CO_2	Carbon Dioxide
COVID-19	Coronavirus Disease 2019
CuO	Copper Oxide
Eg	Optical Band Gap
eV	Electron Volt
Fe_3O_4	Ferrosoferric Oxide
FeSEM	Field-emission Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas chromatography–mass spectrometry
h	Planck's constant
h	Hours
h ν	Photon Energy
H_2O	Dihydrogen Monoxide
HRTEM	High-Resolution Transmission Electron Microscopy
IR	Infrared Ray
min	Minute
ml	Millilitre
NaOH	Sodium Hydroxide

nm	Nanometre
NPs	Nanoparticle
pH	Potential of Hydrogen
PL	Photoluminescence
rpm	Parts Per Million
TEM	Transmission electron microscopes
TiO ₂	Titanium Dioxide
TGA	Thermogravimetric Analysis
UV	Ultraviolet
UV-Vis	Ultraviolet-Visible Spectroscopy
W	Watt
wt%	Percentage by Weight
XRD	X-Ray Diffraction
XRF	X-ray Fluorescence
Zn(CH ₃ CO ₂) ₂ ·2H ₂ O	Zinc Acetate Dihydrate
ZnO	Zinc Oxide
ZnO (DO111)	ZnONPs Synthesized with 1ml of Dayak onions extract with pH 11
ZnO (DO311)	ZnONPs Synthesized with 3ml of Dayak onions extract with pH 11
ZnO (DO611)	ZnONPs Synthesized with 6ml of Dayak onions extract with pH 11
ZnO (R311)	ZnONPs Synthesized with 3ml of roselle extract with pH 11
ZnO (R312)	ZnONPs Synthesized with 3ml of roselle extract with pH 12
ZnO (R611)	ZnONPs Synthesized with 6ml of roselle extract with pH 11
ZnO (R612)	ZnONPs Synthesized with 6ml of roselle extract with pH 12
ZnO NPs	Zinc Oxide Nanoparticles

CHAPTER 1

INTRODUCTION

1.1 Structure of the thesis

Chapter 1 discusses the background of the study, zinc oxide nanoparticles (ZnO NPs), their production, the resulting problem statement, and the research objectives. Chapter 2 describes the ZnO NPs literature review and the green synthesis method. Chapter 3 details the experimental procedure and characterization of ZnO NPs. Then, Chapter 4 discusses the results of ZnO NPs characterisation using XRD, FTIR, UV-Vis, PL, TGA, and GC-MS, as well as the cytotoxicity of ZnO NPs. Finally, Chapter 5 summarises the entire thesis, including its findings, the difficulties encountered during the research, and recommendations for future research.

1.2 Background of study

Nanotechnology has developed into a significant area of research in nearly every sector over the last several decades (Anand, Theophil et al., 2019). It is concentrating on particles with a diameter of less than 100nm. Thus, the particle is referred to as nanomaterials. Nanomaterials are also referred to as critical components of human development due to their extraordinary applications in material science, medicine, engineering, and pharmacy. This is because of their morphology and size, which enable them to react effectively

in a larger area and provide an effective result for humans and animals (Rathnasamy et al., 2017). This property, according to Selim et al. (2020), enables their use in a variety of fields, including material science and biotechnology. Apart from nanomaterials, nanoparticles have a significant impact on nanotechnology fields. Metal oxide nanoparticles, for example, have been widely used as biological agents in a variety of applications (Anand, Theophil et al., 2019). Zinc oxide (ZnO), ferrosferric oxide (Fe_3O_4), cadmium oxide (CdO), and copper oxide are all examples of metal oxides, as Soto-Robles et al. (2019) mention (CuO).

1.3 Zinc Oxide Nanoparticles (ZnO NPs)

Zinc oxide is a found naturally inorganic substance having the chemical formula ZnO. It is a white powder that is water insoluble (Siddiqi et al., 2018). Numerous researchers have expressed interest in ZnO with nanoscale, that why ZnO NPs were developed. This is due to the unique optical and chemical properties of ZnO NPs, which can be altered by altering their shape (Bala et al., 2015). As a result, ZnO was chosen as the metal oxide in this research's nanoparticle synthesis. According to Selim et al. (2020), ZnO NPs already distinguish themselves from other metal nanoparticles due to their unique properties, which include a broad bandgap and a high binding energy. Additionally, Awwad et al. (2020) reported that ZnO NPs are environmentally friendly, simple to synthesise, non-toxic, biosafe, and biocompatible, making them an excellent choice for biological applications.

1.4 Synthesis of ZnO NPs

ZnO NPs can be synthesised using a variety of techniques practised in nanotechnology fields. Because metal oxide nanoparticles possess unique physical, chemical, and biological properties, they are more promising than other nanoparticles (Demissie et al., 2020). This is why ZnO was chosen as the material for this research study. Following that, Pillai et al. (2020) stated that there are two distinct types of nanoparticle synthesis techniques: top-down and bottom-up approaches.

In comparison to top-down approaches, the favourable outcome demonstrates a bottom-up approach. Bottom-up approaches achieve the best results in terms of defect-free nanoparticles (Raj & Lawrence, 2018). Figure 1.1 illustrate and summarises the synthesis of ZnO NPs.

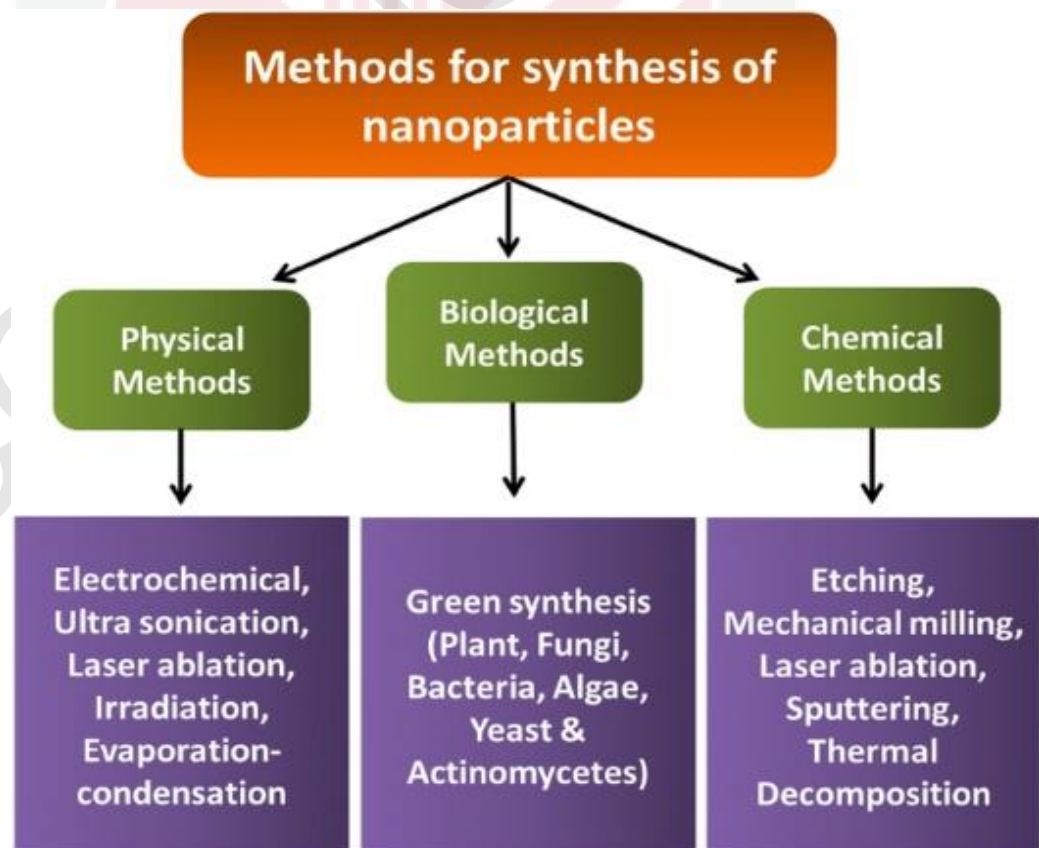


Figure 1.1 : Method for synthesis of nanoparticle (Khandel et al., 2018)

So, as from the figure, physical techniques are top-down approaches, whereas chemical and biological procedures are bottom-up approaches for the synthesis of nanoparticles. Some of the most physical processes for the synthesis of NPs include evaporation-condensation, electrolysis, irradiation, laser ablation, and ultra-sonication. However, the main disadvantages of these technologies are their low output rate, costly operations, and significant energy consumption (Salih et al., 2021). Chemical synthesis techniques, on the other hand, such as chemical reduction, laser ablation, sputtering, etching, mechanical milling, and thermal decomposition, are the most common and commonly utilised methods for the production of metallic nanoparticles. Due to the ease of operation and equipment requirements, chemical reduction of nanoparticles from their respective metal salt precursors by adding specific reducing agents is one of the most extensively used methods for NPs chemical synthesis (Kumari et al., 2021).

These methods are suitable for synthesising ZnO NPs. When hazardous chemicals are used to reduce and cap nanoparticles, a number of undesirable consequences occur, including toxicity. According to Fakhari et al. (2019), chemical and physical methods that employ the toxic material as a capping and reducing agent that result in toxicity. To avoid the negative effects associated with nanoparticle synthesis, the use of biological agents as a substitute in nanoparticles is recommended. Demissie and colleagues (2020) stated that this technique is more environmentally friendly than conventional chemicals and has a faster reaction time with living organisms' body systems. In recent years, the use of biological techniques (green synthesis) as reducing and capping agents has gained popularity (Yuvakkumar et al., 2014). According to

Vijayakumar et al. (2018), the green synthesis method was simple to use, low in cost, and also safe for the environment. Several additional advantages include the ease of handling, the short time required, the absence of intermediate substances (base groups), the low cost of precursors, the use of inexpensive equipment, and the high purity and quantity of the product obtained (Bhuyan et al., 2015).

1.5 Problem Statements

Due to their small size and high surface area, metal oxide nanoparticles have shown promise in nanotechnology. Vijayakumar et al. (2018) established that metal oxides have a high atom fraction, which accounts for their antibacterial, magnetic, and catalytic properties. As a result, metal oxide nanoparticles may be a solution to a problem that has arisen in the field of nanotechnology. Nanoparticles are used in a variety of consumer industries, including health, food, feed, space, chemical, and cosmetics, according to Agarwal et al. (2017). As a result, a method for producing metal oxide nanoparticles such as ZnO NPs was developed.

ZnO NPs have been synthesised via a variety of methods, including physical and chemical methods. Vijayakumar et al. (2018) describe the synthesis of ZnO NPs using spray pyrolysis, thermal evaporation, microwave technique, and sol-gel processing. However, Ahmad and Kalra (2020) stated that all of these methods involved the use of hazardous chemicals that are extremely toxic and harmful to the environment and human beings. Following that, the researcher employed another technique, known as biological synthesis or green synthesis. It is one of the possible solutions to the problem

encountered during the synthesis process. Green synthesis utilises plant extracts, which ostensibly has no adverse effect on the environment. Additionally, green synthesis can be used in place of physical and chemical synthesis due to its low cost, ease of fabrication, and non-toxicity (District, 2021; Fakhari et al., 2019; Jamdagni et al., 2018a). As a result, the roselle and Dayak onions extracts were used in the current study as the plant extracts, acting as a stabilising, capping, and reducing agent.

1.6 Thesis Objectives

The main objectives of this research were to synthesis the ZnO NPs using the roselle and Dayak onion extract. The specific objectives of this research were:

- To optimise the synthesis parameter of Zinc Oxide Nanoparticle (ZnO NPs) using Taguchi approach.
- To synthesis and optimise ZnO NPs using roselle and Dayak onion extract as capping and reducing agent.
- To characterize the structural, chemical, optical and thermal properties of ZnO NPs by using X-Ray Diffraction (XRD), Fourier – transform infrared spectroscopy (FTIR), Ultraviolet-Visible Spectroscopy (UV-Vis), Photoluminescence spectroscopy (PL), Thermogravimetric Analysis (TGA), Gas chromatography–mass spectrometry (GC-MS).
- To evaluate cytotoxicity of biogenic ZnO NPs toward normal and cancer breast cells.

CHAPTER 2

LITERATURE REVIEW

2.1 Nanoparticles

Nanoparticles are small particles with a diameter of 1 to 100 nanometres (Agarwal et al., 2017). Nanoparticles have a large surface area due to their small size. Nanoparticles exhibited increased catalytic reactivity, thermal conductivity, non-linear optical performance, and chemical stability as a result of their high surface area to volume ratio (Agarwal et al., 2017). Additionally, nanoparticles play a role in nanomaterials. This is because nanoparticles have the ability to modify the nanomaterial. As Selim et al. (2020) indicate, nanomaterials have a greater nanoscale dimension and biological properties than bulk nanoparticles. Along with nanomaterials, nanoparticles exhibit unique properties. The unique property of nanoparticles is their small size, excellent optical properties, and suitability for a wide variety of applications. Due to the unique properties of nanoparticles, they are the optimal choice for application-specific performance (Demissie et al., 2020). Additionally, Ahmad and Kalra (2020) stated that nanoparticles are widely used in pharmaceuticals, cosmetics, and biomedical devices.

The shape, size, and distribution of nanoparticles are all unique (Khandel et al., 2018). Following that, nanoparticles are discovered as a trace amount of a toxic chemical, or a significant amount of energy devoted to biological synthesis. As a result, the manufacturing process becomes more cost

effective and environmentally friendly (Bandeira et al., 2020). Additionally, this research thoroughly examines the use of biological substrates in the synthesis of metal oxide nanoparticles that is ecologically acceptable. This is an attempt to replace chemical and physical processes that are frequently used in industry.

2.2 Zinc Oxide Nanoparticles (ZnO NPs)

2.2.1 Crystal structure of ZnO NPs

Wurtzite and zinc blende are the two most common crystalline forms of zinc oxide. The structure of zinc blende is depicted in Figure 2.1, which also depicts the crystal structure of wurtzite. While zinc blende is more stable than wurtzite under normal conditions, wurtzite appears to be more stable in ambient conditions (La Porta et al., 2014). Wurtzite has a hexagonal structure, which is composed of a hexagonal close-packed array of oxygen anions, with metal cations occupying approximately half of the tetrahedral interstices. Zinc blende has a cubic structure, with anions and cations occupying one-half of the tetrahedral holes. Each ion is 4-coordinated and has a local tetrahedral geometry. However, the most crystalline structure formed for ZnO NPs is the

hexagonal crystal structure of wurtzite (Abbas & Bidin, 2017; Anbuvarannan et al., 2015; Manohar et al., 2021).

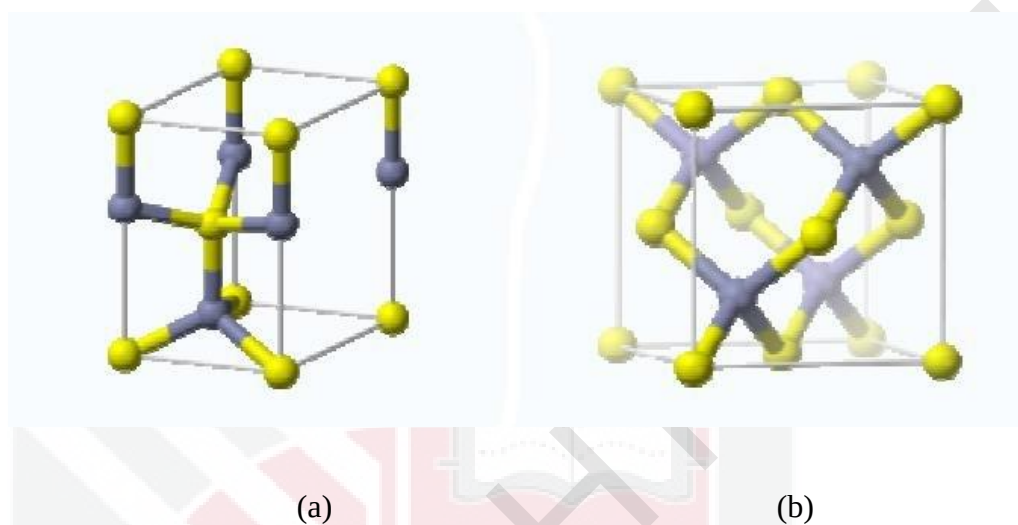


Figure 2.1: The crystal structure of ZnO NPs ; (a) wurtzite structure (b) zinc blende structure (Hammad, 2013)

2.2.2 Properties of ZnO NPs

ZnO NPs is one of inorganic metal oxide that can satisfy the requirements for nanotechnology (Siddiqi et al., 2018). This is because ZnO allowing it to be used safely as a medication, packaging, and antibacterial agent. The properties of ZnO are influenced by many factors such as particle size, shape, concentration, and period of contact with the bacterial cell (Siddiqi et al. 2018). After that, ZnO is the metal oxide that stands out from another metal oxide. This is because of its unique properties, such as a huge energy band gap (3.37 eV) at surrounding temperature, and also huge binding energy (60 MeV). Besides that, ZnO also has biodegradability, can easily modify the electric

conductivity, and have high thermal and chemical resistance (Abbas & Bidin, 2017). Based Alrajhi et al. (2021) observed, the properties of ZnO such as electrical, optical, chemical, and chemical are substantial will be affected. This happens when the size is reduced because of morphology.

2.3 Green synthesis of ZnO NPs

Due to their simplicity, low cost, and environmental friendliness, green synthesis nanoparticles are gaining popularity (Anbuvaran et al., 2015; Bala et al., 2015; Hussain et al., 2016a). Additionally, green synthesis is a simple and effective method for synthesising a variety of bioactive compounds with a variety of different molecular structures. This technique is environmentally friendly because it avoids the use of toxic chemicals and instead makes use of renewable raw materials (Banik et al., n.d.). For instance, biogenic reduction is a "Bottom-Up" technique in which a reducing agent is replaced with a natural product extract that imparts nanoparticles with stabilising, growth terminating, and capping properties. Additionally, the size and morphology of NPs are influenced by the nature of biological entities when they interact with reducing organic agents at various concentrations (Hussain et al., 2016b). That is why, when ZnO NPs are synthesised using a green method, they can exhibit a wide variety of nanostructures. Apart from that, it is completely safe, non-toxic, and biocompatible. This technique can be used in a wide variety of industries, including optoelectronics and magnetic sensors, biodiagnosis, biological labelling, ceramic and rubber manufacturing, environmental protection, biology, and the pharmaceutical industry (Selim et al., 2020). Additionally, Khandel et al. (2018) asserted that green synthesis methods such

as biological synthesis may actually produce nanoparticles with a more precise size and shape compared to other physicochemical methods.

2.3.1 The role of plant extracts in the synthesis of biogenic ZnO NPs

Numerous studies have demonstrated the use of plant extracts to synthesise ZnO NPs. Multiple studies on ZnO NPs using various parts, plant extracts, and particle sizes are summarised in Table 2.1.

Table 2.1 : Green synthesis ZnO NPs utilising various plant extracts.

No	Plants	Parts	Particle size (nm)	References
1	<i>Carica papaya</i>	Leaf	36.90	(Rathnasamy et al., 2017)
2	<i>Lippia adoensis</i>	Leaf	18.50 - 26.70	(Demissie et al., 2020)
3	<i>Atalantia monophylla</i>	Leaf	33.01	(Vijayakumar et al., 2018)
4	<i>Rosa indica</i>	Leaf	34.00 -36.00	(Raj & Lawrence, 2018)
5	<i>Theobroma cacao</i> L.	Fruit	81.00	(District, 2021)
6	<i>Scutellaria baicalensis</i>	Root	50.00	(Chen et al., 2019)
7	<i>Ailanthus altissima</i>	Fruit	20.00	(Awwad et al., 2020)
8	<i>Melia azedarach</i>	Leaf	33.00- 96.00	(Dhandapani et al., 2020)
9	<i>Cayratia pedata</i>	Leaf	52.24	(Jayachandran et al., 2021)
10	<i>Hibiscus sabdariffa</i>	Fruit	38.63	(Soto-Robles et al., 2019)

2.3.2 Factors affecting green synthesis of ZnO NPs

The synthesis, characterization, and use of nanoparticles are all influenced by a number of variables. The pH of the solution, temperature, concentration of the extracts utilised, concentration of the raw materials used, size, and, most all, the procedure followed for the synthesis process was the most important aspects that impact nanoparticles production (Mohamed et al., 2021)

2.3.3 The effect of pH in synthesis of ZnO NPs

pH is one of the variables and parameters that must be considered during the synthesis process. This is because the pH parameter can affect the structural and optical properties of the material. Additionally, this has an effect on the particle size of the ZnO NPs. Thus, Table 2.2 summarises several articles that demonstrate the effect of pH on particle size.

Table 2.2 : The effect of pH on the particle size of ZnO NPs varies according to the type of plant extract.

Plant	pH	Particle size (nm)	References
<i>Nyctanthes arbor-tristis</i>	9.00 – 13.00	12.00–32.00	(Jamdagni et al., 2018b)
<i>Laurus nobilis L</i>	12.00	21.49- 25.26	(Fakhari et al., 2019)
<i>Citrus aurantifolia</i>	3.70 & 4.00	50.00–200.00	(Samat & Md Nor, 2013)
<i>Moringa oleifera</i>	10.50	12.27-30.51	(Matinise et al., 2017)

2.4 Roselle

The scientific name for the roselle flower is *Hibiscus sabdariffa*. Roselle is a dark reddish-purplish medicinal flower that is well-known throughout the world. *Hibiscus Sabdariffa* (*H. Sabdariffa*) is another *Malvaceae* family shrub with red calyx-shaped flowers (Thovhogi et al., 2015). The concentration of phenolic compounds and flavonoids in roselle blooms is quite high. The phenolic compounds found in this plant are a significant but under-reported class of chemicals. Additionally, roselle contains phenolic chemicals such as citric acid, hydroxycitric acid, and hibiscus acid. Flavonoids such as quercetin, luteolin, and gossipetin had been discovered, as well as their glycosides. Anthocyanins, which are abundant in the calyces, give the calyces their red colour (Thovhogi et al., 2015). According to study by Lau et al. (2020), the phytochemicals in roselle flower extracts are effective at reducing size. The chemical compound influenced the size and shape of ZnO NPs during their production by acting as a stabilising and reducing agent.



Figure 2.2: Roselle flowers

2.5 Dayak Onion

Despite their appearance, Dayak onions, botanically known as *Eleutherine palmifolia*, are not onions. Instead of those, they are members of the Iridaceae family, which includes the iris and the spice saffron (Wicaksono et al., 2018). The Dayak onion, also known as 'Bawang Dayak', 'Bawang Sabrang', and Diamond onion, is endemic to Indonesia and has been used for millennia by the Dayak people of Borneo. The Dayak onion has traditionally been used to treat a variety of ailments, including high blood pressure, high cholesterol, diabetes, ulcers, constipation, and stroke, as well as a herbal beverage for postpartum mothers (Abdullah et al., 2021). Dayak onions, renowned for their medicinal properties, are primarily grown for medicinal purposes, with only a few varieties being used in culinary preparations (Yuanita et al., 2020). According to Wicaksono et al. (2018), Dayak onions contain a high concentration of antioxidant flavonoids and phenols, such as anthocyanin. Additionally, they contain compounds that are anti-inflammatory, antibacterial, and antiviral, such as alkaloids, tannins, glycosides, and quinines.



Figure 2.3: Dayak onions

2.6 Taguchi software

The Taguchi method is a statistical technique for conducting empirical research that combines mathematics and statistics (Kim et al., 2007). It is capable of identifying the experimental condition with the fewest variables as the optimal condition. To quantify variability, the signal to noise (S/N) ratio can be used. The optimal experimental condition is defined as one with the highest S/N (Semioshkina & Voigt, 2006). Additionally, Kim et al. (2007) suggested that the Taguchi robust design approach was used to identify the optimal conditions and parameters having the greatest influence on the particle size and size distribution of ZnO NPs. Individual parameters have a significant effect on the characteristics, and their interrelationships are extremely complicated (Jang et al., 2015). As Jang et al. (2015) demonstrated, an efficient analytical method must be chosen to achieve the optimal deposition conditions. Numerous studies have been conducted to determine the optimal conditions for synthesising ZnO NPs using the Taguchi design approach (Adeoye & Lateef, 2022).

2.7 Applications of ZnO NPs

For almost a decade, nanoparticles have been continually examined and employed in a variety of industrial applications. Nanomaterials based on ZnO are being considered for a variety of potential applications, including energy storage, cosmetics, nanooptical devices, and nanoelectronic devices, among others (Kalpana & Devi Rajeswari, 2018). Moreover, Mohamed et al. (2021) reported that the used of ZnO NPs in a variety of sectors, including sunscreen

and burning ointments, antibacterial treatments, lotions, self-cleaning coating material on ceramic and glass surfaces, diodes, transistors, and display screens, due to its unique physical and chemical characteristics. In addition, the unique set of characteristics of ZnO NPs such as catalysis, photochemical capability, medicinal effects, antibacterial and UV filtration has made ZnO NPs as a multi-functional agent and more promising for wastewater remediation (Rambabu et al., 2021).

2.7.1 Biomedical application

Nanobiotechnology is the use of nanotechnology to diverse biological systems. It is the confluence of biology and nanotechnology (Skheel et al., 2021). Besides that, Skheel et al. (2021) also revealed that nanobiotechnology is also involved in the development of biocompatible, environmentally friendly, and biogenic nanomaterials and nanoparticles. Based on Pattanayak et al. (2017), due to an increase in surface area, green bio-nanoparticles have better biological efficiency and hence greater catalytic activity. According to Dulta et al. (2021), ZnO's superior pharmacological properties have facilitated its application in healthcare fields such as anticancer, antibacterial and antioxidant. Although nanobiotechnology has numerous applications, this research focuses on the anticancer and toxicity assessment of green synthesis.

2.7.2 Anti-cancerous

Standard cancer treatments include radiation, therapy, and surgery, which not only kill cancerous cells but also harm healthy cells (Khan et al., 2021). Nanomedicine, tailored drug delivery, and multi-target inhibitors have rendered these therapies obsolete in cancer therapy (Sathappan et al., 2021). Conventional anti-cancer medicines include drawbacks such as water solubility, targeting ability, multidrug resistance, and systemic toxicity (Skheel et al., 2021). Based on Verma et al. (2021), they stated that among the NPs, ZnO has the capacity to target and destroy cancer cells. Besides that, Zhang et al. (2020) stated that the specific cytotoxicity of ZnO NPs may be related to abnormal metabolism and increased cell growth. Several studies have shown that ZnO NPs have an antiproliferative effect on cancer cell lines while having no effect on normal cell lines (Norouzi Jobie et al., 2021). The findings revealed cytotoxicity of green produced ZnO NPs on cancer cells which is explained by their high surface to volume ratio, generating reactive oxygen species (ROS) generation, interfering with cellular signalling pathways, causing DNA damage, decreasing membrane bound enzyme function, and inducing poptosis.

2.8 Toxicity assessment of green synthesis nanoparticles

Cytotoxicity is described as the capacity of manmade medications, naturally occurring poisons, or immune-mediator cells to damage cells in the body (Arumugam et al., 2021). Besides that, cytotoxicity refers to the amount to which a chemical can damage a cell. According to Hassan et al. (2021), Cytotoxic refers to a chemical or method that damages or kills cells, with

"cyto" meaning cell and "toxic" meaning poison. Cytotoxicity testing is essential for nanoparticles since it assists in determining the intended biomedical utilisation and the method for measuring the cytotoxicity and viability of cells (Verma et al., 2021). Based on Hegazy et al. (2020), the cytotoxicity test is one of the biological evaluation and screening procedures that uses tissue cells *in vitro* to examine the effects of medical devices on cell growth, reproduction, and morphology. The cytotoxicity of ZnO NPs is correlated to cell apoptosis, mitochondrial damage and cell cycle shift that depends on the size, shape and surface modifications of ZnO NPs (Umamaheswari et al., 2021). As entered into delivery systems of human body, ZnO NPs would undergo a variety of complex processes such as digestion and absorption, and their toxicity has not been completely examined yet. However, several *in vitro* cytotoxic research indicated that ZnO NPs displayed dose-dependent toxicity on different kinds of human cells (Gao et al., 2020). Furthermore, the IC_{50} is the concentration of an inhibitor at which the response (or binding) is reduced by half. The most generally used and useful indicator of a drug's effectiveness is the half-maximal inhibitory concentration (IC_{50}). It reflects the amount of medication required to block a biological process by half, and so serves as a measure of antagonist drug potency in pharmacological research (Aykul & Martinez-Hackert, 2016).

Table 2.3: The value of IC₅₀ for different types of cell lines and plant extracts.

No	Plant extracts	Type of cell lines	Value of IC ₅₀	References
1	<i>Amygdalus scoparia</i> Spach stem bark extract	Hela, MCF-7, LS180, and Vero cell	69, 64.3, 71.5, and 301.2 mg/ ml	(Norouzi Jobie et al., 2021)
2	<i>Clitorea Ternatea</i> Flower	MCF-7	44.30 µg/mL	(Alahmdi, 2022)
3	<i>Orobanche crenata</i> methanolic	HepG2, PC3, MCF-7	30.00, 111.00, 89.60 µg/mL	(Hegazy et al., 2020)
4	<i>Achiella millefolium</i> L	A549, HT29	46.47, 42.82 µg/mL	(Aydın, 2021)
5	Pine pollen	Macrophages J774	769.00 µg/mL	(Haghighat et al., 2022)

CHAPTER 3

METHODOLOGY

3.1 Introduction

The materials and method related to green synthesis, characterization and toxicity assessment of ZnO NPs were discussed in this chapter.

3.2 Synthesis of ZnO NPs using roselle and Dayak onions extract

3.2.1 Materials

Zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$) and sodium hydroxide (NaOH) are the chemical reagents used in this experiment. These chemical reagents were purchased from R&M, UK. Besides that, Ethanol with 95% purity was purchased that get from R&M Chemicals. Distilled water was used to dilute the solution. The roselle flowers are provided by collaborator from Institute of Medical Research (IMR). Dayak onions were obtained from Johor, Malaysia. The plant sample was identified and authenticated by the Biodiversity Unit, Institute of Bioscience, UPM Serdang, Selangor, Malaysia.

3.2.2 Preparation of plant extract (Roselle)

To remove impurities, the roselle flower was cut into small pieces and washed with distilled water. The roselle flower was then dried in an oven at 120°C until the moisture was gone. The dried roselle was then mashed with a mortar and pestle until it was powdered. The powdered roselle was mixed with 100 ml of distilled water after it was obtained. The solution was then placed in the sonicator water bath for 30 minutes at 40°C agitate the particles using the sound waves provided by the sonicator water bath. This action produced the reddish-colored solution. The extract solution was created by filtering it through filter paper and a filter funnel. The solution extract was kept in the refrigerator to preserve humidity and prevent contamination

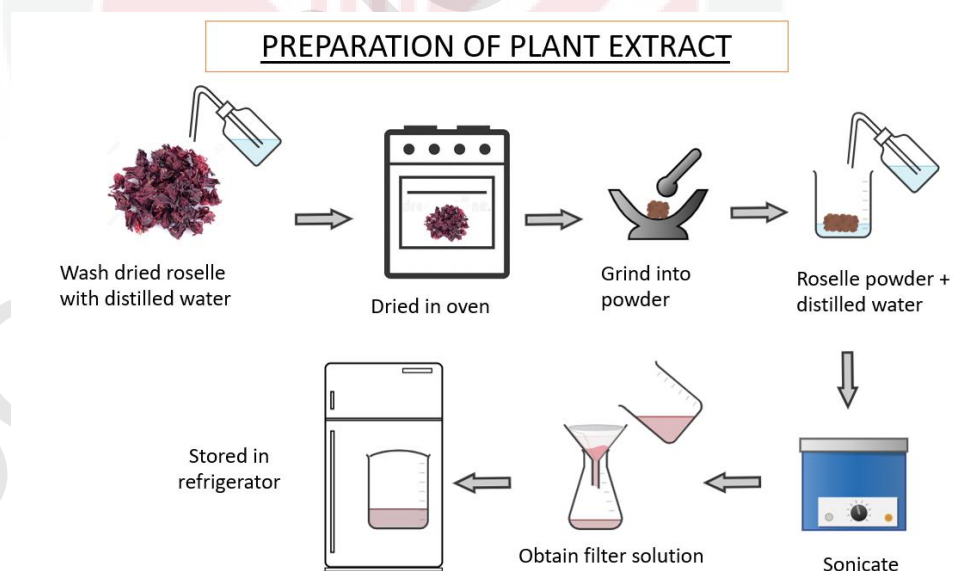


Figure 3.1 : The preparation of roselle flower extract.

3.2.3 Preparation of plant extract *Eleutherine bulbosa* (Dayak onion)

Following the identification of the plant sample, the bulbs were carefully washed under running tap water, sliced, and dried overnight in a 40°C warm air oven. After crushing the dried bulbs into a fine powder, they were sieved through a 400 m sieve and stored at 4°C until extraction.

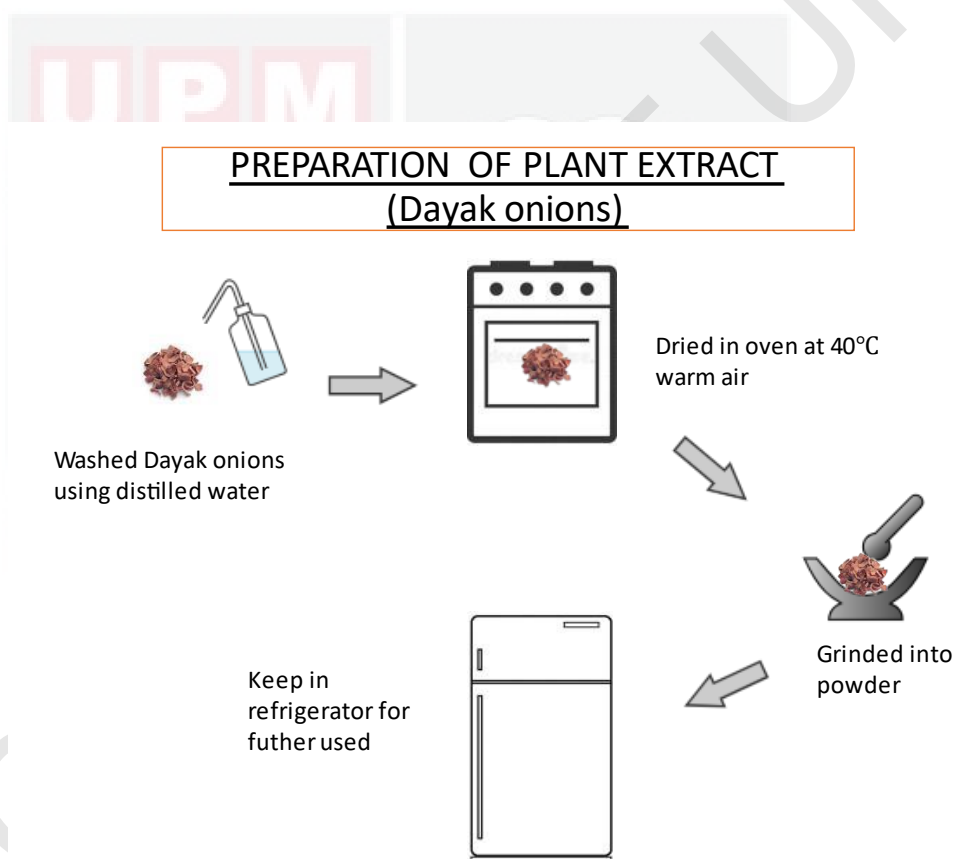


Figure 3.2 : The preparation of Dayak onion extract.

3.2.3a Plant Extraction

Following that, the mixtures were placed in a water bath and extracted for 70 minutes at 45°C. After filtering with Whatman No. 1 filter paper, the aqueous extract was evaporated in a rotary evaporator and dried overnight in a 40°C oven. The dried samples were kept at -20°C for future analysis.

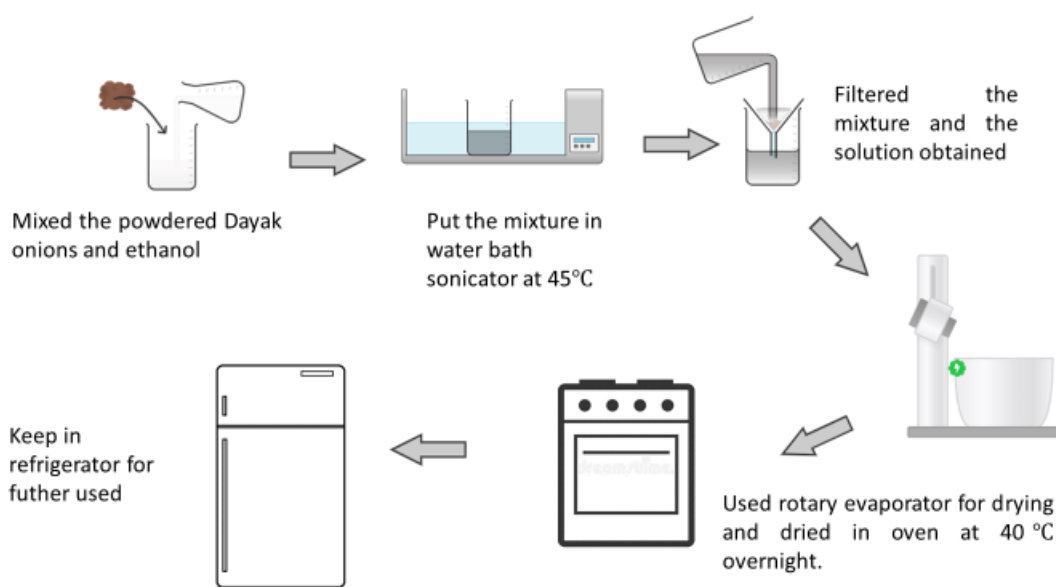


Figure 3.3: The preparation of Dayak onion extract using hot water extraction technique.

3.2.4 Synthesis of ZnO NPs

The weighing balance was used to weigh 0.2 g of zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$). The mixture was then combined with 100 ml of distilled water. A magnetic stirrer was used to stir the solution for 10 minutes at 2000 rpm. Following that, 1ml, 3 mL and 6 mL of plant extract were mixed with the solution. The mixture of plant extract was then stirred for 2 hours with a magnetic stirrer at 200 rpm. While stirring, the pH was measured with a pH metre, and 2M of NaOH was carefully added until the solution reached 11, and then 12 in pH, indicating that the solution had become a basic solution. For the remainder of the time, the stirring was maintained. After finishing the stirring, the mixture was transferred to a centrifuge tube with 40ml allocated in each centrifuge tube. The mixture was then centrifuged for 15 minutes at 6000 rpm in a centrifuge. After centrifugation, a white precipitate formed on the walls of the centrifuge tubes. The eh precipitated were washed twice with distilled water and once with ethanol. To avoid impurities in the sample, the precipitated solution had to be centrifuged each time the solution was washed. The precipitate was then dried in an oven at 120°C for 24 hours or overnight. Following that, the sample was kept for further analysis such as XRD, FITR, SEM, UV-Vis, PL, TGA and GC-MS.

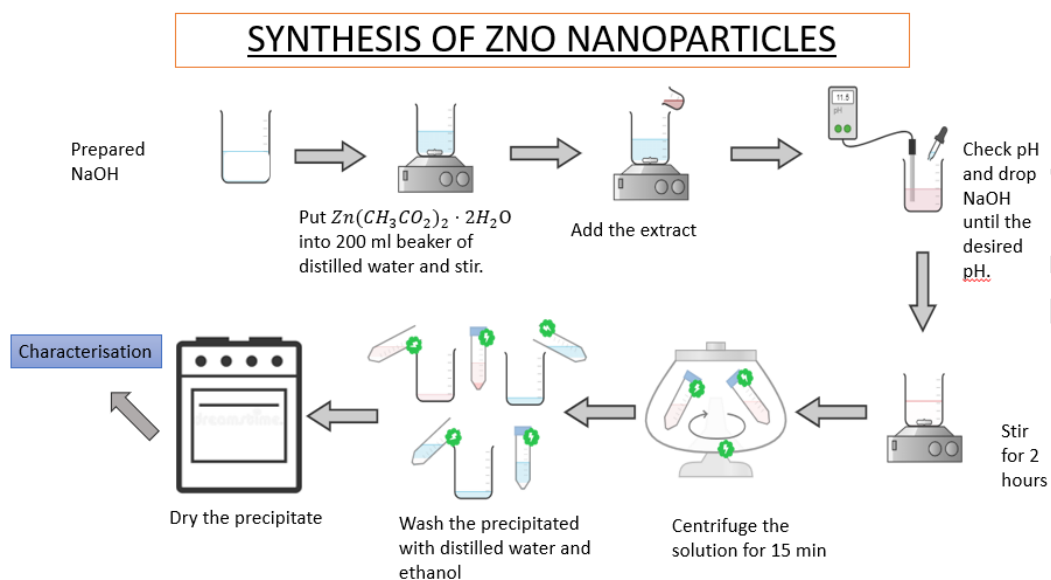


Figure 3.4 : Step by step of green synthesis of ZnO NPs using plant extract.

3.3 Characterization of ZnO NPs

The structural and morphological properties of the materials were characterised using XRD, SEM, and FTIR. While UV-Vis and PL were used to examine the optical properties of the materials. TGA was used to observe and characterise the thermal properties of the materials.

3.3.1 X-ray Diffraction (XRD)

X-ray diffraction (XRD) is a non-destructive technique for obtaining detailed information about the crystal structure, chemical composition, and physical properties of materials or samples (Sima et al., 2016). XRD was also used to determine the particle size and phase structure of the material (Fakhari et al., 2019). According to Theophil Anand et al. (2019), the sharpness of narrow peaks at different phase angles confirms the crystalline nature and phase purity of ZnO NPs. According to Pillai et al. (2020), the particle size of the nanoparticles can be calculated using the Debye-Scherrer equation.

$$\tau = K\lambda/(\beta\cos\theta) \quad (\text{Equation 3.1})$$

Where τ = average size of the crystals

K = constant approximately equal to 0.9

λ = the X-ray wavelength

β = full width at half-maximum peak intensity

θ = is the Bragg angle

The samples' XRD data were characterized using a PW 3040/60 MPD X'Pert High Pro Panalytical based at Universiti Putra Malaysia's Faculty of Science (UPM).

3.3.2 Fourier transmission infrared (FTIR) spectroscopy

Fourier transmission infrared spectroscopy (FTIR) is an analytical technique for determining the chemical compound present in a sample (Ahmad & Kalra, 2020). This characterization provided information for the chemical bonding in the compound, which was used in the synthesis of ZnO NPs. Furthermore, Vijayakumar and colleagues (2018) suggested that FTIR analysis can reveal the functional group responsible for stabilisation reduction and capping agent in nanoparticles. Following that, FTIR works due to the absorption of IR radiation, which stimulated the molecules to a higher vibrational state. This occurred when the material was exposed to infrared radiation (Sadiq et al., 2021). As a result of a specific molecule absorbing the wavelength of light, the difference in the function of energy at excited and rest vibrational states will be presented. Furthermore, according to Dobrucka and Dugaszewska (2016), this is the potential to discover biomolecules in plant extracts. This is due to the fact that this technique can identify which biomolecules are important during the reduction and stabilisation stages of green nanoparticle production. On a Perkin Elmer Spectrum GX, the samples were characterised using the KBr method. The FTIR machine is housed at UPM's Department of Chemistry, Faculty of Science.

3.3.3 Photoluminescence (PL) spectroscopy

In most cases, photoluminescence spectroscopy is used in research to characterise the optical and electrical properties of nanoparticles and molecules (Ramanarayanan et al., 2018). When light is focused onto a sample and absorbed, the PL technique works. The photo-excitation process can then begin. The material jumps to a higher electronic state as a result of the photo-excitation. Then, when it relaxes and returns to a lower energy level, it releases energy (photons), according to Ishikawa-Ankerhold et al. (2012). As a result of this process, photoluminescence (PL) used the emission of light or luminescence. Furthermore, Vijayakumar et al. (2018) reported that the PL test can be used to confirm that the produced nanoparticles were pure and defect-free, as demonstrated by XRD analysis. The Perkin Elmer LS 55 Fluorescence Spectrometer was used to analyse the sample. The PL machine located in Laboratory 305 of the Faculty of Science at Universiti Putra Malaysia.

3.3.4 Ultraviolet - Visible Spectroscopy (UV- Vis)

For this study, UV-Vis analysis was used to characterise and study the optical properties of ZnO NPs. UV-Vis was created to analyse the absorption spectra of ZnO NPs between the wavelength ranges of 200 to 800 nm (Demissie et al., 2020). UV-Vis also reveals the formation of nanoparticles and investigates the sample's band gap value. As a result, ZnO NPs have a bandgap value of 3.37 eV (Elumalai & Velmurugan, 2015; Soto-Robles et al., 2019). The UV-Vis analysis machine is located in the Faculty of Science 2, Universiti Putra Malaysia (UPM). For UV-Vis, UPM uses the UV-3600 SHIMADZU model.

3.3.5 Thermogravimetric analysis (TGA)

Thermogravimetric Analysis (TGA) is a technique for observing the mass or weight of a sample as it changes with temperature and time. These measurements can reveal a lot of information about a material, including its thermal stability, weight changes, oxidation response, composition, breakdown, moisture content, and other properties. Demissie et al. (2020) discovered that TGA characterization determined the decomposition and thermal stability of biosynthesized ZnO NPs. At a heating rate of 10°C/min, ZnO NPs were measured. TGA is a technique for determining the properties of materials used in environmental, food, and pharmaceutical applications, according to Finkelstein et al., (2015). The TGA machine is located in the Faculty of Science 2, Universiti Putra Malaysia. Mettler Toledo TGA/SDTA 851 and Mettler Toledo Model TGA/DSC1 were used to characterise the sample.

3.3.6 Gas chromatography–mass spectrometry (GC-MS)

GC-MS is used to investigate liquids, gases, and solids (Karunakaran et al., 2020). The sample is vaporised into the gas phase before being separated into its constituents using a capillary column covered with a stationary (liquid or solid) phase. The chemicals are propelled by a carrier gas such as helium, hydrogen, or nitrogen. Depending on their boiling point and polarity, compounds elute from the column at different rates. The elution time of a chemical is its retention time. GC is capable of resolving complex mixtures or extracts containing hundreds of chemicals. When the components exit the GC column, the mass spectrometer ionises and fragments them. The ionised

molecules and fragments are accelerated by a mass analyser, which is typically a quadrupole or ion trap. Ions are separated based on their mass-to-charge (m/z) ratios. SIM mode collects data for specific masses of interest, whereas full scan mode covers a wide range of m/z ratios. The procedure concludes with ion detection and analysis, which produces fragmented ions based on their m/z ratios. Peak areas are proportional to the amount of compound. The gas chromatogram of complex samples separated by GC-MS shows several peaks, each with its own mass spectrum. Using commercial mass spectrum libraries, unknown substances and target analytes can be discovered and quantified (Vijayakumar et al., 2021). According to Vijayakumar et al. (2021), the organic chemical compounds identified and characterised in various crude extracts were based on GC retention time.

3.4 In vitro cytotoxicity evaluation of ZnO NPs

3.4.1. Cell lines and reagents

The human breast adenocarcinoma cell lines MCF7 (ATCC® HTB-22TM) and human mammary epithelial cell line (MCF-10A) were used in this study. The cells were obtained from the human cell culture collection of UPM-MAKNA Cancer Research Laboratory, Institute of Bioscience, Universiti Putra Malaysia. The cells were incubated at 37°C in 5% CO_2 incubator (Thermo Fisher Scientific, USA) in their respective complete growth media (Table 3.1).

Table 3.1 Complete growth medium components for MCF-7 and MCF-10A cell lines

Component	MCF-7	MCF-10A
Basal medium	Roswell Park Memorial Institute (RPMI) 1640 Medium	Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12
Serum	Fetal Bovine Serum (10%) v/v	Horse serum (2%) v/v
Antibiotics	Penicillin/Streptomycin (1%) v/v	Penicillin/Streptomycin (1%) v/v
EGF	-	(20 ng/ml)
Hydrocortisone	-	(0.5 mg/ml)
Insulin	-	(10 µg/ml)

The cells were cultured in T-25 sterile tissue culture-treated flasks (Corning, USA) with initial density of 5×10^5 cells/ml. The cells were passaged every two or three days or when the cells were 80-90% confluent.

3.4.2 Subculturing of cells

When the cells reached 80 % confluence, they were splitted into several flasks. The supernatant from the cell culture was discarded, and adhered cells were washed twice with 1 phosphate buffered saline (PBS) to remove any traces of growth medium. Cell detachment was then accomplished through trypsinization, which involved adding 0.25% trypsin/EDTA (Hyclone, USA) to the cells and incubating them at 37°C for 1-3 minutes. When the cells were

partially detached from the plastic surface, complete media was added to stop trypsin activity before centrifuging the cell suspension at 1000 rpm for 5 minutes. The supernatant was then discarded, and the cell pellet was resuspended in 1 mL of pre-warmed growth medium. The cells were then splitted into new sterile cell culture flasks and incubated at 37°C in a 5% CO₂ incubator.

3.4.3 Cryopreservation of cells

When the cells were healthy and had reached 80-90% confluence, they were cryopreserved. Cells were handled as previously described in the subculturing section. Cells were resuspended in cryopreservation medium, which was complete growth medium containing 10% (v/v) dimethyl sulfoxide (DMSO) and 20% (v/v) FBS, after centrifugation. The cell suspension was aliquoted into several 1.8 mL cryovials, each with 1x10⁶ cells/mL. The cryovials were then placed in a cryopreservation 24 container, CoolCell® Cell Freezing Container (Biocision, USA), to achieve an optimal cooling temperature rate of -1°C/min. Overnight, the freezing container was placed in a -80°C freezer. The cryovials were then frozen and placed in a liquid nitrogen tank for long-term storage.

3.4.4 Thawing of cells

A cryovial containing cells was collected from liquid nitrogen storage and thawed rapidly in 37 °C water bath for 1 minute. The cells were then transferred into a 15 mL centrifuge tube containing pre-warmed cell culture

medium, centrifuged at 1000 rpm for 5 minutes, and then the supernatant containing DMSO was discarded. The cell pellet was resuspended in pre-warmed cell culture medium and transferred into a cell culture flask. The cells were incubated in a 5% CO₂ incubator (Thermo Fisher Scientific, USA) at 37°C.

3.4.5 MTT Assays

One of the measures that can be used to evaluate the toxicity is the MTT test (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide). Cell culture was prepared at a concentration of 2×10^3 cells/ml and cells were plated onto 96-well plates. The diluted ranges of sample extracts were added to each well at the specified concentrations of 1.56 µg/ml, 3.125 µg/ml, 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml and incubated for an additional 72 hours at room temperature. At the end of the incubation period, the MTT solution was added to the cells, and the cells were left to incubate for another 3 hours in the incubator.

The Optical Density (OD) was measured using an ELISA reader at a wavelength of 570 nm after the purple formazan crystals were solubilized with DMSO. The results were compared to the control groups. Cell growth inhibition was determined by calculating the medication concentration that resulted in a 50% reduction in cell growth (IC₅₀ value) using the following formula:

$$\text{Cell viability} = \frac{\text{Absorbance sample (mean)}}{\text{Absorbance control (mean)}} \times 100\% \quad (\text{Equation 3.2})$$

After the determination of the percentage of cell viability, graphs were plotted with the percentage of cell viability versus their respective concentrations (Van Tonder et al., 2015).



CHAPTER 4

RESULT AND DISCUSSIONS

4.1 Introduction

A variety of techniques were used to determine the morphology and structural properties of these ZnO NPs. XRD, and FTIR are the techniques used. The optical properties and cytotoxicity of ZnO NPs toward MF-7 cells were investigated using UV-Vis. The results of ZnO NPs synthesised from (a) ZnO (R311), (b) ZnO (R312), (c) ZnO (R611), and (d) ZnO (R612) roselle extract extracts. For reference, 'R' stands for roselle extract, '3' and '6' stand for the volume of plant extract, which are 3 ml and 6 ml, respectively, and '11' and '12' stand for the pH value that was used to synthesise ZnO NPs. The same is true for ZnO NPs, which were synthesised using Dayak onion as a reducing and capping agent. The 'DO' represents Dayak onion extract, the '1', '3', and '6' represent the parameters for volume of plant extract, which are 1ml, 3 ml, and 6 ml, respectively, and the '11' represents the parameter for pH value. It has also been expanded and researched further in the subtopics listed below.

4.2 X- Ray Diffraction (XRD) analysis for ZnO NPs

The XRD patterns of ZnO (R311), ZnO (R312), ZnO (R611), and ZnO (R612) demonstrated that the ZnO NPs prepared using roselle extract are metal oxides with a pure hexagonal structure. Figure 4.1 depicts this obtained the diffraction pattern from 20° to 80° . Diffraction peaks are shown in Figure 4.2 at 31.75° , 34.31° , 36.16° , 47.44° , 56.50° , 62.75° , 66.28° , and 67.84° with miller indices of (010), (002), (011), (012), (110), (013), and (020). (112). According to the spectra of all samples, the highest intensity peak was (011) planes of ZnO. As a result, all of the planes depicted were applied to ZnO NPs. All of the diffraction patterns agreed well with the Inorganic Crystal Structure Database (ICSD) with the code number (98-002-5767) displaying the hexagonal wurtzite ZnO pattern. As a result, this study supported the findings of Chakraborty et al (2020). Based on the FWHM of the most significant diffraction peaks, the average crystallite size for ZnO (R311), ZnO (R312), ZnO (R611), and ZnO (R612) is 21.01 nm, 68.5 nm, 16.9 nm and 22.76 nm, respectively.

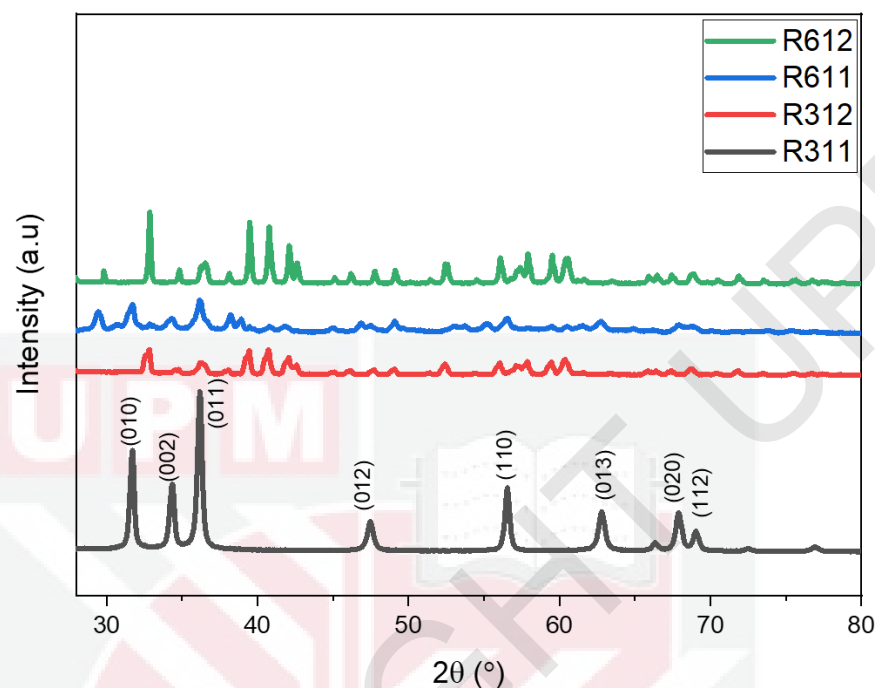


Figure 4.1 : XRD spectra of synthesized ZnO NPs using roselle extract:
(a) ZnO (R311); (b) ZnO (R312); (c) ZnO (R611); (d) ZnO (R612).

4.3 Fourier-transform infrared spectroscopy (FTIR)

The functional group of ZnO NPs was identified in this FTIR spectra based on the graph. The FTIR analysis offers information on the interaction of ZnO NPs with the EM biomolecules. These interactions function as reducing-stabilizing agents. All of the main peaks for both ZnO NPs that used plant extract roselle and Dayak onions can be seen in Figures 4.2 and 4.3, with the exception of ZnO (R312) sample, which is ZnO NPs that used parameter 3ml for roselle extract and pH is 12. The peaks that are around 470 cm^{-1} to 480 cm^{-1}

correspond to ZnO NPs. Furthermore, Khan et al. (2021) stated that the peaks below 500 cm^{-1} show formation, which is thought to be caused primarily by flavonoid (polyphenol) compounds and stabilised by the protein molecule. The bands corresponding to O-H stretching vibrations from alcohols and phenols range from 3230 cm^{-1} to 3250 cm^{-1} . The bands centred at 710 cm^{-1} to 720 cm^{-1} represent C-H bending in the alkene group, while the bands centred at 1020 cm^{-1} to 1030 cm^{-1} represent C-O-C glycosidic linkage and N-H aromatic group stretching vibrations. In conclusion, the presence of phytochemical substances in ZnO NPs causes all of the vibration bands. It also helps with the reduction and stability of ZnO NPs. As a result, these findings agree with those of the previous researcher (lashin et al., 2021).

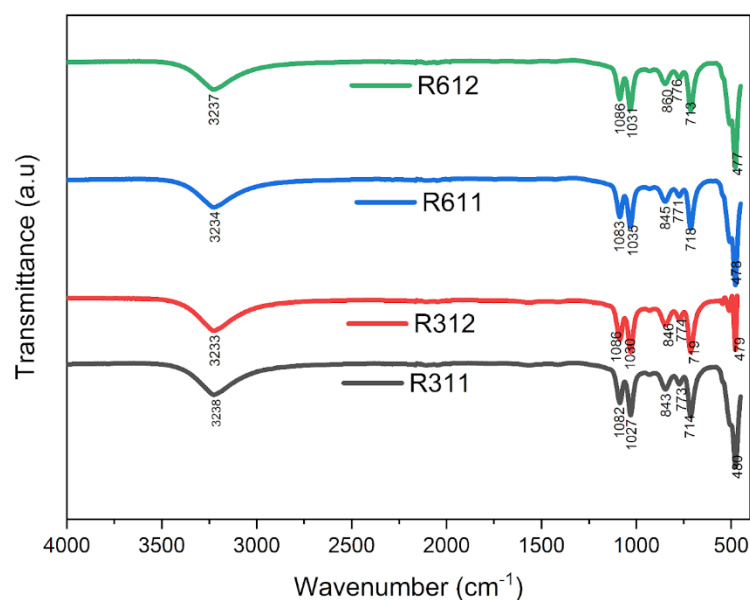


Figure 4.2 : The FTIR spectrum of ZnO NPs with different volumes of roselle extract.

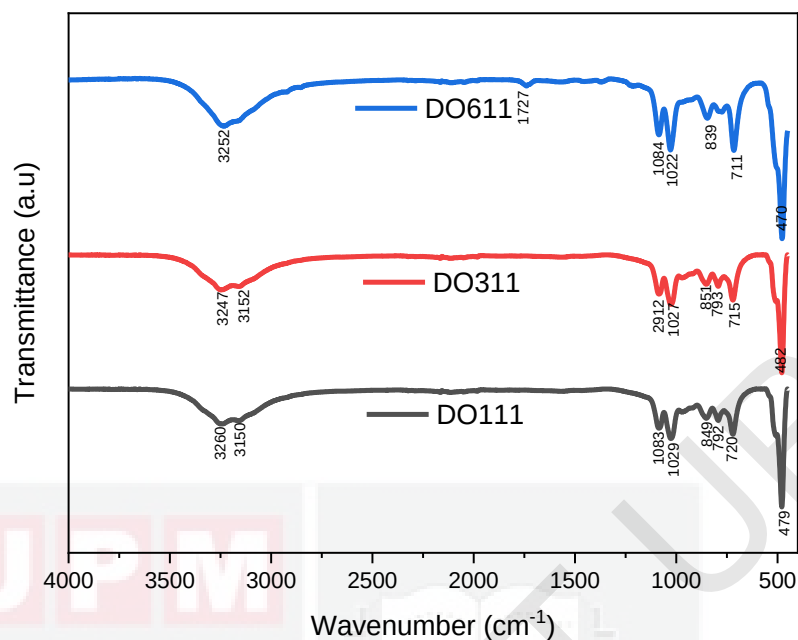


Figure 4.3: The FTIR spectrum of ZnO NPs with different volumes of Dayak Onions extract.

Table 4.1: The optical information of FTIR spectrum for ZnO NPs with different volumes of Dayak onions and roselle extract.

Sample ZnO NPs	O-H stretching (nm)	C-O-C Glycosidic linkage (nm)	C-H Bending (nm)	Zn-O stretching (nm)
BD111	3260	1029	849	479
BD311	3247	1027	851	482
BD611	3252	1022	839	470
R311	3238	1027	843	477
R312	3233	1035	846	479
R611	3234	1033	845	478
R612	3237	1031	869	478

4.4 Ultraviolet-Visible Spectroscopy (UV-Vis)

The surface plasmon resonance effect of the UV–Vis spectral analysis provides information on the actual synthesis of metal oxide nanoparticles (Ezealisiji et al., 2019). A UV–Visible spectrometer was used to monitor the production of green synthesised ZnO NPs. The wavelength ranged between 200 and 800 nm. ZnO NPs have a significant absorption band at approximately 369 nm for ZnO NPS with roselle extract with parameter 6ml and pH is 11, and ZnO NPs with Dayak onions extract with parameter 1ml for volume of extract and pH is 11 is at characteristic peaks at 332 nm, confirming the formation of ZnO NPs.

The band gap for ZnO NPs with roselle extract is 3.68 eV for ZnO (R311), 3.89 eV for ZnO (R312), 3.22 eV for ZnO (R611), and 3.12 eV for ZnO (R612). While the band gaps of ZnO NPs with Dayak onions extract are 3.42 eV, 3.67 eV, and 3.89 eV, respectively, the band gaps of ZnO (DO111), ZnO (DO311), and ZnO (DO611) are 3.42 eV, 3.67 eV, and 3.89 eV, respectively. This band gap had been calculated by gathering absorbance and transmittance data, the absorption coefficient may be expressed in the light absorption area using the Tauc model:

$$(\alpha h\nu)^2 = Ah\nu - E_g \quad (\text{Equation 4.1})$$

Where α = optical absorption coefficient

h = Planck's constant

ν = frequency of the incident photon

E_g = optical band gap

A = a constant for a direct transition

This findings is comparable to observations previously published by Soltanian et al. (2021). The peak in the UV spectrum, according to Chakraborty et al. (2020), is caused by the coupling of incoming electromagnetic radiation into a surface plasmon at the interface. Furthermore, the surface plasmon resonance phenomena of ZnO NPs vary with particle size, shape, and the dielectric constant of the reaction medium.



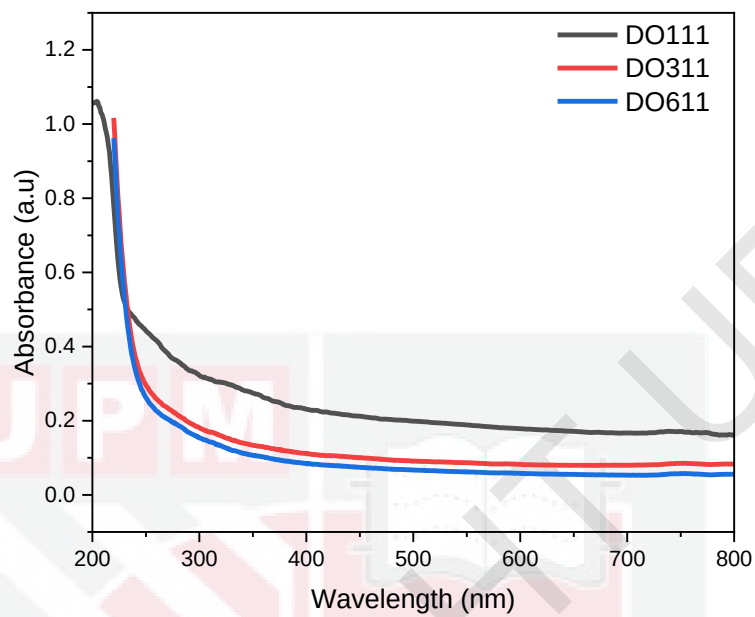


Figure 4.4 : UV-Vis spectrum of ZnO NPs with different volumes of Dayak Onions extract.

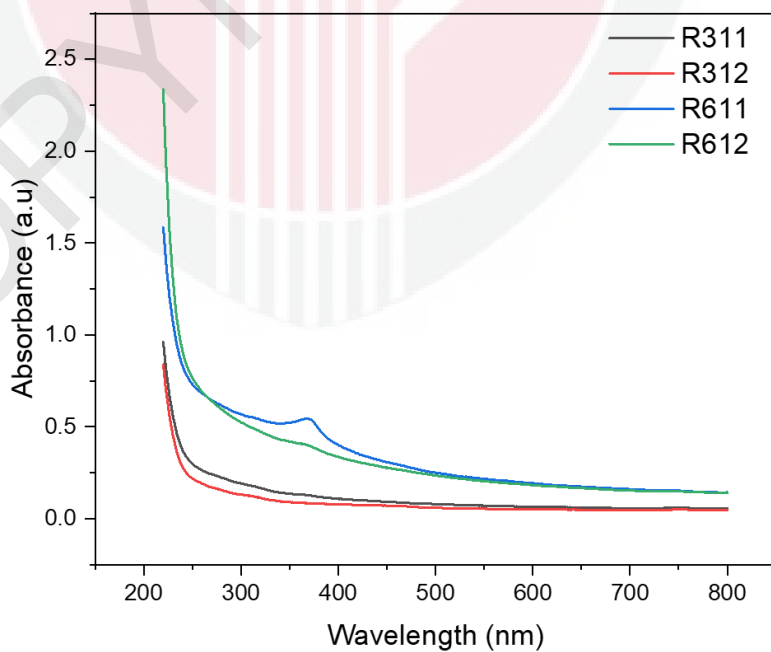


Figure 4.5 : UV-Vis spectrum of ZnO NPs with different volumes of Roselle extract.

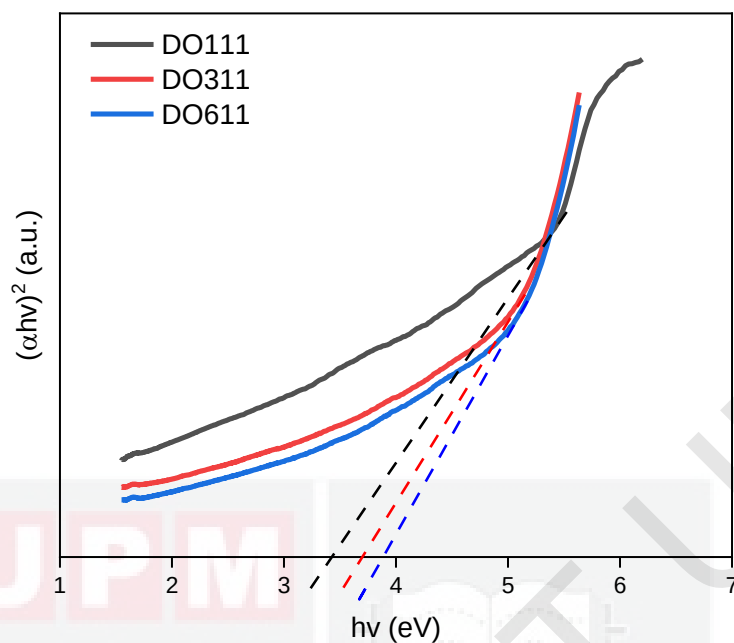


Figure 4.6 : Graph of $(\alpha h\nu)^2$ against photon energy ($h\nu$) of ZnO NPs with different volume of Dayak onions extract.

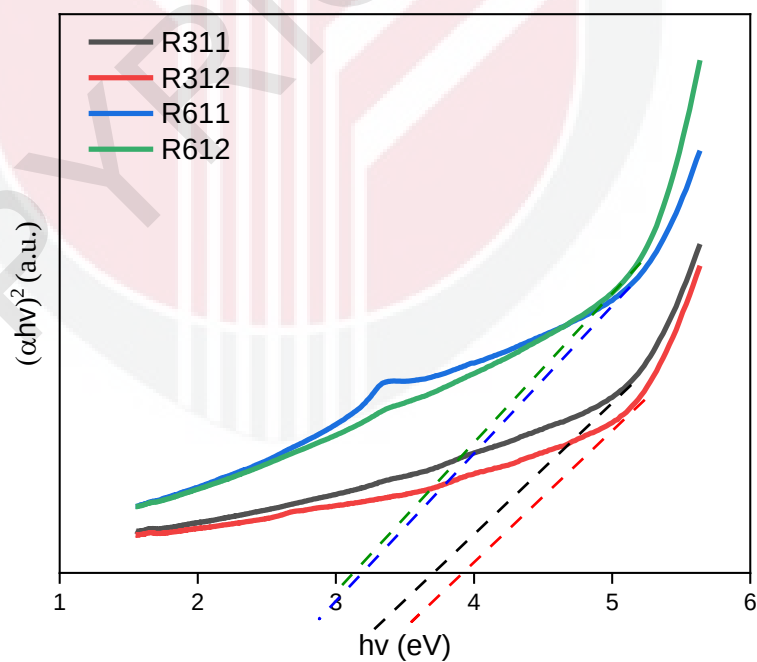


Figure 4.7 : Graph of $(\alpha h\nu)^2$ against photon energy ($h\nu$) of ZnO NPs with different volumes of Roselle extract.

4.5 Photoluminescence Spectroscopy (PL)

Photoluminescence (PL) used the emission of light or luminescence as a result of this process. Furthermore, Vijayakumar et al. (2018) reported that the PL test can be used to confirm that the nanoparticles produced were pure and defect-free, as demonstrated by XRD analysis. According to the graph, the excitation range of ZnO NPs is from 200 nm to 800 nm, and the spectra show significant emission bands at 600 nm to 686 nm for ZnO NPs with roselle extract and at 600nm to 654nm for Dayak onion. This result is consistent with the findings of (Markevich et al., 2016).

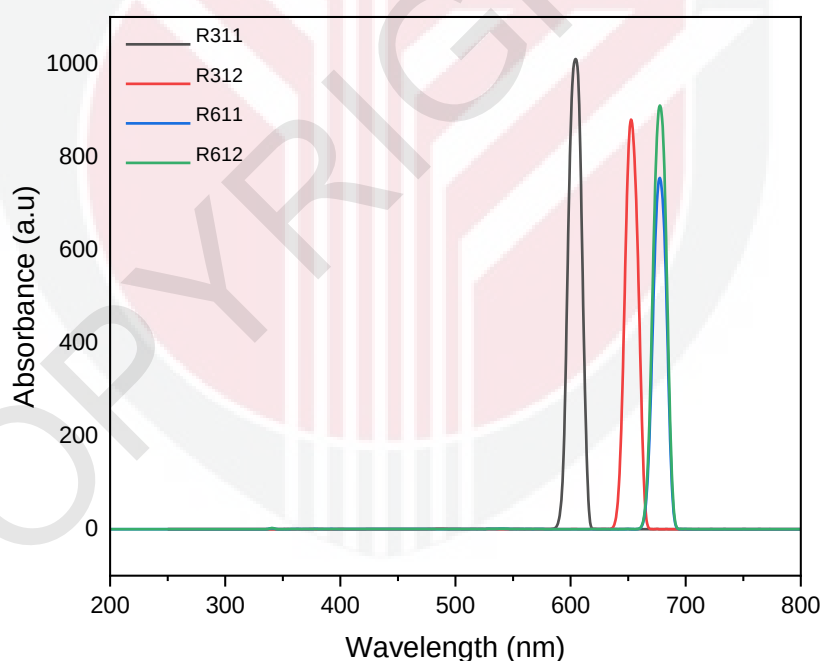


Figure 4.8 : PL spectra of ZnO NPs using roselle extract.

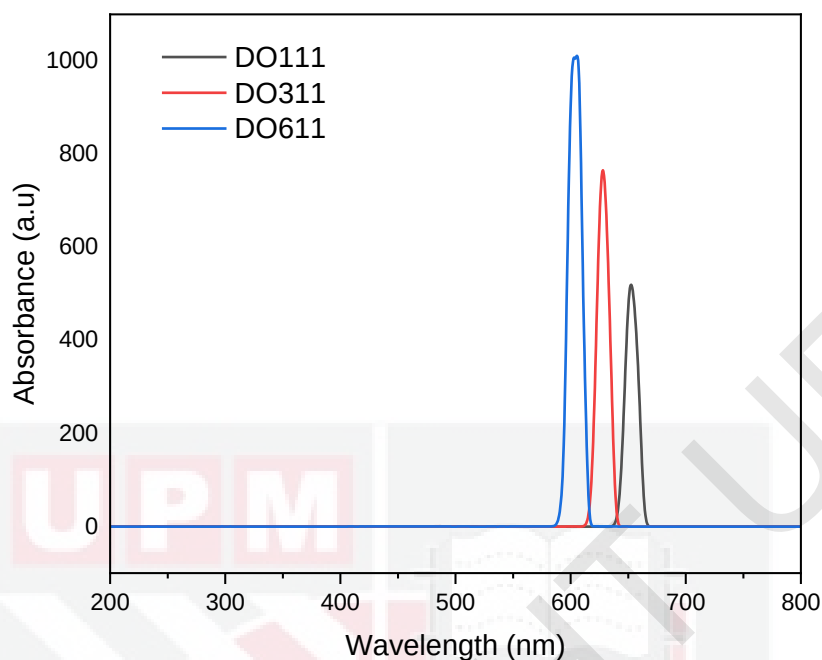


Figure 4.9 : PL spectra of ZnO NPs using Dayak onion extract.

4.6 Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) is a technique for observing the mass or weight of a sample as it changes with temperature and time. Thermal research of the entire extract, ZnO NPs, was conducted to determine the effect of plant extract in reducing metals to nano size. The temperature ranged from 50 to 1000 ° C. The plant extract has low thermal stability, according to the major aspects, organic compounds with certain salts included in the extract as a result of the extraction procedure (lashin et al., 2021). From 97.93 to 153.35 °C, the sample was completely lost. TGA transition shows a loss of 10.13 % up to 200°C and no significant loss of mass after this temperature up to 1000°C. This shows that the biosynthesized ZnO NPs are thermally stable after 200°C. As

shown in Figure 4.10, the graph depicting the ZnO NPs as a result of TGA analysis was performed in nitrogen gas (N_2) and did not involve the combustion of organic for the sample. According to Fatimah et al. (2016), ZnO NPs derived from an aqueous extract of *Mimosa pudica* leaves and coffee powder reduced weight loss in a similar manner.

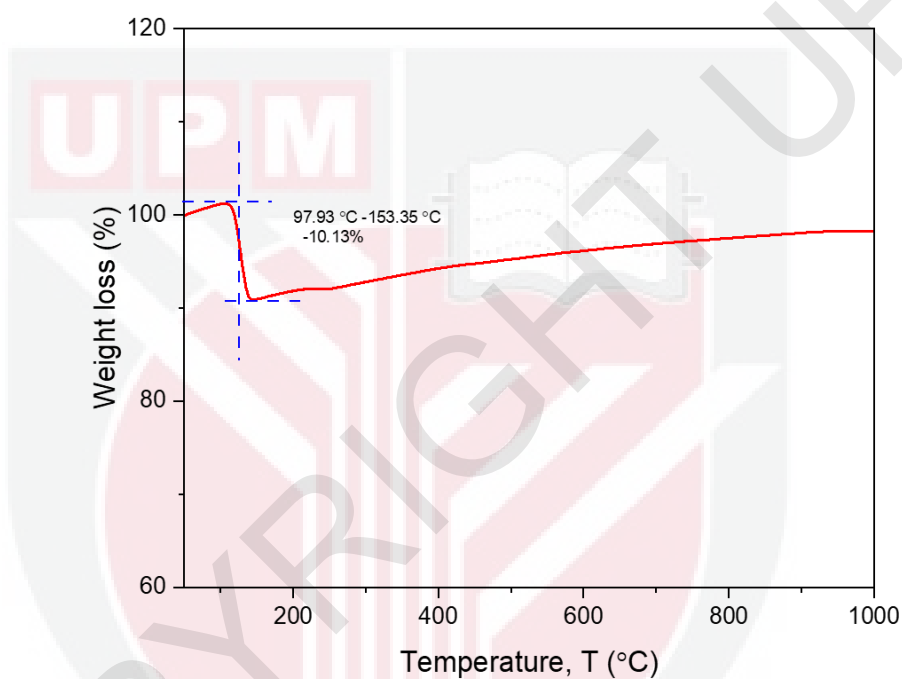


Figure 4.10 : TGA spectrum of ZnO NPs using Roselle extract as capping and reducing agent.

4.8 Gas chromatography–mass spectrometry (GC-MS)

GC–MS analysis was used to identify the component of Roselle Extract and Dayak onions extract to prove the present of capping agents and reducing agents in the plants. The phytochemical compound that present in Dayak onions extract were Propanoic acid, Hexadecanoic acid, Ethyl ester (CAS) Ethyl palmitate, Coumarine, Cis-12-Octadecadienoic acid, Benzeneethanal, 4-[1,1-dimethylethyl] and 9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid. These chemical compounds could act as reducing and capping agents in ZnO NPs. After that, for roselle extract, there were 7 chemical compound that detected present in the extract. The seven chemical compounds were Furfural, 2-Carboxylic acid-2, N-[Carboxymethyl]maleamic acid dimethyl ester, Hexadecanoic acid, Linoleic acid, Cis-13-Eicosenoic acid and Stearic acid. As mention by Agasti and Kaushik (2018), they stated that stearic acid can acted as capping agents and reducing agents that can protect nanoparticle from separation. Other than that, Linoleic acid also can acted as reducing and capping agents (Liu et al., 2020).

Table 4.2 : Gas Chromatography/ Mass Spectrometry of Dayak onions extract.

No	Retention time, R_T (min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1.	4.1663	Propanoic acid	$C_5H_{10}O_3$	118	0.38
2.	4.3425	Propanoic acid	$C_5H_{10}O_3$	118	0.47
3.	50.8420	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.49
4.	51.9710	Hexadecanoic acid, ethyl ester (CAS) Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.42
5.	55.4617	Coumarine, 8-allyl-7-hydroxy-6-ethyl-4-methyl-	$C_{15}H_{16}O_3$	244	2.36
6.	56.3982	Cis-9,cis-12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280	0.56
7.	56.8070	Benzeneethanal, 4-[1,1-dimethylethyl]-	$C_{12}H_{16}O$	176	0.78
8.	57.3080	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	$C_{18}H_{32}O_2$	280	1.02
9.	59.5095	-	-	-	36.69
10.	60.8740	-	-	-	17.25

Table 4.3: Gas Chromatography/ Mass Spectrometry of Roselle extract.

No	Retention time (R_T) (min)	Compound	Molecular formula	Molecular weight (g/mol)	Peak area (%)
1.	5.3073	Furfural	$C_5H_4O_2$	96	1.39
2.	16.1040	2-Carboxylic acid-2	$C_7H_{10}O_3$	142	7.27
3.	19.8790	N-[Carboxymethyl]maleamic acid dimethyl ester	$C_8H_{11}NO_5$	201	2.14
4.	50.9272	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.52
5.	56.5488	Linoleic acid	$C_{18}H_{32}O_2$	280	21.66
6.	56.7555	Cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310	48.45
7.	57.3642	Stearic acid	$C_{18}H_{36}O_2$	284	5.57

4.8 Cytotoxicity assessment of ZnO NPs.

The dosage of ZnO NPs required to reduce cell survival to 50% (IC₅₀) in comparison to untreated cells was used to determine cytotoxicity (Arumugam et al., 2021). ZnO NPs caused 64% of cell death at 100 μ g/ml; increasing the concentration of ZnO NPs may cause a protein imbalance and an excess of ROS, which destroys the mitochondrial electron transport chain (Weigelin et al., 2021). MTT assay of MCF-7 and MCF-10A cell viability after 24 h treatment with different concentrations of ZnO NPs (1 to 100 μ g/ml). The results show that increasing the concentrations of ZnO NPs reduces the survival rate of MCF-7 and MCF-10A cells. The viability of the cells was dependent on the concentration of the applied ZnO NPs, as shown in Fig. 4.11

and Figure 4.12, and the survival rate of MCF-7 cells after exposure to of 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, 12.5 $\mu\text{g/ml}$, 6.25 $\mu\text{g/ml}$, 3.125 $\mu\text{g/ml}$ and 1.56 $\mu\text{g/ml}$ of the ZnO NPs with roselle extract was 4.104 %, 4.299 %, 9.462 %, 12.429 %, 21.798 %, 36.179 %, and 90.395 %, respectively. The viability of MCF-10A cells was 3.753 %, 3.683 %, 3.1276 %, 74.670 %, 84.401 %, 90.675 %, and 96.195 %. MCF-7 viability was 3.116 %, 3.249 %, 5.464 %, 6.531 %, 44.611 %, 74.879 %, and 81.698 % when treated with 100 $\mu\text{g/ml}$, 50 $\mu\text{g/ml}$, 25 $\mu\text{g/ml}$, 12.5 $\mu\text{g/ml}$, 6.25 $\mu\text{g/ml}$, 3.125 $\mu\text{g/ml}$ and 1.56 $\mu\text{g/ml}$ of ZnO NPs with Dayak onions, respectively. MCF-10A viability with Dayak onions was 2.794 %, 2.136 %, 7.667 %, 68.166 %, 90.597 %, 94.834 %, and 99.879 %. As a result, the IC_{50} for MCF-7 for ZnO NPs with roselle extract and ZnO NPs with Dayak onions was 2.503 $\mu\text{g/ml}$ and 5.540 $\mu\text{g/ml}$, respectively. While for MCF-10A, the IC_{50} for ZnO NPs containing roselle extract was 16.47 $\mu\text{g/ml}$ and 15.77 $\mu\text{g/ml}$ for ZnO NPs containing Dayak onions extract.

The cell was observed by using the phase contrast mode of microscopy as shown by Figure 4.13 (a) and Figure 4.13 (b) for MCF-7, and Figure 4.14 (a) and Figure 4.14 (b) for MCF-10A. By the result that, had been obtained, this cytotoxicity test can be conclude that ZnO NPs prepared using roselle extract is more compatible for medical applications and less toxic than the ZnO NPs prepared using Dayak onions. As in the figures shown, when the concentration of ZnO NPs was increased, the viability of cell decreased. So, the survival rate for the cells decreased and it shown in the Figure (g) with the concentration of ZnO NPs which was 100 $\mu\text{g/ml}$, the cell was totally dead.

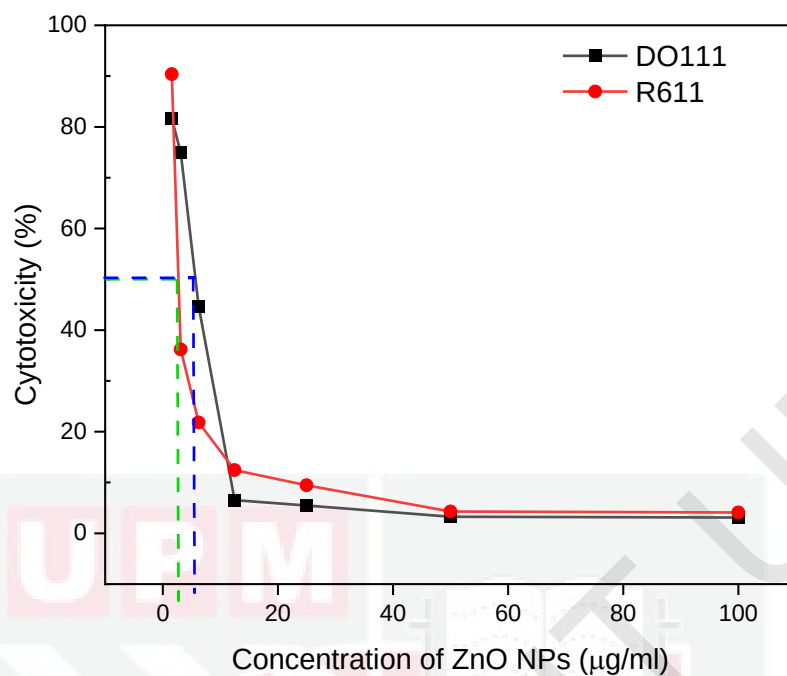


Figure 4.11 : The viability of MCF-7 cell after treatment with different of concentration of ZnO NPs

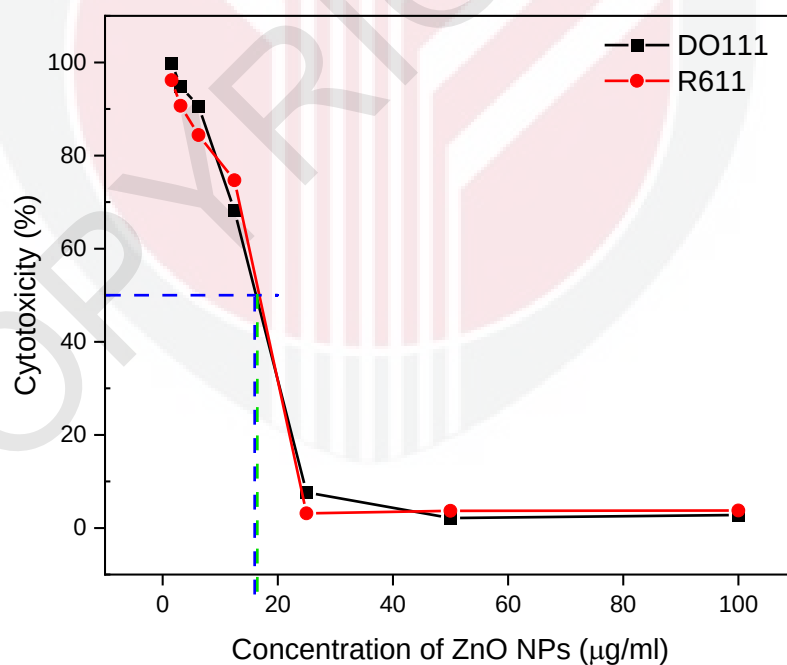


Figure 4.12 : The viability of MCF-10A cell after treatment with different of concentration of ZnO NPs

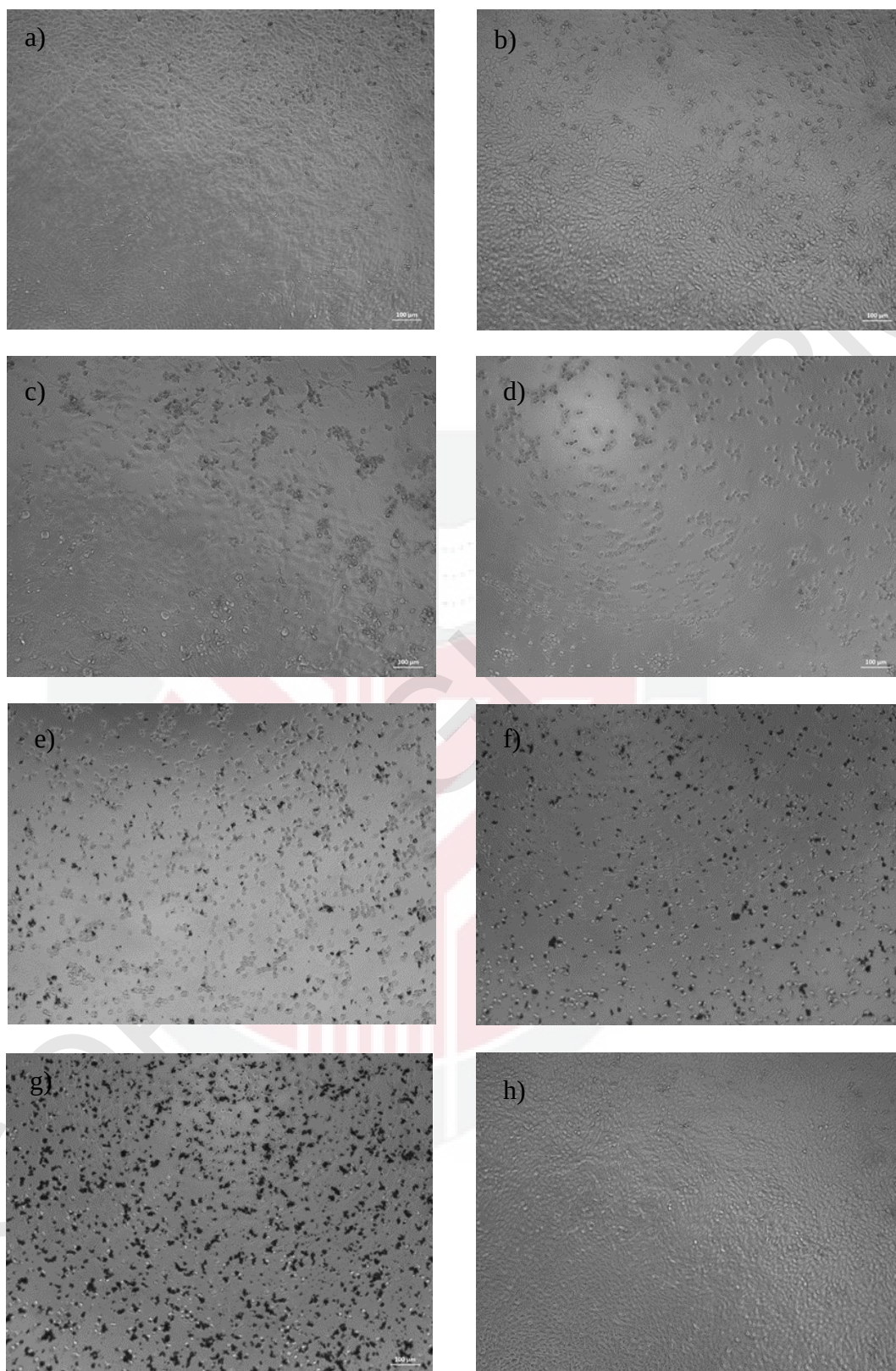


Figure 4.13 (a) : The viability of the cells with the different concentration of ZnO NPs with Dayak onions extract for MCF-7 : a) concentration of 1.56 $\mu\text{g/ml}$; b) concentration of 3.125 $\mu\text{g/ml}$; c) concentration of 6.25 $\mu\text{g/ml}$; d) concentration of 12.5 $\mu\text{g/ml}$; e) concentration of 25 $\mu\text{g/ml}$; f) concentration of 50 $\mu\text{g/ml}$; g) concentration of 100 $\mu\text{g/ml}$; h) untreated cells.

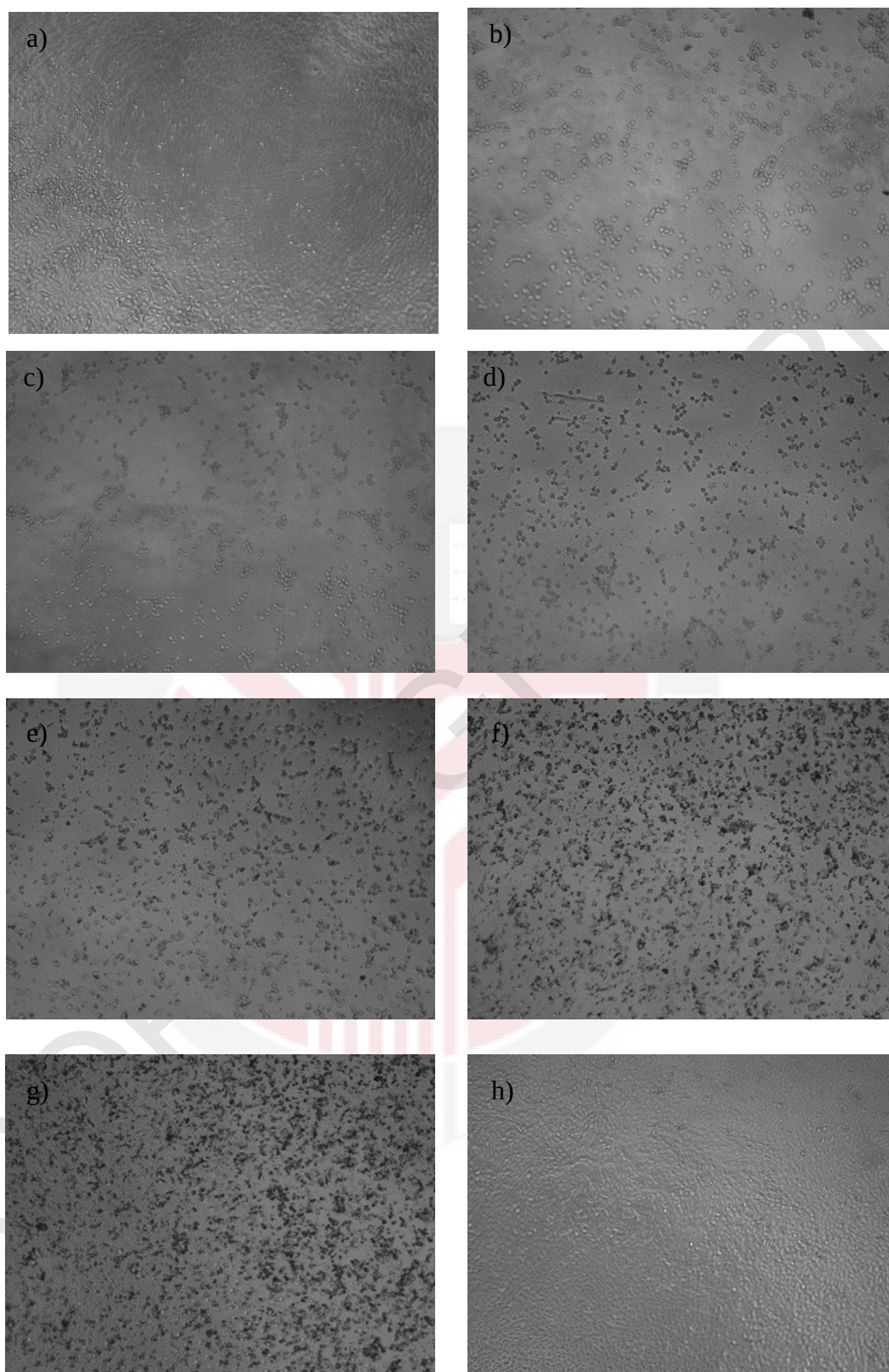


Figure 4.13 (b): The viability of the cells with the different concentration of ZnO NPs with roselle extract for MCF-7: a) concentration of 1.56 $\mu\text{g/ml}$; b) concentration of 3.125 $\mu\text{g/ml}$; c) concentration of 6.25 $\mu\text{g/ml}$; d) concentration of 12.5 $\mu\text{g/ml}$; e) concentration of 25 $\mu\text{g/ml}$; f) concentration of 50 $\mu\text{g/m}$; g) concentration of 100 $\mu\text{g/ml}$; h) untreated cells.

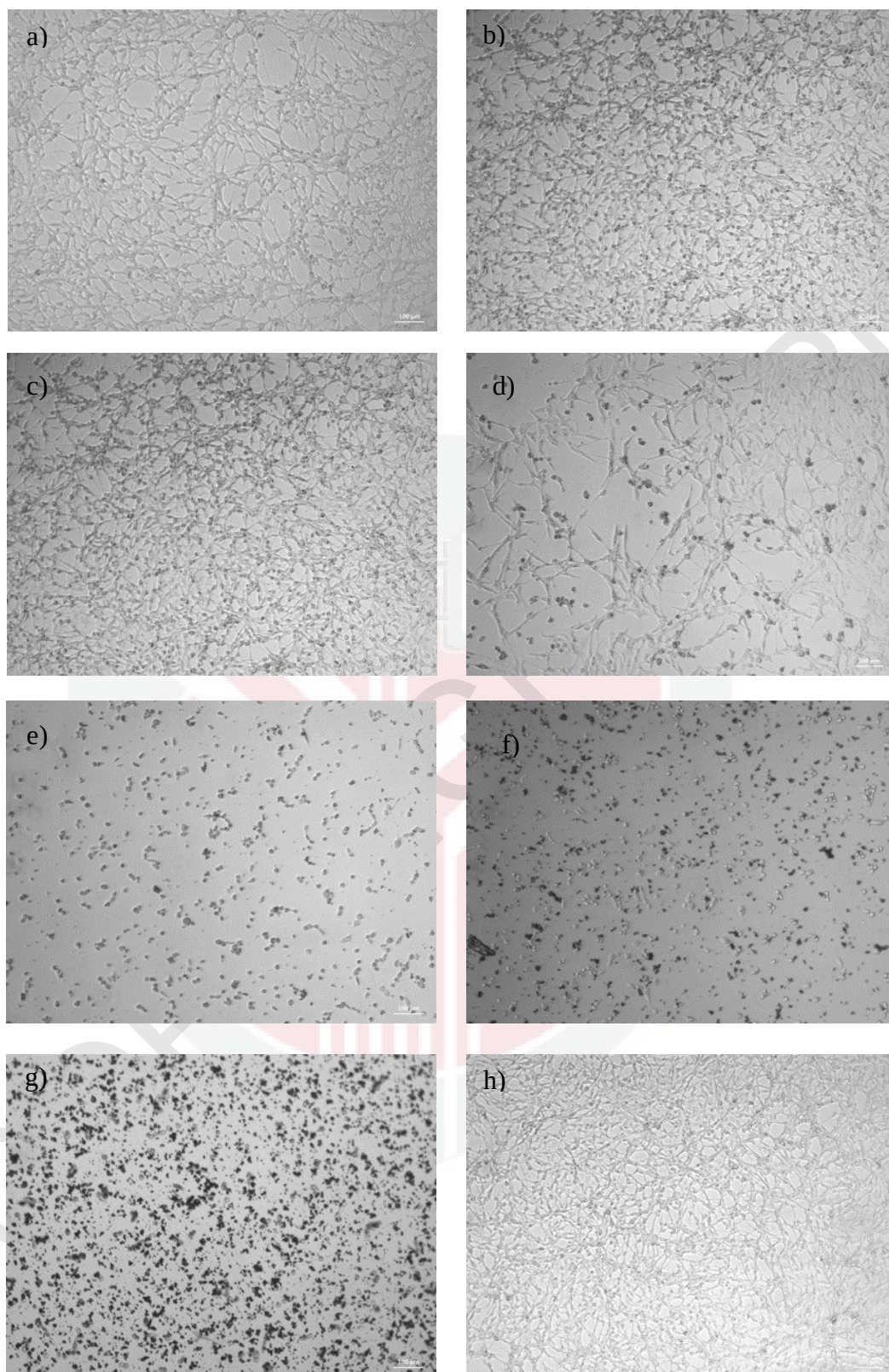


Figure 4.14 (a) : The viability of the cells with the different concentration of ZnO NPs with Dayak onions extract for MCF-10A : a) concentration of 1.56 $\mu\text{g/ml}$; b) concentration of 3.125 $\mu\text{g/ml}$; c) concentration of 6.25 $\mu\text{g/ml}$; d) concentration of 12.5 $\mu\text{g/ml}$; e) concentration of 25 $\mu\text{g/ml}$; f) concentration of 50 $\mu\text{g/ml}$; g) concentration of 100 $\mu\text{g/ml}$; h) untreated cells.

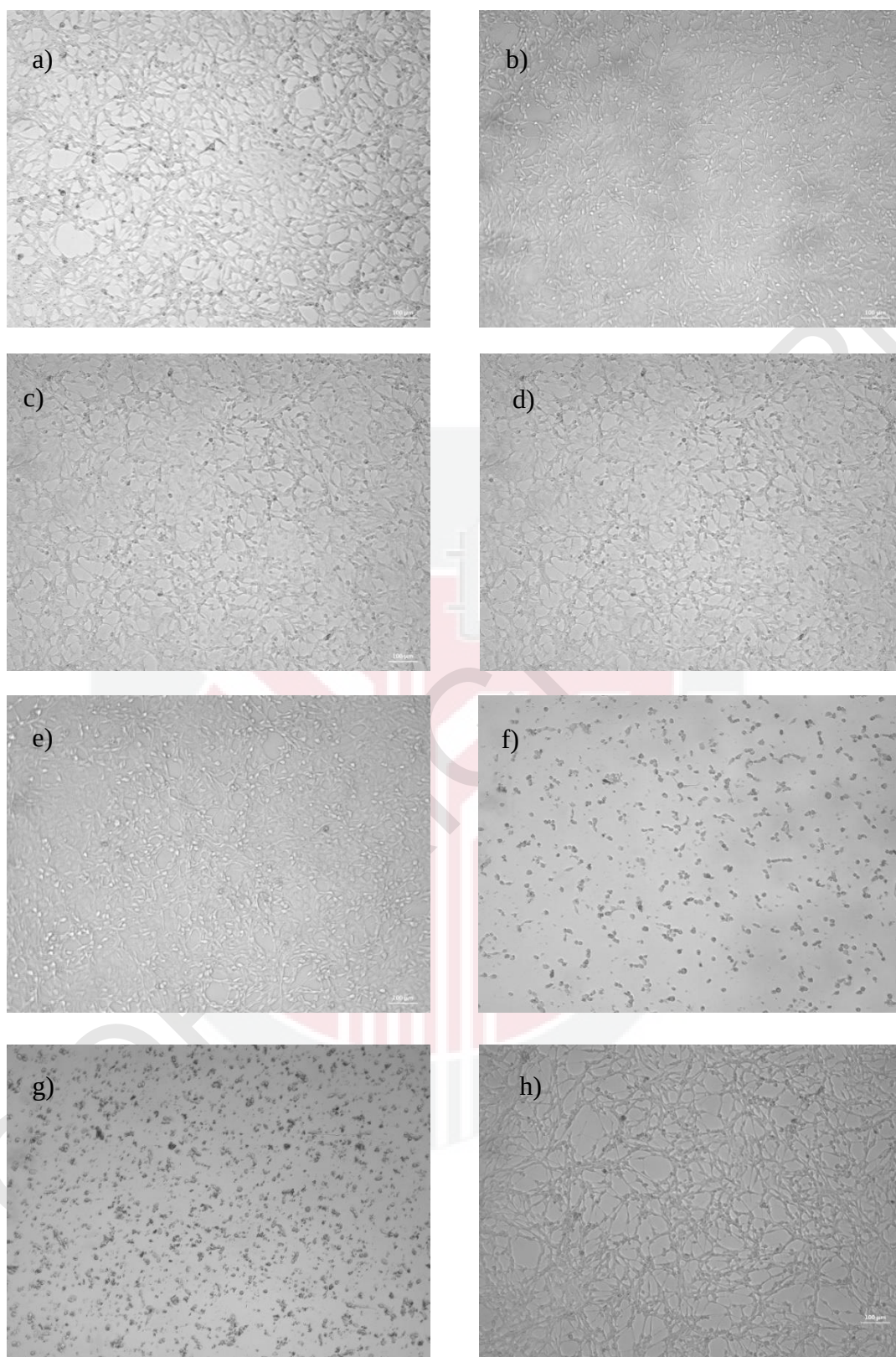


Figure 4.14 (b) : The viability of the cells with the different concentration of ZnO NPs with roselle extract for MCF-10A: a) concentration of 1.56 µg/ml; b) concentration of 3.125 µg/ml; c) concentration of 6.25 µg/ml; d) concentration of 12.5 µg/ml; e) concentration of 25 µg/ml; f) concentration of 50 µg/m; g) concentration of 100 µg/ml; h) untreated cells.

CHAPTER 5

CONCLUSION

5.1 Introduction

In summary, the ZnO NPs was successfully synthesized based on the optimised parameters and using two different plant extract which were roselle extract and Dayak onions extract. The optimised parameter used for synthesized ZnO NPs was ZnO (R611) for roselle extract and ZnO (DO111) for Dayak onions extract.

This research also successfully characterizes structural, optical and thermal properties of ZnO NPs from roselle and Dayak onions extract. For the structural properties which is XRD analysis, the crystal structure had been successfully identified. The crystal structure was wurtzite crystal structure. The highest peaks for crystal plane for the structure which are at (011) with the crystalline size was around 21nm until 68nm. Then, from FTIR analysis, ZnO NPs had successfully revealed the peaks of formation of Zn-O stretching vibration which at 470 cm^{-1} to 480 cm^{-1} for all the sample. and them represent the phytochemical of roselle and Dayak onions that act as reducing and capping agent. For optical properties, UV-Vis graph shown the characteristic peaks with roselle and Dayak onion extract are 369 nm and 334 nm respectively. After that, the band gap for ZnO NPs with roselle extract had eV values of 3.68 eV, 3.89 eV, 3.22 eV, and 3.12 eV for ZnO (R311), ZnO (R312), ZnO (R611), and ZnO (R612). While the band gaps of ZnO NPs containing Dayak onions extract, the band gaps of ZnO (DO111), ZnO (DO311), and ZnO (DO611) are 3.42 eV, 3.67 eV, and 3.89 eV, respectively.

This was calculated by the result that had been extracted from UV-Vis analysis. After that, as for PL, the graph shown the excitation range of ZnO NPs are from 200 nm to 800 nm, the spectral show significant emission bands at 600nm to 686nm for ZnO NPs with roselle extract and for Dayak onion is at 600nm to 654nm. As for TGA, the analysis represented the thermal properties respectively. This analysis shown the weight loss of the sample which is 10.13%. It is shown the sample is stable after the temperature at 200°C in the nitrogen (N₂) gas combustion. While, GC-MS analysis, it confirms the present of capping agents and reducing agents in the synthesis of ZnO NPs for both extract, which the phytochemical compounds were Linoleic acid, stearic acid, Hexadecanoic acid and more chemical compounds.

For the cytotoxicity studies was successfully evaluated for both ZnO NPs from both roselle and Dayak onions using breast normal and cancer cell lines. As for the IC₅₀ for ZnO NPs with roselle extract and Dayak onions were 16.47 µg/ml and 15.77 µg/ml for MCF-10A while for MCF-7 were 2.503µg/ml and 5.540 µg/ml respectively. It also revealed that ZnO NPs with roselle extract is more compatible and less toxic as compared to the ZnO NPs with Dayak onions extract. This is because the value of IC₅₀ for ZnO NPs with roselle extract in MCF-7 cell lines much smaller than ZnO NPs that synthesis with Dayak onions. But the value is higher when in MCF-10A cell lines.

5.2 Future work

While conducting this research, a number of challenges and problems were encountered. One of the scientific issues is impurity, which may have an impact on the sample's outcome. Because there are too many unwanted species in the laboratory, the humidity of the plant extract is uncontrolled, and there are other issues. Furthermore, during the study period, COVID-19 is the most difficult task. As a result, the decision to continue with the experiment is unpredictable. As a result, numerous characterisations and additional research cannot be conducted.

Transmission electron microscopes (TEM) can be used in future studies to obtain better results for characterization because this technique has the ability to magnify things up to 2 million times. As a result, the images for sample shape and size will be more accurate than Scanning Electron Microscopy (SEM) techniques. In addition, at high magnifications and with a nearly infinite depth of focus, Field-emission Scanning Electron Microscopy (FESEM) can be used to examine topography, molecular surface structures, and detail information. X-ray Fluorescence is another method for determining the elemental composition of a sample (XRF). Furthermore, the parameter can be changed and examined for future studies. For example, the temperature of the solution during stirring, the concentration of plant extract that can be varied, and the change of the precursor that is used are all variables that will be considered for this study.

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APPENDIXES

Name and formula

Reference code: 98-010-6787

Compound name: Zinc Oxide
Chemical name: Zinc Oxide
Common name: Zinc Oxide
ICSD name: Zinc Oxide

Chemical formula: O_1Zn_1
Second chemical formula: ZnO

Crystallographic parameters

Crystal system: Hexagonal
Space group: P 63 m c
Space group number: 186

a (Å): 3.2530
b (Å): 3.2530
c (Å): 5.2110
Alpha (°): 90.0000
Beta (°): 90.0000
Gamma (°): 120.0000

Calculated density (g/cm³): 5.66
Volume of cell (10⁶ pm³): 47.76
Z: 2.00

RIR: 5.56

Subfiles and quality

Subfiles: ICSD Pattern
Inorganic
Quality: Calculated (C)

Comments

ICSD collection code: 155780
Creation Date: 1/1/1970
Modification Date: 1/1/1970
Calculated Pattern Original Remarks: Rietveld profile refinement applied
Structures: ZnS(2H)
Original ICSD space group: P63MC
ICSD Collection Code: 155780

Original ICSD space group: P63MC

No R value given in the paper

X-ray diffraction (powder)
Structure type:

ZnS(2H)

Rietveld profile refinement applied

Structure type: ZnS(2H)

Recording date: 8/1/2007

ANX formula: AX

Z: 2

Calculated density: 5.66

Pearson code: hP4

Wyckoff code: b2

Publ. title: The relationships between microstructure and
crystal
structure in zincite solid solutions

References

Primary reference:

Hayes, P.C. Hansson, R. Yamashita, A., *Journal of Materials Science*, **41**, 5559, (2006)

Peak list

No.	h	k	l	d [Å]	2Theta[deg]	I [%]
1	0	1	0	2.81718	31.737	51.2
2	0	0	2	2.60550	34.392	38.7
3	0	1	1	2.47821	36.218	100.0
4	0	1	2	1.91283	47.494	21.0
5	1	1	0	1.62650	56.536	32.9
6	0	1	3	1.47855	62.797	31.3
7	0	2	0	1.40859	66.304	4.6
8	1	1	2	1.37973	67.876	24.0
9	0	2	1	1.35979	69.011	12.7
10	0	0	4	1.30275	72.497	1.9
11	0	2	2	1.23910	76.875	3.9
12	0	1	4	1.18244	81.302	2.0
13	0	2	3	1.09407	89.509	9.1

Structure

No.	Name	Elem.	X	Y	Z	Biso	sof	
<u>Wyck.</u>								
1	ZN1	Zn	0.66670	0.33330	0.50000	0.5439	1.0000	2b
2	O1	O	0.33330	0.66670	0.37500	0.2737	1.0000	2b

Stick Pattern

