



UNIVERSITI PUTRA MALAYSIA

***SYNTHESIS AND CHARACTERIZATION OF BIOGENIC ZINC OXIDE
NANOPARTICLES PHOTOCATALYSTS AND EVALUATION OF ITS
ANTIOXIDANT, ANTIBACTERIAL AND NANOTOXICITY
PROPERTIES***

LAU GEE EEN

ITMA 2021 1



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By

LAU GEE EEN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

January 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

SYNTHESIS AND CHARACTERIZATION OF BIOGENIC ZINC OXIDE NANOPARTICLES PHOTOCATALYSTS AND EVALUATION OF ITS ANTIOXIDANT, ANTIBACTERIAL AND NANOTOXICITY PROPERTIES

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January 2021

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During the process of dyeing and printing in textile industry, more than 8000 chemicals are taking part. Methyl orange (MO) and Methylene blue (MB) are synthetic dyes commonly used in the dyeing process. Inappropriate usage and disposal of these synthetic dye may lead to negative impacts on the environment. Photocatalysis of nanoparticles is a pioneering wastewater treatment technique, notably for those containing organic compounds which are difficult to remove. The fascinating physical and chemical properties made zinc oxide nanoparticles (ZnONPs) famous in nanotechnology. The research highlights the potential of new nano-agents on antioxidant and photocatalytic activity. The roselle flower and oil palm leaf extracts act as reducing agents, while Sodium hydroxide (NaOH) is used as a precipitating agent during the synthesis of ZnONPs. Synthesis of ZnONPs without the addition of plant extracts, ZnO (B) was used as blank control of the study. Characterization and analytical studies were conducted on the synthesized ZnONPs. GC-MS discovered that saturated and unsaturated fatty acids were the main constituents in both extracts. EDX revealed the synthesized ZnONPs constituent two elements, Zn and O. The FTIR analysis showed the presence of phenolic compounds and Zn-O stretching bond in green synthesized products. In terms of morphology, the size of the synthesized ZnONPs was 10–15 nm. They are found in agglomerated spherical-shaped. XRD spectra revealed the ZnONPs have 20-30 nm crystallite size. ZnONPs have wurtzite structure in Raman spectrum. UV Vis discovered that ZnONPs have relatively large band gap, which are 2.9 eV and 3.2 eV. TGA proved high thermal stability of ZnONPs. ESR revealed the presence of oxygen vacancies in ZnONPs, which play a vital role in the dye degradation process. The presence of 5% of ZnONPs as catalysts under exposure of 10 W UV lamp could degrade 10 ppm MO in 5 hours and MB in 3 hours efficiently. ZnONPs exhibit high antioxidant and antibacterial properties, especially with the presence of phenolic compounds. *Artemia* sp.

assay discovered that low toxicity of ZnONPs has the potential to work as water treatment agents. To conclude, for pharmaceutical and industry use, ZnONPs can be further developed.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**SINTESIS DAN PENCIRIAN ATAS BIOGEN ZINK OKSIDA NANOPARTIKEL
FOTOKATALIS DAN PENILAIAN ATAS CIRI-CIRI ANTIOKSIDA,
ANTIBAKTERIA DAN NANO-KETOKSIKAN.**

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Dalam proses pewarnaan dan percetakan dalam pembuatan tekstil, lebih daripada 8000 bahan kimia terbabit. Metil Oren (MO) dan Metilena biru (MB) merupakan pewarna sintetik yang biasa digunakan dalam proses pewarnaan. Kesalahan penggunaan dan pembuangan pewarna sintetik ini akan mendorong kepada impak negatif terhadap alam sekitar. Fotomangkin nanopartikel merupakan teknik yang perintis untuk rawatan air, terutamanya untuk yang mengandungi sebatian organik yang susah dihapuskan. Keunikan ciri-ciri fizikal dan sifat kimia menjadikan zink oksida nanopartikel (ZnONPs) terkenal dalam nanoteknologi. Kajian ini menonjolkan potensi ejen nano yang baru atas antioksidan dan fotomangkin aktiviti. Ekstrak bunga asam belanda (Rosel) dan daun kelapa sawit memainkan peranan sebagai agen penurunan manakala natrium hidroksida (NaOH) digunakan sebagai ejen pemendakan dalam proses sintesis ZnONPs. Sintesis ZnONPs tanpa tambahan ekstrak tumbuhan digunakan sebagai kawalan kosong dalam penyelidikan. Pencirian dan kajian analisis dijalankan atas ZnONPs. GC-MS menemukan bahawa asid lemak tepu dan tidak tepu merupakan unsur utama dalam kedua-dua jenis ekstrak. EDX menunjukkan ZnONPs merangkumi dua element, Zn dan O, dalam ketulenan yang tinggi. Analisis FTIR menunjukkan kewujudan sebatian fenolik dan ikatan Zn-O dalam produk sintesis hijau. Dari segi morfologi, saiz ZnONPs adalah 10-15 nm. Nanopartikel ini didapati dalam bentuk bulat bertimbunan. Spektro XRD menunjukkan ZnONPs mempunyai 20-30 nm kristalit saiz. ZnONPs didapati dalam struktur wurtzit melalui spektral Raman. UV-Vis menemukan ZnONPs mempunyai jurang jalur yang besar, iaitu 2.9 eV dan 3.2 eV. TGA membuktikan kestabilan terma yang tinggi daripada ZnONPs. ESR mendedahkan kehadiran kekosongan oksigen dalam ZnONPs, yang memainkan peranan penting dalam proses pelunturan pewarna sintetik. Kehadiran 5% ZnONPs sebagai pemangkin bawah pendedahan kepada 10 W lampu UV dapat melunturkan 10 ppm MO dalam 5 jam dan MB dalam 3 jam secara efektif. Seterusnya, ZnONPs

menunjukkan ciri-ciri anti-oksidasi dan anti-bakteria yang tinggi, terutamanya dengan kewujudan sebatian fenolik. Asai Artemia sp. menemukan ZnONPs mengandungi toksik yang rendah mempunyai potensi untuk kerja sebagai agen rawatan air. Kesimpulannya, untuk kegunaan farmasi dan industry, ZnONPs dapat diperluaskan.



ACKNOWLEDGEMENTS

It is a genuine pleasure to acknowledge my sincerest thanks and gratitude to my supervisor, Assoc. Prof Dr. Che Azurahaman Che Abdullah, senior lecturer of Faculty of Science, Universiti Putra Malaysia, for her kind supervision. Her dedication and keen interest above all her overwhelming attitude to help her students had been solely and mainly responsible for completing my master study. It is a great honour to work under her supervision. Thanks for her encouragement, creative and comprehensive advice until this work came to existence.

I would like to express my deepest thanks and sincere appreciation to my co-supervisors for my master's study, Dr. Nurul Husna Shafie from Department of Nutrition, Universiti Putra Malaysia and Dr. Wan Amir Nizam Wan Ahmad from School of Health Sciences, Universiti Sains Malaysia. Thanks for their supervision and generous bits of advice throughout the study. Their scholarly advice and scientific approach helped me to a very great extent to accomplish my master's study. Nonetheless, profusely thanks for allowing me to complete my research. Thanks for their kind help and guidance throughout my study period.

I greatly appreciate the assistance and support from laboratory staff assistants in Physics Department, Faculty of Science, Universiti Putra Malaysia. Besides, I also offer my gratitude to all staff from Institute of Advanced Technology, Universiti Putra Malaysia, for their help during my master's research.

I owe a deep sense of gratitude to my labmates and friends for guiding me and giving supports physically and mentally at every stage of my research. Their prompt inspirations and timely suggestions with kindness have enabled me to complete my research study successfully.

Lastly, it is my privilege to thank my lovely families and friends for their constant encouragement throughout my research period. Their supports lead to becoming a determined person who has a firmness of purpose and the resolve to achieve goals throughout my study period.

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xiv
LIST OF ABBREVIATIONS	xvi
CHAPTER	
1 INTRODUCTION	1
1.1 Background of Study	1
1.2 Problem Statement	2
1.3 Significant of Research	3
1.4 Objectives	3
1.5 Structure of the Thesis	4
2 LITERATURE REVIEW	5
2.1 Nanoparticles	5
2.2 Properties of Zinc Oxide Nanoparticles (ZnONPs)	5
2.2.1 Structural Properties	6
2.2.2 Physical and Chemical Properties	8
2.3 Methods to Synthesize ZnONPs	8
2.3.1 Physical Method	9
2.3.2 Chemical Method	10
2.3.3 Biological Method	12
2.4 Description of Plants Used	13
2.4.1 Oil Palm Leaf	13
2.4.2 Roselle Flower	14
2.5 Characterization of ZnONPs	15
2.5.1 Characterization Techniques	15
2.5.2 Findings of ZnONPs by SEM Characterization	16
2.5.3 Findings of ZnONPs by FE-SEM Characterization	18
2.5.4 Findings of ZnONPs by TEM Characterization	18
2.5.5 Mechanism of DLS	19
2.5.6 Finding of ZnONPs by UV-Vis Characterization	20
2.5.7 Finding of ZnONPs by FTIR Characterization	21
2.5.8 Finding of ZnONPs by TGA Characterization	21

	2.5.9	Advantages and Limitations of Characterization Techniques	22
2.6		The Advancing Activities of ZnONPs	24
	2.6.1	Photocatalytic	24
	2.6.2	Antioxidant	29
	2.6.3	Antibacterial	31
	2.6.4	Antifungal	34
2.7		Brine Shrimp Toxicity Test	34
2.8		Methods for Removal of Dyes	35
	2.8.1	Physical Method	36
	2.8.2	Chemical Method	36
	2.8.3	Biological Method	37
3		MATERIALS AND METHOD	38
	3.1	Materials	38
	3.2	Synthesis of ZnONPs	38
	3.2.1	Preparation of Oil Palm Leaf Extract	38
	3.2.2	Preparation of Roselle Flower Extract	38
	3.2.3	Synthesis of ZnONPs	39
	3.3	Characterization of Synthesized ZnONPs	40
	3.3.1	Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX)	40
	3.3.2	Field Emission Electron Microscope (FE-SEM)	40
	3.3.3	Transmission Electron Microscope (TEM)	40
	3.3.4	X-ray Diffraction (XRD)	41
	3.3.5	Ultraviolet- visible Spectroscopy (UV-Vis)	41
	3.3.6	Fourier Transform Infrared Spectroscopy (FTIR)	41
	3.3.7	Thermogravimetric Analysis (TGA)	41
	3.3.8	Raman Spectroscopy	42
	3.3.9	Electron Spin Resonance (ESR)	42
	3.3.10	Gas Chromatography-Mass Spectroscopy (GC-MS)	42
	3.4	Photocatalytic Activity	42
	3.5	Antibacterial Activity	43
	3.6	Antioxidant Activity	44
	3.7	Brine Shrimp Toxicity Test	44
	3.8	Statistical Analysis	45
4		RESULTS AND DISCUSSION	46
	4.1	Characterization of ZnONPs	46
	4.1.1	Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDX)	46
	4.1.2	Field Emission Scanning Electron Microscopy (FE-SEM)	48
	4.1.3	Transmission Electron Microscopy (TEM)	49
	4.1.4	X-ray Powder Diffraction (XRD)	51
	4.1.5	Ultraviolet-visible Spectroscopy (UV-Vis)	52

4.1.6	Fourier-Transform Infrared Spectroscopy (FTIR)	55
4.1.7	Thermogravimetric Analysis (TGA)	57
4.1.8	Raman Spectroscopy	58
4.1.9	Electron Spin Resonance (ESR)	59
4.1.10	Gas Chromatography- Mass Spectroscopy (GC-MS)	61
4.2	Photocatalytic Activity	63
4.3	Antibacterial Activity	70
4.4	Antioxidant Activity	73
4.5	Brine Shrimp Toxicity Test	74
5	CONCLUSION	77
5.1	Conclusion	77
5.2	Future Work	78
	REFERENCES	79
	APPENDICES	103
	BIODATA OF STUDENT	105
	LIST OF PUBLICATIONS	106

LIST OF TABLES

Table		Page
2.1	Physical and Chemical Properties of ZnO	8
2.2	Characterization techniques to determine corresponding parameters	16
2.3	Advantages and limitations of characterization techniques.	22
2.4	Photocatalytic degradation of selected organic contaminants by ZnONPs	28
2.5	Summary of antioxidant activity of ZnONPs	30
2.6	Summary of antibacterial activity of ZnONPs on <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	32
2.7	Summary of toxicity test using brine shrimp lethality assay for ZnONPs	35
2.8	Methods for removal of dyes from textile water	35
4.1	Band gap energy and the wavelength maximum of synthesized ZnONPs: (a) ZnO (R); (b) ZnO (O); ZnO (B)	54
4.2	Phytochemicals identified in oil palm leaf extract by GC-MS	62
4.3	Phytochemicals identified in roselle flower extract by GC-MS.	63
4.4	Kinetic data of MO dye.	67
4.5	Kinetic data of MB dye	70
4.6	Diameter of inhibition zone on <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	71

LIST OF FIGURES

Figure	Page
2.1 ZnO with hexagonal wurtzite structure model. Tetrahedral coordination of Zn-O is presented	6
2.2 The cubic zinc blende structure model of ZnO	7
2.3 Synthesis techniques of ZnONPs	9
2.4 Schematic diagram for the green synthesis of ZnONPs. Nucleation happened once the plant extract is added into the zinc salt solution. The small neighboring nanoparticle will accumulate, and form stabilized and capped nanoparticles	13
2.5 Corresponding mapping images for O and Zn in ZnONPs	17
2.6 DLS analysis on suspension with different sizes of nanoparticles. The larger particles settle to the bottom over time, while the smaller particles remain suspended and are detected by the device	19
2.7 The advancing activities of ZnONPs	24
2.8 Schematic Representation of ZnONPs Photocatalytic Process.	25
2.9 Factors affecting degradation efficiency	26
3.1 Step by step method to synthesize ZnONPs	39
3.2 Set up of apparatus for photocatalytic activity	43
4.1 SEM mapping image and EDX spectrum of synthesized ZnONPs: (a) ZnO (B); (b) ZnO (O); (c) ZnO (R). The weight percentage and atomic percentage of three variants ZnONPs were obtained	47
4.2 FE-SEM image and average size of synthesized ZnONPs: (a1) ZnO (B), (b1) ZnO (R), (c1) ZnO (O) under x100,000; (a2) ZnO (B), (b2) ZnO (R), (c2) ZnO (O) under x200,000	49
4.3 TEM image and average size of synthesized ZnONPs:	50
4.4 Particle size distribution from TEM image analysis: (a) ZnO (B), (b) ZnO (R), (c) ZnO (O). ZnO (R) has the smallest average size	51

4.5	XRD of synthesized ZnONPs and SAED pattern from TEM: (a) ZnO (B); (b) ZnO (R); (c) ZnO (O). The XRD spectra and the SAED pattern revealed the ZnONPs are in polycrystalline structure	52
4.6	UV-Vis Spectra of synthesized ZnONPs: (a) ZnO (R); (b) ZnO (O); (c) ZnO (B)	54
4.7	UV-Vis and band gap spectra of synthesized ZnONPs: (a) ZnO (R); (b) ZnO (O); (c) ZnO (B). UV-Vis spectrums for three variants ZnONPs are in direct transition	55
4.8	FTIR of synthesized ZnONPs: (a) ZnO (B); (b) ZnO (R); (c) ZnO (O). Zn-O bond was found in three variant ZnONPs. Three bonds (C-O-C, C-O, and C-H) which indicate the presence of plant extract were revealed in ZnO (R) and ZnO (O)	56
4.9	Thermogravimetric Analysis (TGA) for synthesized ZnONPs: (a) ZnO (B); (b) ZnO (R); (c) ZnO (O). The total weight loss percentage was 4%, 6% and 9% respectively	57
4.10	Raman Spectra of synthesized ZnONPs: (a) ZnO (B); (b) ZnO (R); (c) ZnO (R). The narrow strong band, E ₂ high modes occurred at 440 cm ⁻¹	59
4.11	ESR spectra for synthesized ZnONPs: (a) ZnO (R); (b) ZnO (B); (c) ZnO (O). The g-factor associated to the native defects of ZnONPs and showed no significance difference	60
4.12	GC-MS Spectrum of oil palm leaf extract	61
4.13	GC-MS Spectrum of roselle flower extract	62
4.14	(a), (b): Calibration curves of: (a), (b) Methyl orange (MO) dye solution; (c), (d) Methylene blue (MB) dye solution	64
4.15	Photocatalytic Activity of MO dye: (a) Control; (b) ZnO (B); (c) ZnO (O); (d) ZnO (R). The decrement of absorbance indicated the concentration is getting lower	65
4.16	(a), (b): Process of degradation of MO dyes with irradiation in the UV region. ZnO (B) has the highest kinetic constant, while the photolysis set (control) has the lowest	66
4.17	Photocatalytic Activity of MO dye: (a) Control; (b) ZnO (B); (c) ZnO (O); (d) ZnO (R). The decrement of absorbance indicated the concentration is getting lower	68

4.18	(a), (b): Process of degradation of MB dyes with radiation in the UV region. ZnO (O) has the highest kinetic constant, while the photolysis set (control) has the lowest	69
4.19	Antibacterial activity of plant extract and ZnONPs on (a) <i>Staphylococcus aureus</i> , (b) <i>Escherichia coli</i>	71
4.20	Zone of inhibition on <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> . The larger the zone of inhibition, the stronger the antibacterial properties	72
4.21	DPPH scavenging activity of synthesized ZnONPs. The higher the antioxidant properties, the mixture of DPPH solution and ZnONPs will turn to more milky or yellowish	74
4.22	(a) Brine Shrimp Cysts and Hatching Cysts (b) Microscopy photograph of control brine shrimp (c) Microscopy image of brine shrimp with synthesized ZnONPs	75
4.23	Brine Shrimp Toxicity Test: (a) Hatching Test (b) Lethality Test. The lower the toxicity of the ZnONPs, the higher the hatching percentage. Conversely, the lethality will be getting lower	76

LIST OF ABBREVIATIONS

UPM	Universiti Putra Malaysia
UKM	Universiti Kebangsaan Malaysia
Kyutech	Kyushu Institute of Technology
ZnONPs	Zinc Oxide Nanoparticles
ZnO (O)	Synthesized Zinc Oxide Nanoparticles with Oil Palm Leaf
ZnO (R)	Synthesized Zinc Oxide Nanoparticles with Roselle Flower
ZnO (B)	Synthesized Zinc Oxide Nanoparticles without Extract
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
MO	Methyl Orange
MB	Methylene Blue
CB	Conduction Band
VB	Valence Band
1D	One-Dimensional Structure
2D	Two-Dimensional Structure
3D	Three-Dimensional Structure
SEM	Scanning Electron Microscope
EDX	Energy Dispersive X-ray
FE-SEM	Field Emission Electron Microscope
TEM	Transmission Electron Microscope
XRD	X-ray Diffraction
DLS	Dynamic Light Scattering
UV-Vis	Ultraviolet- visible Spectroscopy
FTIR	Fourier Transform Infrared Spectroscopy

TGA	Thermogravimetric Analysis
ESR	Electron Spin Resonance
GC-MS	Gas Chromatography-Mass Spectroscopy
KBr	Potassium bromide
Cu K- α	Copper K-Alpha
UV	Ultraviolet



CHAPTER 1

INTRODUCTION

1.1 Background of Study

A large number of researches had been done on the field of nanotechnology. They have diverse applications in medicine, biology, physics, chemistry, and material sciences (Chaudhuri & Malodia, 2017). Nanoparticles define as particles having at least one dimension with the size range of 1 nm to 100 nm (Boholm & Arvidsson, 2016). High surface area and nanoscale size increased their exposure as part of daily life. They were used as drug delivery, cosmetics, food packaging, therapeutics, and to name a few (Gatoo *et al.*, 2014).

Zinc oxide, an inorganic compound, is said to be one of the most exploited metal oxides. It was explored extensively by researchers due to simplicity, rapidity, and unique properties (Pholnak *et al.*, 2011). They are found in white powder form and insoluble in water (Chaudhuri & Malodia, 2017). In the biological field, zinc oxide nanoparticles (ZnONPs) are applied as drug delivery, nanomedicine, biological sensing, and biological labelling. They have antibacterial, antifungal, antidiabetic, and variety potential (Bala *et al.*, 2015).

Fascinating researches had done to synthesize nanoparticles in the most simply and cost-effectively way. It can be synthesized in conventional and non-conventional methods with the theory of top-down and bottom-up. In these decades, green synthesis is desirable compared to chemical and physical synthesis. Green synthesis provides high efficiency, ecologically, and reproducible (Jin & Jin, 2019). In green synthesis, the production of ZnONPs is accomplished with plant extracts or living organisms, such as algae and fungi (Zelechowska, 2014). During the synthesis process, plant extract or biological agents act as reducing agents.

Medicinal plants are frequently used as antimicrobial and antifungal agents. They are introduced to replace the usage of antibiotics that may bring adverse effects to the environment. Oil palm leaf was chosen as one of the green products in this research. They pose high antimicrobial, anticancer, and antifungal potential (Rajoo *et al.*, 2013). Malaysia is the second-largest producer of palm oil in the world. The crop is nominated as "golden crop" in Malaysia (Pohl & Loong, 2016). However, the biowaste of the oil palm tree which include leaves were left aside without further management (Nordin *et al.*, 2016). In this research, oil palm leaf was used to synthesize ZnONPs with a green method. From the findings, a new achievement was successfully approached.

Besides, roselle flower, also known as *Hibiscus sabdariffa* Linn, is a common bright red shrub. It is facile to find worldwide. Roselle flowers contain high phenolics, flavonoids, and anthocyanidins (Villasante *et al.*, 2019). Riching of active compounds made them used in various sectors. It can be a source of natural colouring, antimicrobial, antifungal, antidiabetic and anti-cholesterol agents (Bariyyah *et al.*, 2019). They can inhibit Gram-negative and Gram-positive bacteria (Borrás-Linares *et al.*, 2015).

The aims of this study were synthesizing and characterizing ZnONPs with and without plant extracts. Besides, the potentials of ZnONPs photocatalytic, antibacterial and antioxidants were tested. Their acute toxicity had also been investigated to ensure the safety of use.

1.2 Problem Statement

Bacteria is abundant in nature. Harmless bacteria colonized on human skin. However, overgrowth of these bacteria may risk human health. For instance, *Staphylococcus aureus* will cause skin disease and infections (Hay & Morris - Jones, 2016). Besides, living organisms like *Escherichia coli*, *Aspergillus*, and *fusarium* will lead to water contamination (Graça *et al.*, 2016; Richardson & Rautemaa-Richardson, 2019). These phenomena bring rapid and large fluctuations to the environment and health effects. In this case, these culprits frequently fight by antibiotics. However, extensively used of antibiotics is risky to human health. It may enhance the selective pressure on infectious bacteria. It will also have adverse effects on natural bacterial diversity (Bighiu *et al.*, 2019).

Methyl orange (MO) dye, $C_{14}H_{14}N_3NaO_3S$, is an orange colour synthetic dye used in various textile industries, leather industries, and paper printing. Nevertheless, MO has adverse effects on the environment and human body (Bhatia & Verma, 2017). Methylene blue (MB), $C_{16}H_{18}ClN_3S$, is a blue colour basic synthetic dye that is extensively utilized in medical treatment. Principally, MB is used to eliminate malaria due to its attractive features (Weitzel *et al.*, 2017). Indeed, the toxicity of MB is low. However, the complex aromatic in dye provides stability against biodegradation in aquatic ecosystem. The sunlight will be obstructed and not manage to penetrate the water. This circumstance will retard the growth of aquatic living (Cengiz & Cavas, 2008). To date, various nanoparticles developed as photocatalyst in photocatalytic activity.

In this case, a shortage of researches looks over the impact and the risk of nanoparticles, especially towards aquatic systems. Most of the materials will release into marine systems. This action will indirectly cause the aquatic system as inevitable receptacles (Rekulapally *et al.*, 2019). One of the efficient and straightforward acute toxicity tests of nanoparticles is using brine shrimp,

Artemia species. This assay was implemented by estimating the mortality and hatching rate of brine shrimps.

Most of the common physical and chemical methods implemented to synthesize metal oxide are complicated. In chemical synthesis, toxic and expensive chemical might be involved during the synthesis process. The produced ZnONPs by this non-environmentally friendly path may lead to pollution and detrimental to human being health. Moreover, most of the physical synthesis of metal oxide nanoparticles requires higher energy compared to chemical and biological methods. These include higher-energy ball milling and calcination at high temperature. The steps involve are not energy-saving and are relatively more expensive compare to green synthesis. Therefore, numerous researches undergo producing metal oxide nanoparticles, which is environmentally friendly.

1.3 Significant of Research

A significant way to produce ZnONPs with plant extract with a shorter time and simpler way had been introduced. Environmentally benign materials and renewable of reaction materials had been implemented. The process requires low cost to produce small size with high purity nanoparticles within short reaction times. Besides, synthesized ZnONPs may develop for pharmaceutical uses and wastewater treatment.

1.4 Objectives

The objectives of this research are:

1. To synthesize ZnONPs with and without plant extracts by co-precipitation method.
2. To study the morphological, optical and structural properties of synthesized ZnONPs.
3. To elucidate the potential of synthesized ZnONPs in antioxidant, antibacterial and photocatalytic activity.
4. To evaluate the toxicity of synthesized ZnONPs using brine shrimp (*Artemia* sp) lethality and hatching assay.

1.5 Structure of the Thesis

Chapter 1 gives a brief introduction to the research, significance and structure of thesis study. In **Chapter 2**, reviews of properties of zinc oxide nanoparticles (ZnONPs), the way to synthesize the metal oxide and detailed information on the plants used in the research were presented. This chapter discusses the advancing activities of the synthesized ZnONPs to show their potential on antioxidant, photocatalytic, antibacterial and antifungal.

Chapter 3 presents the materials, analytical instruments used and the experimental procedures during the research. **Chapter 4** discusses the outcome from each of the analytical characterizations and exhibits the potential of ZnONPs in antioxidant and photocatalytic activity. In the final chapter, **Chapter 5** encapsulates the findings of the whole thesis.

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