SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING GALLIC ACID: CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY

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by

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LIST OF SYMBOLS

A_c	Absorbance of the control
A_s	Absorbance of the samples
℃	Degree Celsius
E_{gb}	Bulk band gap
E_{gn}	Band gap at a strong absorption edge
eV	Electron volts
g	Gram
h	Planck's constant
hr	Hour
keV	Kiloelectron volts
kV	Kilovolt
M	Molarity
m^*	Effective mass of the specimen
meV	Milli electron volts
min	Minute
R	Radius
rpm	Revolutions per minute
μ	Micro
V	Volume
W	Watt
%	Percentage

LIST OF ABBREVIATIONS

AA	Ascorbic acid
AA: Zn	Ratio of ascorbic acid to zinc precursor
Ag	Silver
AgNPs	Silver nanoparticles
ABTS	2,2’-azino-bis-3-ethylbenzthiazoline-6 sulphonic acid
BT	Black tea
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDX	Energy dispersive X-Ray
FTIR	Fourier transform infrared spectroscopy
GA	Gallic acid
GA: Zn	Ratio of gallic acid to zinc precursor
HO^-	Hydroxyl group
IC50	Half-maximal inhibitory concentration
KBr	Potassium bromide
LE	Leaf extract
NaOH	Sodium hydroxide
NPs	Nanoparticles
O	Oxygen element
OFAT	One factor at a time
PVP	Polyvinyl pyrrolidone
PVA	Polyvinyl alcohol
PGE	Polyethylene glycol
SEM	Scanning electron microscopy
SDG	Sustainability development goal
UV-Vis	Ultraviolet-Visible
Wt.%	Weight percent
Zn	Zinc
Zn^{2+}	Zinc cations
ZnO	Zinc oxide
ZnO NPs	Zinc oxide nanoparticles
$Zn(OH)_2$	Zinc hydroxide

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SINTESIS ZINK OKSIDA NANOPARTKEL MENGGUNAKAN ASID GALLIK: PENCIRIAN DAN PENILAIAN AKTIVITI ANTIOKSIDAN

ABSTRAK

Nanopartikel zink okida (ZnO) digunakan secara meluas dalam industri bioperubatan kerana sifat antioksidan, anti-kanser, anti-radang, antibakteria dan antikulatnya. Sintesis nanopartikel ZnO melalui kaedah sintesis hijau telah menjadi tumpuan penyelidik sejak beberapa tahun lalu kerana ia mesra alam sekitar, kurang berbahaya dan tidak toksik. Dalam kajian ini, sintesis nanopartikel ZnO melalui kaedah sintesis hijau kimia dengan menggunakan teknik pemendakan kimia telah dilakukan. Prekursor zink asetat dihidrat dan asid gallik sebagai agen pengurangan dan penyekat telah digunakan. Kesan mempelbagaikan pH larutan dari 7 hingga 11 telah dikaji untuk mensintesis nanopartikel ZnO. Selain itu, nisbah molar asid gallik kepada zink asetat dihidrat juga dikaji dengan menukar daripada 1.0% hingga 4.0% semasa proses sintesis. Nanopartikel ZnO yang disintesis dicirikan di bawah teknik yang berbeza termasuk spektrometer utlralembayung/nampak (UV-Vis), sinaran inframerah jelmaan fourier (FT-IR), sinar-X penyerakan electron (EDX) dan pengimbasan mikroskop electron (SEM). Morfologi dan saiz partikel ditentukan menggunakan SEM di bawah kedua-dua keadaan sintesis. Saiz partikel dianggarkan pada 269.6 ± 58.23 nm, 234.91 ± 7.51 nm dan 118.76 ± 1.27 nm masing-masing untuk pH 9, 10 dan 11. Keputusan menggambarkan bahawa sintesis di bawah pH 7 dan 8 membawa kepada penggumpalan. Selain itu, saiz partikel dianggarkan pada 118.76 ± 1.27 nm, 201.85 ± 16.06 nm, 198.49 ± 15.25 nm, 174.86 ± 6.93 nm dan 99.70 ± 2.78 nm masing-masing untuk pelbagai nisbah molar asid gallik terhapap zink prekursor 1.0, 1.5 3.0, 3.5 dan 4.0%. Keputusan juga mendedahkan bahawa nisbah molar yang berbeza-beza telah mempengaruhi pelbagai jenis bentuk nanopartikel ZnO. Akhirnya, optimum daripada kedua-dua keadaan sintesis (pH 11 dan GA: Zn sebanyak 4.0%) digunakan untuk mengkaji aktiviti antioksidan. Nanopartikel ZnO mempamerkan aktiviti antioksidan yang baik, dengan nilai IC50 masing-masing 28.88 μg/ml dan 25.40 μg/ml untuk kaedah DPPH dan ABTS. Ini mencadangkan bahawa nanopartikel ZnO boleh mempunyai potensi kegunaan dalam bidang bioperubatan dan bertindak sebagai elisitor abiotik untuk meningkatkan biosintesis metabolit sekunder dalam kultur sel tumbuhan.

SYNTHESIS OF ZINC OXIDE NANOPARTICLES USING GALLIC ACID: CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT ACTIVITY

ABSTRACT

Zinc oxide (ZnO) nanoparticles are widely used in biomedical industries due to their antioxidant, anti-cancer, anti-inflammatory, antibacterial and antifungal properties. The synthesis of ZnO nanoparticles via the green synthesis pathway has become the focus of the researcher over the past years due to its being environmentally benign, less harmful and non-toxic. In this present study, the synthesis of ZnO nanoparticles via a chemically green synthesis pathway using the chemical precipitation technique was done. A precursor of zinc acetate dihydrate and gallic acid as a reducing and capping agent was used. The effect of varying the pH of solutions from 7 to 11 was studied to synthesize ZnO nanoparticles. Besides, the molar ratios of gallic acid to zinc acetate dihydrate were also studied by changing from 1.0% to 4.0% during the synthesis process. The synthesized ZnO nanoparticles were characterized under different techniques, including Ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), Energy-dispersive X-ray spectroscopy (EDX) and Scanning electron microscope (SEM). The morphology and particle size were observed using SEM under both synthesis conditions. The particle sizes were estimated at 269.65 $\pm$ 8.23 nm, 234.91 $\pm$ 7.51 nm and 118.76 $\pm$ 1.27 nm for pH 9, 10 and 11, respectively. The results illustrated that the synthesis under pH 7 and 8 led to agglomeration. Moreover, the particle sizes were estimated at 118.76 $\pm$ 1.27 nm, 201.85 $\pm$ 16.06 nm, 198.49 $\pm$ 15.25 nm, 174.86 $\pm$ 6.93 nm, and 99.70 $\pm$ 2.78 nm for various molar ratio gallic acid to zinc precursor 1.0, 1.5 3.0, 3.5 and 4.0%, respectively. The results also revealed that varying molar ratios had influenced the different types of shapes of ZnO nanoparticles. Finally, the optimum from both synthesis conditions (pH 11 and GA: Zn of 4.0%) was used to study antioxidant activity. The ZnO nanoparticles exhibited good antioxidant activity, with IC50 values of 28.88 μg/ml and 25.40 μg/ml for DPPH and ABTS, respectively. This suggested that ZnO nanoparticles could have potential uses in biomedical fields and act as an abiotic elicitor to enhance the biosynthesis of secondary metabolites in "in vitro" plant cell cultures.

CHAPTER 1

INTRODUCTION

Chapter 1 introduces the overview of the research background and significance of synthesizing ZnO nanoparticles, especially in industrial applications. Besides, a brief introduction to the synthesis methods and their respective advantages and drawbacks were summarized. The problem statement and the objectives of this present study also were presented.

1.1 Research Background

The area of nanotechnology has been extensively explored throughout the last century and numerous developments have been made to synthesize various materials on a nanoscale. The size of nanoparticles (NPs) is in the range of 1 to 100 nm (Thakkar et al., 2010). The morphology and the size of nanomaterials are highly dependent on the preparation methods and synthesis parameters. The nanoscale size such as nanoparticles have very high surface to volume and aspects ratio which make them ideal for use in various materials (Rane et al., 2018). The unique properties such as high electrical properties, high mechanical and thermal stability, high surface area and high optical and magnetic properties make nanomaterials can be used in various applications (Yaqoob et al., 2020). Some of the nanomaterials especially metal oxide (e.g., titanium oxide, silver oxide, copper oxide and zinc oxide) nanoparticles have been widely used in pharmaceutical and biomedical fields (Yaqoob et al., 2020).

1.2 Zinc Oxide Nanoparticles

Zinc oxide (ZnO) nanoparticles are the most relevant metal oxides nanoparticles due to their low cost, lox toxicity and outstanding biomedical applications such as good properties in anticancer, drug delivery, antibacterial, and anti-inflammation, antioxidant and bioimaging (Jiang et al., 2018). Besides, ZnO nanoparticles are known as semiconductors because of their distinctive physical features, including a large energy band gap (3.3 eV) at room temperature, high excitonic binding energy (60 meV) and natural n-type electrical conductivity (Wang, 2004; Wellings et al., 2008). This makes ZnO nanoparticles widely used in the semiconductor and electronics industries.

Recently, ZnO nanoparticles have been widely investigated in the field of agricultural, biotechnological and plant sciences. For instance, ZnO nanoparticles act as an abiotic elicitor to enhance the yield of bacoside A component in *Bacopa monnieri* (Bhardwaj et al. 2018) and enhance the secondary metabolite in "in vitro" plant cultures such as *Trigonella foenum-graecum L., Linum usitatissimum*, *Capsicum annuum*, and *Luffa acutangular* (Abbasi et al., 2019a; Asgari-Targhi et al., 2021a; Tariverdizadeh et al., 2021a; Tanveer et al., 2022). Figure 1.1 summarizes the application of zinc oxide nanoparticles in several industries.

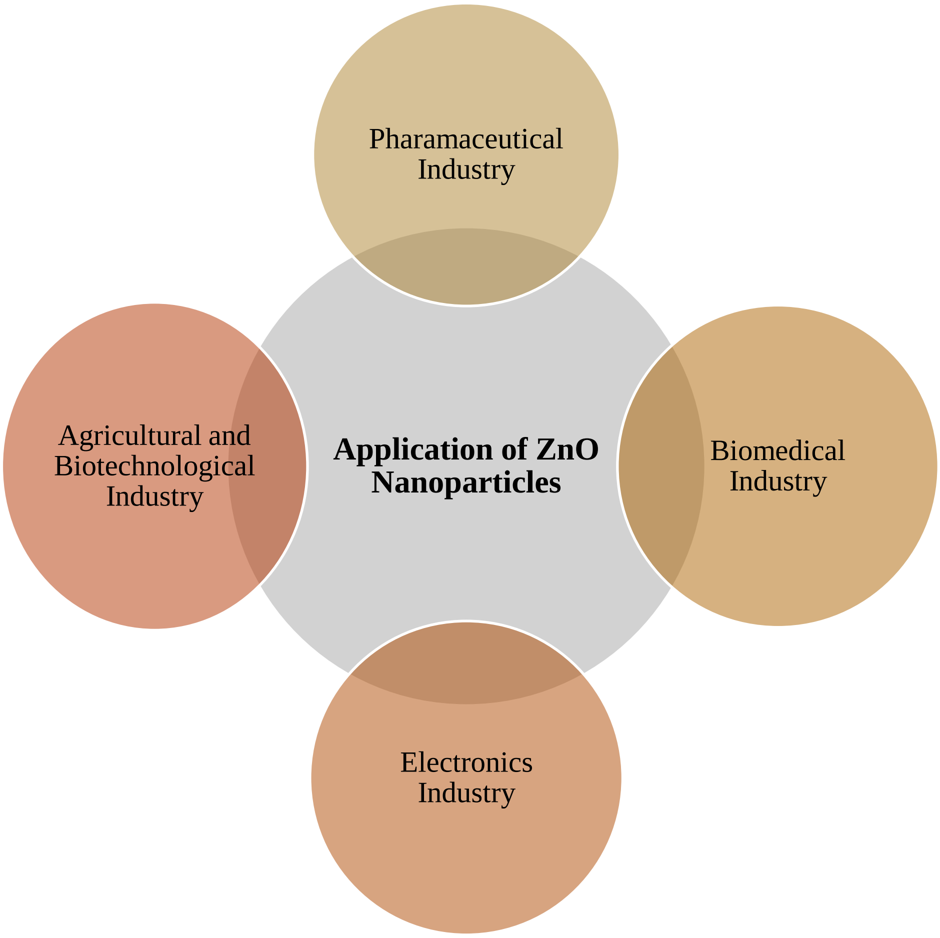


Figure 1.1 Application of ZnO nanoparticles in various industries (Wang, 2004; Wellings et al., 2008; Bhardwaj et al., 2018; Jiang et al., 2018).

1.3 Synthesis of Zinc Oxide Nanoparticles

Various studies have been conducted in recent years to promote biological synthesis methods for the production of ZnO nanoparticles since they are environmentally benign, non-toxic, and less harmful (Jeyabharathi et al., 2022). However, biological synthesis is still fundamentally reliant on chemical processes. The term “biological method” is due to the reducing agent and capping agent extracted from waste materials, plants, and microorganisms, which then was used to synthesize ZnO nanoparticles. Previous studies by Dulta et al., (2022a); Jeyabharathi et al., (2022); Rafique et al., (2022) utilized plants such as *Wattakaka volubilis* leaf, *Syzygium cumini* leaf and *Carica papaya* leaf, which were then extracted and afterwards used in the synthesis of ZnO nanoparticles using precipitation procedure. The chemical method can be further divided into precipitation, sol-gel, hydrothermal and microemulsion. Each technique has its advantages and disadvantages, as shown in Table 1.1.

Table 1.1 Classification of chemical synthesis methods with their respective advantages and drawbacks (Rane et al., 2018).

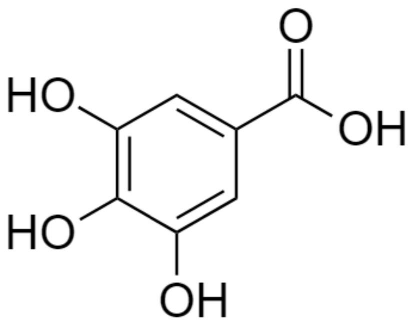
Chemical method	**Advantages**	**Drawbacks**
Precipitation	• Simple and rapid preparation • Easy to control particle size and shape • Low temperature and energy-efficient process • Does not use any hazardous organic solvents	• Trace impurities might precipitate as well • Time consuming
Sol-gel	• High product purity • Easy to prepare in various sizes and shapes • Low temperature synthesis condition	• Longer reaction time • Use of organic solvents that ate detrimental to the environment
Hydrothermal	• Size, shape distribution and crystallinity are easily controlled • Heating and pressurizing at its critical point makes any material can be soluble in a solvent	• Autoclaves are expensive • Concerns about safety during the reaction process • Unable to observe the reaction process
Microemulsion	• Easy to prepare • Particle in nanometer-	• Require a significant amount of surfactant

	sized with less agglomerated • Crystalline nanoparticles with a high specific area	• High melting point compounds have a limited solubilizing capacity

1.4 Gallic Acid

Gallic acid (3,4,5-Trihydroxybenzoic acid) is a natural phenolic compound that can be found in various natural sources, including plants (e.g., *Boswellia dalzielii*), fresh fruits (e.g., strawberry, grape, banana, apple, avocado, mulberry and green tea) and vegetables (Badhani et al., 2015; Panoff, 2020). Table 1.2 provides information on the molecular structure, molecular formula, molecular weight and chemical properties of gallic acid.

Table 1.2 Chemical information and properties of gallic acid (National Center for Biotechnology Information, 2022).

Compound	**Gallic Acid**
Molecular formula	$C_7H_6O_5$
Molecular structure	
Molecular weight (g/mol)	170.12
Water solubility (mg/L)	1.19×10^4
Relative density (water = 1.0)	1.7
Dissociation constants	pKa_1 = 4.39, pKa_2 = 8.50, pKa_3 = 10.38, pKa_4 = 13

Formerly, gallic acid has been used as a reducing agent and capping agent for producing silver nanoparticles (AgNPs) because of its capability to dissolve in water, contain more than two hydroxyl groups to reduce Ag ions to Ag(0) and control the size of particles (Ahani and Khatibzadeh, 2022). Besides, Pauzi et al., (2018) have revealed that gallic acid can be used as a reducing agent and stabilizing agent to synthesize ZnO nanoparticles via microwave irradiation. It also showed that the synthesized ZnO nanoparticles using gallic acid have good antibacterial activity on *Bacillus subtillis* and *Escherichia coli*. Moreover, Lee et al., (2017) reported that ZnO NPs conjugated with gallic acid (GA) were produced using a coating method that exhibits good antioxidant activity via ABTS decolorization assay. The latest study by Yadav et al., (2021a) showed that adding ZnO nanoparticle-loaded gallic acid into chitosan films improved the mechanical and physical properties and also had antibacterial and antioxidant activity, suggesting it could be used as active food packaging.

1.5 Problem Statement

Recently, the green biological synthesis of ZnO nanoparticles from waste materials, plants extract, and microorganism have received a lot of interest since it is less hazardous, non-toxic, uses less energy and produces environmentally friendly product and by-products. Despite its positive impact on the environment and fulfilled green chemistry principle, there are several drawbacks, such as the uncontrollable size and shape of synthesized ZnO nanoparticles, as well as the difficulty of scaling up to be used in large-scale production (Seabra and Durán, 2015). Therefore, chemically green synthesis of ZnO nanoparticles can be applied to solve the addressed issues from the biological synthesis method.

Few studies have proved that ZnO nanoparticles can be synthesized and fabricated using gallic acid, where gallic acid acts as a reducing agent and capping agent for the synthesis process (Lee et al., 2017; Pauzi et al., 2018; Yadav et al., 2021a). Until now, the synthesis of ZnO nanoparticles using gallic acid via the chemical precipitation method has not been reported. Some methods, such as microwave irradiation and the hydrothermal method, were used, but the process required expensive equipment and was unsuitable for reaction monitoring (Rane et al., 2018). Therefore, it is advantageous to complete the synthesis method via the chemical precipitation method and resolve the sophisticated method of obtaining ZnO nanoparticles.

Besides, previous studies that have been reported provide roughly the method to synthesize ZnO nanoparticles using gallic acid, antioxidant activity, and antibacterial activity. In other words, there is no in-depth study about the synthesis parameters that will affect the morphology and particle size of synthesized ZnO nanoparticles using gallic acid. Therefore, in this present study, ZnO nanoparticles were synthesized using a chemically green synthesis method in a one-step and rapid process using gallic acid as both reducing and capping agent without using any other chemicals and examined the effect of pH value and gallic acid concentration on the prepared ZnO nanoparticles. Subsequently, the average particle size and morphology of ZnO nanoparticles were also determined. Using this approach, we can obtain ZnO nanoparticles of various sizes by adjusting the synthesis parameters (i.e., pH and GA: Zn molar ratio). To meet the requirements as an abiotic elicitor, antioxidant activity evaluation of the optimally synthesized ZnO nanoparticles is required; thus, the radical scavenging activity and the half-maximal inhibitory concentration (IC50) of ZnO nanoparticles are determined.

1.6 Research Objectives

The main objectives of this research are:

1. To synthesizes and characterize zinc oxide nanoparticles by using chemically green synthesis methods.
2. To investigate the morphology and size of zinc oxide nanoparticles under various pH of solution and molar ratio of GA: Zn.
3. To evaluate the antioxidant activity of the synthesized zinc oxide nanoparticles using DPPH and ABTS methods.

1.7 Sustainability Element in Research Work

The United Nations developed 17 Sustainable Development Goals (SDGs) in 2015, intending to end poverty, protect the planet and ensure people can enjoy peace and prosperity by 2030. Furthermore, the SDGs serve as a guide for achieving a balance between social, economic, and environmental sustainability through supporting health and education, eliminating discrimination, fostering economic growth, battling climate change, and working to preserve our forests and oceans. The 17 goals are (United Nations, 2022):

- SDG 1: No Poverty
- SDG 2: Zero Hunger
- SDG 3: Good Health and Well-Being
- SDG 4: Quality Education
- SDG 5: Gender Equality
- SDG 6: Clean Water and Sanitation
- SDG 7: Affordable and Clean Energy
- SDG 8: Decent Work and Economic Growth

- SDG 9: Industry, Innovation and Infrastructure
- SDG 10: Reduced Inequalities
- SDG 11: Sustainable Cities and Communities
- SDG 12: Responsible Consumption and Production
- SDG 13: Climate Action
- SDG14: Life below Water
- SDG15: Life on Land
- SDG16: Peace, Justice and Strong Institutions
- SDG17: Partnerships for The Goals

In this study, some sustainable goals were implemented and achieved. To start, SDG 12, which is "Responsible Consumption and Production," was considered in this research. SDG 12 relates to using products that meet basic needs and improve quality of life while minimizing the use of natural resources and harmful materials and waste and pollutant emissions during the product's life cycle (United Nations, 2022). For instance, the synthesis of ZnO nanoparticles was carried out without using harmful organic solvents. A green solvent (water) was utilized, making the process non-toxic, non-harmful, and environmentally friendly. Thus, this study is aligned with SDG 12.

Moreover, SDG 3 aims to prevent unnecessary suffering from preventable diseases and untimely mortality by focusing on critical targets that improve the overall health of a nation and its people (United Nations, 2022). ZnO nanoparticles can be used in pharmaceutical and biomedical industries due to their antioxidant, anticancer, anti-inflammatory, and antibacterial characteristics. Incorporating ZnO nanoparticles into the medical field can help minimize diseases and improve human health. This suggested that SDG 3, "Excellent Health and Well-Being," is in line with the research work.

Furthermore, ZnO nanoparticles can be utilized to make sunscreen and food packaging, which will help expand the global production of sunscreen and food packaging. Also, ZnO nanoparticles are unlikely to cause harmful effects in humans following dermal application. Thus, the country's economy will be boosted without affecting the environment or people adversely, which is aligned with SDG 8. In conclusion, the research work can achieve some of the critical aims of sustainable development goals, such as responsible consumption and production, good health and well-being, and decent work and economic growth.

CHAPTER 2

LITERATURE REVIEW

Chapter 2 presents the literature review on previous studies on the chemically green synthesis of ZnO nanoparticles. Besides, the effect of pH and concentration of reducing and capping agents for synthesising ZnO nanoparticles was also included. The antioxidant activity evaluation method was thoroughly discussed and the summary of overall findings was presented at the end of the chapter.

2.1 Chemically Green Synthesis ZnO Nanoparticles

The terms “chemically” and “green synthesis” refer to the manufacture of ZnO nanoparticles using a chemical method and the use of non-hazardous reducing and capping agents and environmentally friendly solvents, respectively (Pauzi et al., 2018). Figure 2.1 shows the synthetic phenolic, β-carotene and ascorbic acid compounds derived from natural resources (i.e., plants and fruits), which are utilized as reducing and capping agents in the chemically green synthesis of nanoparticles. The phenolic compounds are classified into polyphenols, phenolic acids, and miscellaneous. Polyphenols can be classified based on phenol rings which include tannins and flavonoids (e.g., quercetin). Tannins are divided into hydrolyzable (e.g., gallic acid) and non-hydrolyzable (e.g., catechin). Phenolic acids contain at least one aromatic ring, where a hydroxyl group substitutes at least one hydrogen. They consist of two groups, hydroxycinnamic acids and hydroxybenzoic acids, derived from caffeic acid and hydroxybenzoic acid, respectively. Miscellaneous contains two or more aromatic rings that are fused. Coumarin is the only type under miscellaneous, which is derived from scopoletin. Other components that are found and can be extracted from natural sources are carotenoids and ascorbic acid.

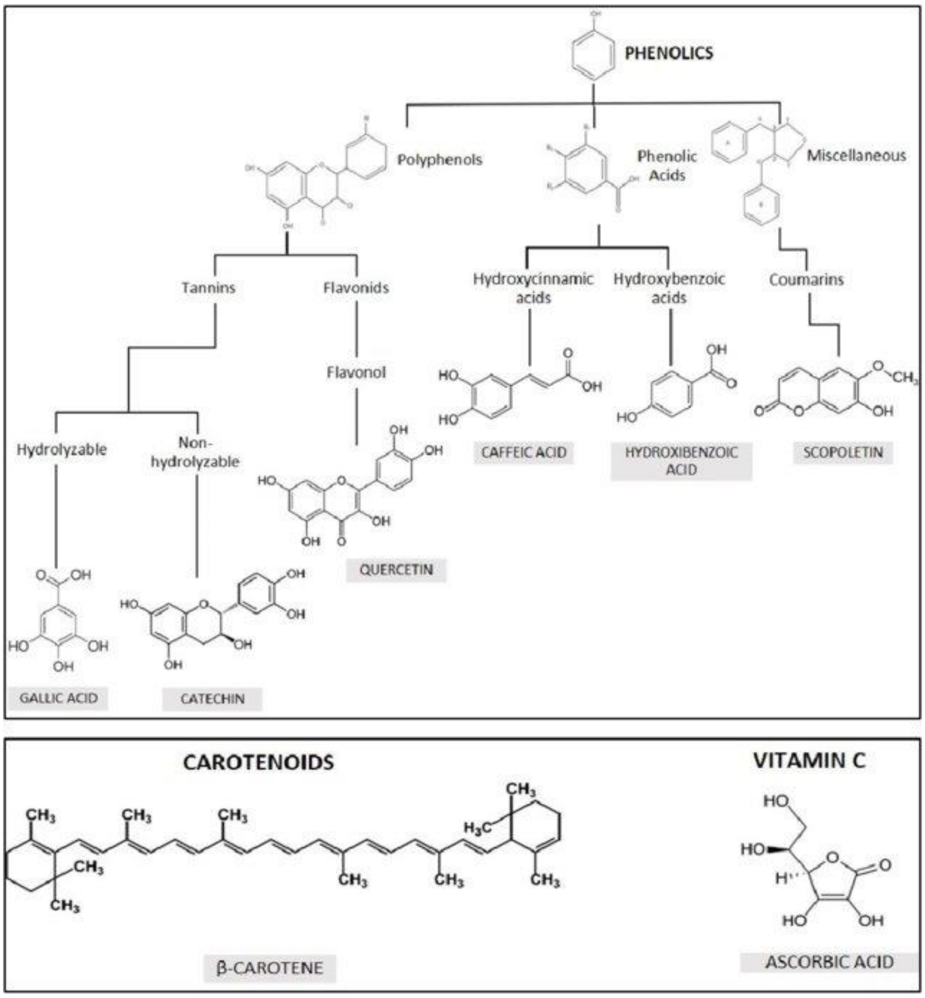


Figure 2.1 Natural resources of plants used as reducing and capping agents (Al-Zahrani et al., 2021).

Table 1.1 summarizes the chemically green synthesis of ZnO nanoparticles using different synthetic reducing and capping agents and synthesis methods by various researchers. Kamaruzaman and Lah, (2021) utilized tannic acid as a capping agent to synthesize ZnO nanoparticles. A hydrothermal process was implemented, and trisodium citrate was used as a stabilizing agent during the synthesis. The average diameter of particles was around 26 nm to 34 nm with a nano-spherical morphology. Tannic acid and trisodium citrate work together to control the synthesis process's nucleation, growth, and stabilization (Ranoszek-Soliwoda et al., 2017).

Jeyaleela et al., (2020) conducted research using quercetin as a reducing and capping agent to synthesize ZnO nanoparticles by homogenous precipitation. The reaction was carried out at 60 ℃ under stirring conditions for 7 hours. Then, it was centrifuged and dried to obtain quercetin mediated ZnO nanoparticles. From scanning electron microscopy (SEM), the ZnO nanoparticles were fiber shaped with an average particle size of 31.24 nm.

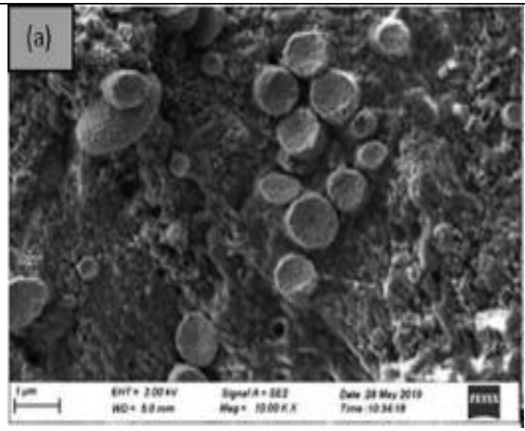
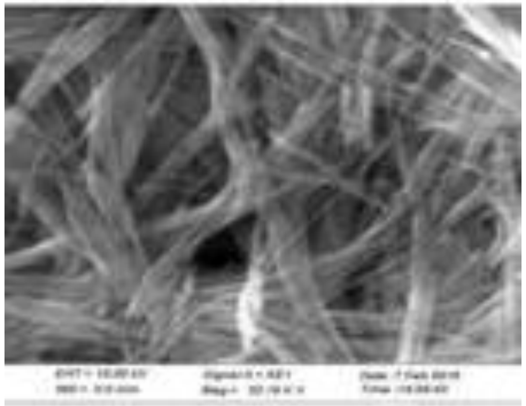
Pauzi et al., (2018) implemented ascorbic acid and gallic acid as reducing and capping agents to synthesize ZnO nanoparticles. The microwave irradiation technique was implemented with 400 W and 800 W power. The Ultraviolet-Visible (UV-Vis) confirmed the formation of ZnO nanoparticles using ascorbic acid and gallic acid with an absorption peak range of 321-381 nm. The average particle size was 6.03 nm and 11.16 nm for ZnO nanoparticles synthesized using gallic acid and ascorbic acid, respectively. The particle size was estimated using the hyperbolic band model described in Equation (2.1).

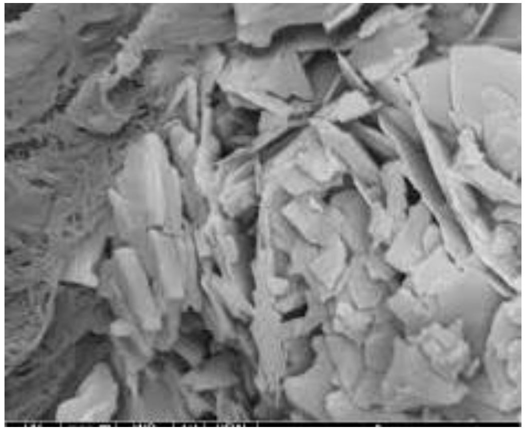
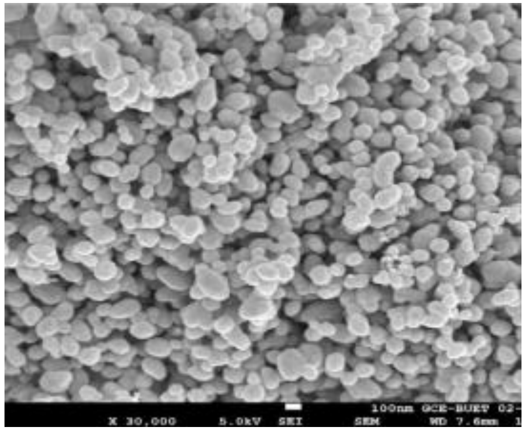
$$R = \sqrt{\frac{2\pi^2 \times h^2 \times E_{gb}}{m^* \times \left({E_{gn}}^2 - {E_{gb}}^2\right)}} \tag{2.1}$$

Whereby the R is radius, $m^*$is the effective mass of the specimen (29.15×10^{-13}kg for ZnO NPs), E_{gb} is the bulk band gap, h is Planck's constant (6.6261×10^{-34} J s) and E_{gn} is the band gap at a strong absorption edge.

Following that, Ahammed et al., (2020) used ascorbic acid as a reducing agent and additional polyvinyl alcohol (PVA) as a capping agent to synthesize ZnO nanoparticles via the microwave irradiation method. The synthesis was carried out at 400 W for 3 minutes using a microwave oven after adding zinc precursor, ascorbic acid, and PVA. The ZnO nanoparticles were spherical through SEM images, and the average particle size was obtained at 70 nm to 90 nm.

Table 2.1 Different synthetic reducing and capping agents are used to produce ZnO nanoparticles with varying morphologies and sizes.

Method	Reducing and capping agents	Synthesis condition	Average size (nm)	Shape	Images of ZnO NPs	Reference
Hydrothermal	Tannic acid	70 ℃ for 1 hr 30 mins	26-34	Nano-sphere		(Kamaruzaman and Lah, 2021)
Precipitation	Quercetin	60 ℃ for 7 hr	31.24	Fiber		(Jeyaleela et al., 2020)

Microwave irradiation	Hydroxybenzoic acid	600 ℃ for 2 hr	30	Large to medium block slices		(Alhadhrami, 2018)
Microwave irradiation	Ascorbic acid and gallic acid	400 W and 800 W for 4 mins			N/A	(Pauzi et al., 2018)
Microwave irradiation	Ascorbic acid	400 W for 3 mins	70-90	Spherical		(Ahammed et al., 2020)

2.2 Factor Affecting Synthesis ZnO Nanoparticles

There are various factors affect the size and morphology of the synthesis of ZnO nanoparticles. These include process parameters such as pH, reaction temperature and time, zinc precursor concentration, concentration of reducing and capping agents, and calcination temperature. The effect of pH and concentration of reducing and capping agents were reviewed in the following subchapters. Most of the reviews are synthesized either from synthetic reducing and capping agents or directly from plant extracts containing abundant phenolic compounds.

2.2.1 Effect of pH

The pH of the solution influenced the size, morphology, and texture of the synthesized ZnO nanoparticles. The optimum pH can be acidic ($pH < 7$), neutral ($pH = 7$) and alkaline ($pH > 7$). Alias et al., (2010) reported that ZnO nanoparticle sizes were 36.65 – 49.98 nm when synthesized under various pH ranges from 6 to 11. The study also revealed that ZnO is highly influenced on the pH as in acidic medium and neutral medium the synthesized ZnO nanoparticles were highly agglomerated while under basic medium the ZnO nanoparticles were found to be less agglomerated and reduced particle size with the optimum pH of 11. Therefore, the ideal pH must be determined to synthesize the smallest ZnO nanoparticles.

Several reported studies on the effect of pH on the synthesis of ZnO nanoparticles are thoroughly discussed in this subchapter. The summary of the effect of pH from different studies mainly using synthetic and directly natural resources reducing and capping agents is tabulated in Table 2.1. Mohammadi and Ghasemi, (2018) studied the effect of pH on the synthesis of ZnO nanoparticles using cherry extract. The pH range in the studies is 4, 6, 7, 8, and 10. In the study, pH 8 was the optimum and a smaller size was expected, while increasing pH 8 to 10 is expected to produce a large particle size due to the red shift of absorption band occurring from pH 8 to 10. As observed from scanning electron microscopy (SEM), the synthesized ZnO nanoparticles were hexagonal.

Kamaruzaman and Lah, (2021) reported that pH influenced the synthesized ZnO nanoparticles using tannic acid. The low pH was used in this study, ranging from 3 to 5. The suspension colour was changed at different pH; deep dark brown, pale dark brown, and light brown were observed at pH 3, 4, and 5, respectively. However, due to low pH synthesis conditions, the formation of ZnO complexes is not achieved and suggested to increase the pH by more than 7. The synthesized ZnO nanoparticles had a polygonal structure ranging from 18 to 23 nm at low pH levels.

Cho et al., (2010) reported that ZnO nanoparticles synthesized using ascorbic acid had a spherical shape in acidic conditions and a flower-like shape in an alkaline medium. The study showed that the different of pH influenced the morphology of ZnO nanoparticles. Similar to Doan Thi et al., (2020) work, the synthesis of ZnO nanoparticles using orange peel extract under different pH values ranging from 4 to 10 influenced the morphologies of ZnO nanoparticles. The spherical-like shape was observed at pH 4 and 6 and increasing the pH from 8 to 10 caused the particle to coagulate into large blocks. The optimum synthesis of ZnO nanoparticles was at acidic condition (pH 4) and it differs from the study by Mohammadi and Ghasemi, (2018) which the optimum pH is basic condition (pH 8).

Abdol Aziz et al., (2019) studied the pH effect of synthesizing ZnO nanoparticles using banana peel extract. The pH range in the studies is 8, 9, 10, 11, and 12. Based on the UV-Vis spectra, the absorption peak was increased as the pH increased from pH 8 to 12. The optimum pH to synthesize ZnO nanoparticles using banana peel extract is 12. The average particle size reported at pH 12 was the smallest, 53.15 nm.

Sarillana et al., (2021) reported that the synthesized ZnO nanoparticles using *Theobroma cacao* L. pod husks have a red-shifted absorption band from pH 7 to 9. Thus, under high pH levels, the phenolics compound was unstable, which caused the particles to be aggregate and produced large particles size (Friedman and Jürgens, 2000). A study on the effect of pH (5 and 8) on the synthesis of ZnO nanoparticles using *Salvadora oleoides* leaf extract was done by Padalia et al., (2017). One significant finding in this research is the formation of ZnO nanoparticles in spherical shape at pH 5 with sizes ranging from 20 to 56 nm and irregular shape ZnO nanoparticles at pH 8 ranging from 15 to 80 nm.

Table 2.2 Effect of pH on the synthesized ZnO nanoparticles using synthetic or natural resources reducing and capping agents.

Methods	**Zinc precursor**	**Reducing and capping agents (from synthetic or natural resources)**	**pH studied**	**Research findings**	**Reference**
Green route	Zinc nitrate hexahydrate	Cherry fruit extract (rich source of phenolic compounds)	4, 6, 7, 8 and 10	• pH effects the electrical charges of biomolecules which alter the capacity of reducing and capping • pH influences the growth of ZnO nanoparticles • The optimal pH was 8, due to fastest rate of reduction when compared to other pH levels	(Mohammadi and Ghasemi, 2018)
Sol-gel	Zinc acetate dihydrate	Tannic acid	3, 5 and 7	• pH influences the size and morphology of ZnO nanoparticles • TEM revealed the shape was irregular and polygonal • Acidic pH (5 and 7) resulted in fewer aggregates with a size ranging from 18 to 23 nm • The smallest particle size was 8 nm which was synthesized at pH 7	(Che Lah et al., 2020)
Precipitation	Zinc nitrate	Ascorbic acid	5 to 12	• Under an acidic medium, the ZnO nanoparticles	(Cho et al., 2010)

	hexahydrate			crystal growth decreased • The ZnO nanoparticles in an acidic medium was spherical • Under alkaline medium, the ZnO nanoparticles in the flower-like shape in a smaller size	
Sol-gel	Zinc acetate dihydrate	Banana peels extract (rich source of phenolic compounds)	8, 9, 10, 11 and 12	• The absorbance peak increased as raised pH from 8 to 12 • From Fourier Transform Infrared spectroscopy (FT-IR), the ZnO nanoparticles bands were observed at a wavenumber of 350-390 cm^{-1} • The crystallite size of ZnO nanoparticles synthesized at pH 8 to 12 was 16.11 – 23.76 nm	(Abdol Aziz et al., 2019)
Green route	Zinc acetate dihydrate	*Theobroma cacao* L. pod husks (rich source of phenolic compounds)	7, 8 and 9	• Formation of precipitate at all pH synthesis conditions • UV-vis confirmed a strong absorption peak at 372 nm, 383 nm, and 391 nm for pH 7, 8, and 9, respectively • The smallest size was obtained at pH seven, as confirmed by the absorption peak	(Sarillana et al., 2021)

				• The red shift and broadening were observed as pH 7 to 9, indicating that the particle size is increased	
Green route	Zinc nitrate hexahydrate	*Salvadora oleoides* Leaf Extract (rich source of flavonoids)	5 and 8	• UV-Vis confirmed a strong absorption peak at 380 nm for pH 5 and 8 • ZnO nanoparticles synthesized at pH 5 were spherical in shape and size of 20 to 56 nm • ZnO nanoparticles synthesized at pH 8 were irregular in shape and size at 26.62 nm	(Padalia et al., 2017)
Green route	Zinc nitrate	Orange fruit peel extract (rich sources of flavonoid, limonoid and carotenoids)	4, 6, 7, 8, 9, 10 and 11	• pH affects the colour of ZnO nanoparticles powder, such as ivory colour obtained at pH 4, 6, and 9, black colour at 7 and 8, and white powder at 10 and 11 • At acidic conditions, the ZnO nanoparticles were spherical, with sizes ranging from 10-20 nm. • pH 8 produced a large, coagulated particle with a size of 400 nm. • pH 11 produced large, coagulated blocks with a size of around 160-370 nm	(Doan Thi et al., 2020)

2.2.2 Effect of Concentration of Reducing and Capping Agents

The concentration of synthetic plant compounds and natural compounds (i.e., from plant or fruit extracts) used as reducing and capping agents can influence the size and morphology of ZnO nanoparticles. Nevertheless, it would not be guaranteed that a higher concentration of reducing and capping agents could result in the smallest ZnO nanoparticles. Therefore, the effect of concentration on reducing and capping agents was discussed in this subchapter and tabulated in Table 2.3.

Fiedot-Toboła et al., (2021) studied the effect of black tea (BT) extract by manipulating the black tea extract to zinc precursor ratio (v:v) at 1:1, 1:6, and 1:12 to synthesize ZnO nanoparticles. The most significant finding revealed in this study is the gallic acid that is highly abundant in the black tea extract interacts with zinc ions to form the chelate complex. In terms of morphology, the ZnO nanoparticles were found to be nanoflakes, nanocones, and quasi-spherical at the ratio of 1:12, 1:6 and 1:1, respectively.

Al-Salem and Seoudi, (2020) manipulated the molar ratio of ascorbic acid to zinc precursor (AA: Zn) from 1.0 to 4.0%. The absorption band was red shifted from the (AA: Zn) ratio of 1.0 to 3.0%, indicating increased particle size. While the absorption band was blue shifted from the (AA: Zn) ratio from 3.0 to 4.0%, indicating the particle size is reduced. The synthesized ZnO nanoparticles at various (AA: Zn) molar ratios were spherical. Chaithanatkun et al., (2015) also studied the effect of molar ratio (AA: Zn) at 0.5%, 1.0% and 4.0%. The important findings from this study are the size and shape of ZnO nanoparticles is influenced by the molar ratio (AA: Zn). As the molar ratio (AA: Zn) increases, the ZnO nanoparticles are more aggregated due to the ascorbic acid binds to zinc ions as chelating ligands.

The study conducted by Kamaruzaman and Che Lah, (2021) utilized tannic acid at concentrations of 0.1 M and 0.4 M. The addition of tannic has influenced the size and enhanced ZnO nanoparticles' aggregation. As reported in the study, a low concentration of tannic acid (0.1 M) produced a smaller particle size than a high concentration of tannic acid (0.4 M). Contradicted with the study from Taşdemir et al., (2022), the high molar ratio of AA: Zn (8.0%) produced the smaller ZnO nanoparticles with a size of 142.3 nm than the low molar ratio of AA: Zn (4.0%), which the size was 707.5 nm. The spherical shape of ZnO nanoparticles was observed at a molar ratio AA: Zn of 4.0%, which in agreement with Al-Salem and Seoudi, (2020) findings.

Rajendran and Sengodan, (2017) studied the effect of plant extract concentration on the synthesis of ZnO nanoparticles using *Sesbania grandiflora* leaf extract. The leaf extract quantity varied from 5, 10, 15, and 20%. It was found that, as increased the amount of leaf extract, the rate of reduction increased and the optimum synthesis was at 20%. The synthesized ZnO nanoparticles at the various amount of leaf extract had a spherical-like shape.

In Abel et al., (2021) research, the various amount of plant extract of 5, 10, 15 and 20% were manipulated to evaluate the effect *of Coffea arabica* leaf extract on the synthesis of ZnO nanoparticles. At room temperature, a strong absorption peak was observed at 360 nm due to significant excitation binding energy. Through Scanning Electron Microscopy (SEM), the synthesized ZnO nanoparticles had an astounding shape and fixed wood-like shape at 10 and 15% and 5 and 20%, respectively.

Table 2.3 Effect of synthetic or natural resource concentrations reducing and capping agents on the synthesized ZnO nanoparticles.

Methods	**Zinc precursor**	**Reducing and capping agents (from synthetic or natural resources)**	**Concentration studied**	**Research findings**	**Reference**
Green route	Zinc acetate dihydrate	Black tea (BT) extract (Rich source of phenolic compounds)	BT to Zn ratio (v:v) of 1:1, 1:6 and 1:12	• At a 1:12 ratio, the ZnO nanoparticles were in nanoflakes, and the length and widths were around 100-550 and 21 nm, respectively • At a 1:6 ratio, the ZnO nanoparticles were in nanocones-like shape, and the length and widths were around 50-300 and 30-175 nm, respectively • At a 1:1 ratio, the ZnO nanoparticles were quasi-spherical and had a diameter of 150 nm	(Fiedot-Toboła et al., 2021)
Precipitation	Zinc nitrate hexahydrate	Ascorbic acid (AA)	AA to Zn molar ratio (%) of 1.0, 1.5, 3.0, 3.5 and	• UV-vis confirmed a strong absorption peak at the wavelength range of 352-368 nm	(Al-Salem and Seoudi, 2020)