GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *Curcuma xanthorrhiza* RHIZOME EXTRACT

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by

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Nathan Deva

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF ABBREVIATIONS

Ag	Silver
AgNPs	Silver nanoparticles
ADM	Arc discharge method
DMF	N-diemthylformamide
EDX	Energy Dispersive X-ray
NADH	Nicotinamide adenine
NPs	Nanoparticles
PDI	Polydispersity index
SEM	Scanning Electron Microscopy
SPR	Surface plasmon resonance
UV-Vis	Ultraviolet visible

SINTESIS HIJAU NANOPARTIKEL PERAK MENGUNAKAN EKSTRAK RIZOM *Curcuma xanthorrhiza*

ABSTRAK

Penggunaan ekstrak tumbuhan dalam proses sintesis nanopartikel perak adalah kemajuan terbaru dalam kimia hijau dan menjana minat yang besar di kalangan penyelidik di seluruh dunia. Kajian ini menekankan pada sintesis hijau nanopartikel perak menggunakan ekstrak rizom *C. xanthorrhiza*, yang bertindak sebagai agen penurunan dan penstabilan. Ion perak dalam larutan nitrat perak dapat diturun menjadi nanopartikel perak valensi sifar, apabila ekstrak rizom dicampurkan dengannya. Penurunan ion perak mengakibatkan perubahan warna dalam campuran dari kuning ke coklat gelap. Oleh itu, beberapa parameter yang mempengaruhi sintesis hijau nanopartikel perak telah dioptimumkan melalui manipulasi pemboleh ubah dalam eksperimen. Kemudian, nanopartikel perak yang disintesis dicirikan menggunakan spektrometer Ultraviolet-Visible (UV-Vis) dan spektrometer Energy Dispersive X-ray (EDX). Spektrum UV-Vis nanopartikel perak diperoleh, dan puncak penyerapan maksimum diperoleh pada panjang gelombang dalam lingkungan 445 hingga 450 nm. Oleh itu, keadaan optimum untuk sintesis nanopartikel perak telah ditentukan dan didapati pada kepekatan ekstrak rizom 8 wt%, kepekatan larutan perak nitrat 8 mM, nisbah isipadu larutan ekstrak rizom dan perak nitrat pada 1: 5 dan masa inkubasi 72 jam. Selanjutnya, analisis EDX nanopartikel perak menunjukkan spektrum komposisi unsur di mana perak mempunyai puncak tertinggi pada 3 keV. Terdapat beberapa sebatian lain dalam spektrum, yang menunjukkan adanya biomolekul dari ekstrak pada permukaan nanopartikel sebagai agen penstabilan. Hasil ini menunjukkan bahawa proses pengurangan ion perak menggunakan ekstrak rizom *C. xanthorrhiza* adalah

kaedah yang lebih hijau dengan kurang toksik dan dapat membentuk nanopartikel yang lebih halus dan stabil.

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ABSTRACT

The employment of plant extract in the synthesis process of silver nanoparticles is the recent advancement in green chemistry and garnering great interest among researchers worldwide. This study mainly emphasizes on green synthesis of silver nanoparticles using *Curcuma xanthorrhiza (C. xanthorrhiza)* rhizome extract, which acts as a reducing and capping agent. Silver ions in the silver nitrate solution can be reduced into zero valence silver nanoparticles when the rhizome extract is mixed. The reduction of silver ions resulted in color changes in the mixture from yellow to dark brown due to the surface plasmon resonance (SPR) phenomenon. Thus, several parameters influencing the green synthesis of silver nanoparticles were optimized by manipulating variables in the experiment. Then, the synthesized silver nanoparticles were characterized via analytical equipment such as UV-Visible spectrometer and Energy Dispersive X-ray (EDX) spectrometer. The UV-Vis spectrum of the silver nanoparticles was obtained, and the maximum absorption peak was acquired at a wavelength in the range of 445 to 450 nm. Hence, the best conditions for synthesizing silver nanoparticles were determined and found to be 8 wt % rhizome extract concentration, 8 mM silver nitrate solution concentration, 1:5 rhizome extract to silver nitrate solution volume ratio, and 72 hours of incubation time. Furthermore, the EDX analysis of silver nanoparticles demonstrated a spectrum of elemental composition in which silver has the highest peak at 3 keV. Several other compounds in the spectrum indicate the presence of biomolecules from the extract on the surface of nanoparticles as a stabilizing agent. These results showed that the reduction process of silver ions

using *C. xanthorrhiza* rhizome extract is a greener method with less toxicity and can form more refined and stable nanoparticles.

CHAPTER 1

INTRODUCTION

1.1 Research Background

In recent years, nanotechnology emerges as a propitious science section in various fields that deals with research and development in material science. Advanced research in nanotechnology results in evolution of nanoparticles, which have a vast application in diverse sectors such as electronics, energy applications, future transport systems, healthcare, and many more. Metal nanoparticles having exceptional qualities in these fields and have attracted intensive research interest owing to their properties which in contrast with their bulk counterparts (He et al., 2017). Palladium, silver, gold, and platinum are fabricated in nano-form in a large scale due to their impacts on living organisms. Among these metal nanoparticles, silver nanoparticle (AgNP) is one of the most commercialized nanomaterials in this decade. The market size of silver nanoparticles is kept increasing due to its distinctive physical, chemical and biological properties (Ahmed et al., 2016). The market size of silver nanoparticles is clearly illustrated in Figure 1.1, as the trend is increasing until 2022, and it is majorly used in healthcare applications.

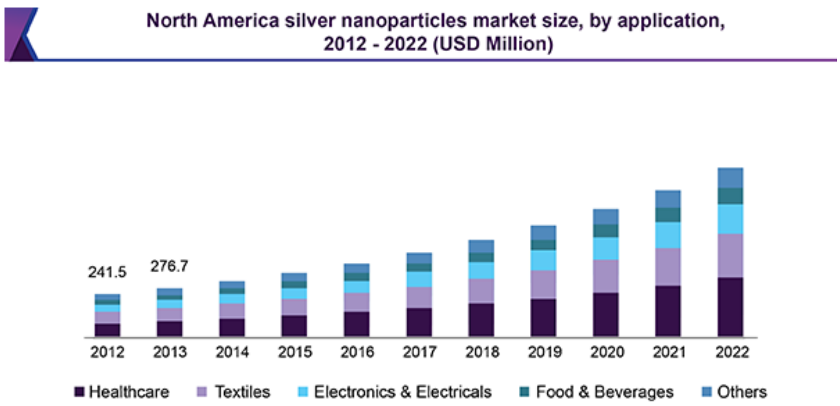

Figure 1:1 Silver nanoparticles market size, by application (Silver Nanoparticles Market Size Global Industry Report, 2017)

These oligodynamic silver nanoparticles have been proven to be the most efficacious antibacterial agent because of their extensive antibiotic inhibition spectra for microorganisms. This property made silver nanoparticles widely used in medical plasters, surgical lint, and medical instruments which were utilized to prevent infectious health problems (Lee et al., 2008).

The synthesizing process of these silver nanoparticles is a vital part as it will be associated with healthcare applications. Conventional physical and chemical routes for synthesizing silver nanoparticles are not preferred due to their drawbacks. The major drawback of chemical route is its toxic behaviour resulting in undesired effects when employed in healthcare applications. Moreover, physical methods require high energy and space, which makes it a cost-prohibitive choice (Pirtarighat et al., 2019).

So, green synthesis of silver nanoparticles found its possible use in the biomedical field, specifically in antimicrobial development. This method is defined as production of silver nanoparticles obtained from different biological mediums such as fungi, algae, bacteria, and plant extract. The green synthesis route has eliminated the vigorous parameters involved in other methods such as inert gases, high temperature, high pressure, laser radiation, and toxic by-products (Ijaz et al., 2020). Among these biological mediums used in this method, using plant extract is the most efficient and propitious way to synthesize silver nanoparticles. This is due to the presence of various phytochemicals in plant extract, which not only reduce Ag^{+} ions into Ag^{0}, but also offer capping effects and reduce toxicity. In addition, these phytochemicals also have good antimicrobial activities, which can give a rise to this silver nanoparticle production usage in healthcare applications (Roy et al., 2019).

In this study, *Curcuma xanthorrhiza (C. xanthorrhiza)* rhizomes are used to synthesize silver nanoparticles. *C. xanthorrhiza* or popularly known as Java ginger or 'temulawak' in Malaysia. This plant is one of the medicinal plants in *zingiberaceae* family, and it is widely used by people in Malaysia and Indonesia. It is cultivated around the world due to its exceptional pharmacological activities such as anticancer, antimicrobial, and anti-inflammatory. Many significant phytochemicals contained in this rhizome's extract like curcumin, phelandren, camphor, tumerol, sineol, borneol, flavonoids, and sesquiterpenes. These metabolites are responsible for the reduction of silver ions (Ag^{+}) into silver nanoparticles (Rohman et al., 2020).

1.2 Problem Statement

Recent advancements in nanotechnology to produce nanoparticles reduce the gap between macro-materials and atomic or molecular level materials. This research and development of nanoparticles with prominent antimicrobial actions against multidrug-resistant pathogens initiate a new platform to combat bacterial mutation arch. However, the main problem here is the toxicity produced by the synthesizing methods of these nanoparticles, which can be detrimental when used in medical sectors. So, the conventional techniques such as physical and chemical synthesizing routes have to be substituted by an effective, less harmful, sustainable, and cost-effective way as the nanoparticles can be widely utilized as an antimicrobial agent in healthcare industries (Roy et al., 2019). In this context, green synthesis methods are rendering themselves as a suitable and proper alternative for these traditional methods as it is employed in medical fields. Numerous biological entities can be used to synthesize silver nanoparticles, such as fungi, algae, bacteria, and plant extract. However, this utilization of microorganisms for nanoparticle fabrication have a slow rate of reaction and not cost-effective as the culture requires stable environmental conditions (Roy et al., 2019). So, plant-mediated synthesis of silver nanoparticles is considered one of the effective ways compared to the utilization of microorganisms.

Various researchers have conducted experiments using multiple plant extracts to synthesize silver nanoparticles such as turmeric, ginger, galangal, and many more. But to date, no studies have been reported on the green synthesis of silver nanoparticles using C. *xanthorrhiza* rhizomes. This choice of plant extract is due to its exceptional biomolecule contents, which are responsible for the reduction of Ag^{+} ions to produce silver nanoparticles. after the selection of plant extract, there are several parameters influencing the production of silver nanoparticles and their application as an antimicrobial agent in the medical field.

1.3 Research Objectives

This research aims to synthesize silver nanoparticles via green synthesis method using *C. xanthorrhiza* rhizomes' extract and characterize the synthesized nanoparticle. In general, the objectives of the present study are as follows:

I. To reduce Ag^{+} ions into silver nanoparticles using *C. xanthorrhiza* rhizomes extract.

II. To study the effect of concentration of *C. xanthorrhiza* rhizomes extract, concentration of silver nitrate solution, ratio between rhizome extract and silver nitrate solution, and incubation time on the synthesis of silver nanoparticles.

III. To characterize the synthesized silver nanoparticles in terms of absorbance and elemental composition.

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Nanoparticles

The emerging research in the nanotechnology fields has established the studies and development of nanoparticles all around the world due to its wide applications in many sectors. Nanoparticles are defined as particles having a size between 1-100 nm in diameter that can alter their physicochemical properties with the bulk counterparts (Pirtarighat et al., 2019). These nanoparticles are the leading edge of the rapidly developing field of nanotechnology because of their novel features and applications such as tissue engineering, bio-detection of pathogens, energy industries, electronics, and many more. Among these applications, healthcare industries are the ones with the most utilization of nanoparticles.

2.2 Overview of Silver Nanoparticles

There are many metal nanoparticles fabricated worldwide, such as gold, palladium, platinum, and silver. Silver nanoparticles stand out to be one of the most commercialized nano-form materials as the market size for it keeps increasing until the year 2024. Silver nanoparticles are extensively used in the medical field due to their high antibacterial activity for a wide range of strains without harming animal cells. Its ability to halt multidrug resistance bacteria makes it a proper alternative for antibiotics in the biomedical field (Pirtarighat et al., 2019). These silver nanoparticles have three different mechanisms against pathogens or microbes such as cell wall and membrane damage, intracellular penetration and damage, and oxidative stress. The bacterial growth inhibition rate of silver nanoparticles is highly influenced by several parameters such as penetration of silver nanoparticles into the bacterial cell, electrostatic interaction, and their surface absorption into the bacterial cell wall (Ijaz et al., 2020). Silver nanoparticles with a greater surface area to volume ratio are garnering high interest due to enhanced microbial action towards gram-negative and positive bacteria and other eukaryotic microorganisms.

Moreover, the morphology of silver nanoparticles also affects their microbial activity, where the hexagonal and truncated triangle-shaped silver nanoparticles exhibit high antibacterial activity (Roy et al., 2019). Over the years, silver nanoparticles are synthesized via conventional physical and chemical routes. But these synthesizing routes having a main drawback, which is toxic by-product formation. Generally used reducing agents such as hydrazine and sodium borohydride are not suitable because of their tendency in in-situ capping and reducing, which could lead to undesired toxicity (Roy et al., 2019). This occurrence makes the physical and chemical routes not preferred to produce silver nanoparticles. Recent advancement in green synthesis of silver nanoparticles using biological medium is gathering high interests among researchers as the route is simple, efficient, eco-friendly, cost-effective and more importantly non-toxic (He et al., 2017).

2.3 Overview of *Curcuma Xanthorrhiza* rhizomes

C. xanthorrhiza (Figure 2.1), also known as Java ginger or 'temulawak'; is Indonesia native medicinal plant belong the *Zingiberaceae* family. Traditionally, this plant's rhizomes have been used as herbal drinks, food supplements, and *jamu* prescriptions (Rohman et al., 2020). This plant's rhizome has also been used as raw material in cosmetics and pharmaceutical industries in Southeast Asia regions. Due to its high demand in these industries, the rhizomes are sold at relatively high prices compared to other traditional herbs. *C. xanthorrhiza* has a deep yellow rhizome and green leaves with brownish-purple veins (Anjusha & Gangaprasad, 2014).

Figure 2:1 *C. xanthorrhiza* plant and rhizome (Anjusha & Gangaprasad, 2014)

C. xanthorrhiza rhizome's extracts containing various phytochemicals and metabolites, which are responsible for reducing Ag^{+} ions into silver nanoparticles and become a capping agent at the surface to prevent agglomeration. Many phytochemicals available in this plant rhizomes extract, such as curcumin, phelandren, camphor, tumerol, sineol, borneol, flavonoids, and sesquiterpenes. Curcumin: a type of phenolic content, is the major constituent in the extract. The list of constituents in the extract is as below (Anjusha & Gangaprasad, 2014):

Table 2:1 Qualitative analysis of phytochemicals in the rhizome's extract extract (Anjusha & Gangaprasad, 2014)

Compounds	**Distilled water**	**Methanol**
Flavonoid	+	+
Tannin	+	-
Saponin	+	-
Carbohydrate	+	+

Reducing sugar	-	-
Quinine	-	-
Terpenoids	+	+
Sterols	+	+
Protein	+	+
Phenols	+	+

+ = indicates the presence of constituents, - = indicates the absence of constituents

2.4 Synthesis of Silver Nanoparticles

There are many methods to synthesize silver nanoparticles, which is practiced for a long time, where research and development of these materials keep garnering many interests. These methods are classified into two main groups, which is either 'top-down' approach or 'bottom-up' approach. The 'bottom-up' approach initiates from atom to new nuclei growth, which will further encounter the self-assembly process. In contradiction, the 'top-down' approach uses initial macroscopic structures to disintegrate into fine particles by using external processes. Some examples of 'top-down' processes are mechanical milling, chemical etching, sputtering, and laser ablation. Meanwhile, green synthesis, chemical precipitation, sol-gel process, and aerosol pyrolysis are the methods classified under the 'bottom-up' approach (Jawaad et al., 2014). The mechanisms involved in these two approaches are well depicted in Figure 2.2.

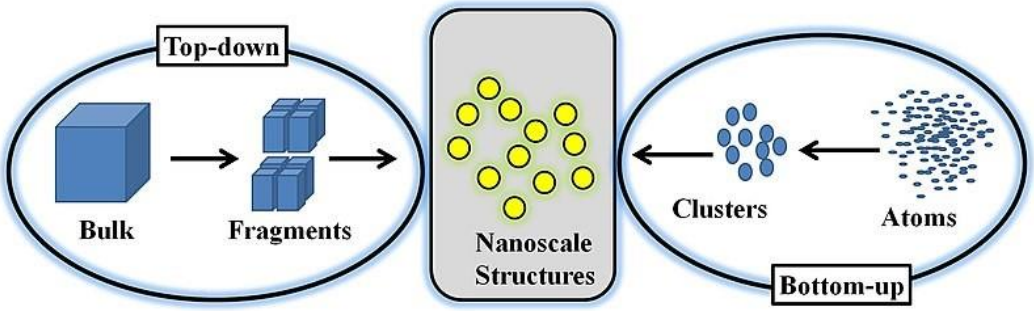

Figure 2:2 'Top-down' and 'bottom-up' approach for the synthesis of silver nanoparticles (Nanoparticle Synthesis - Nanoscience Instruments, 2017).

2.4.1 Physical Synthesis of Silver Nanoparticles

Physical synthesis of silver nanoparticles usually conducted using the vaporization-condensation process, where a fixed vessel is utilized to contain substance within it and evaporate it into the carrier gas at atmospheric pressure. Then it will be condensed rapidly to form silver nanoparticles. But there are several drawbacks associated with this method, where ample space and energy are required. Moreover, this process takes a longer time to achieve thermal equilibrium and makes the reaction slow (Yaqoob et al., 2020).

In addition, another physical synthesis method is the arc discharge method (ADM), which is a productive process to form silver nanoparticles. ADM method utilizes two silver electrodes immersed into inert gas with direct-current arc voltage applied to it. Then, the surface layers of electrodes will disintegrate and condense in water. Eventually, the solution will turn into golden brown indicates the formation of silver nanoparticles (Yusuf, 2019).

Another recent advancement in the physical method is using laser ablation. The pure colloid can be obtained using laser ablation method without using chemical additives. The wavelength of the laser, laser power, and duration of ablation are the parameters influencing the properties of silver nanoparticles produced using this method (Pyatenko et al., 2004).

2.4.2 Chemical Synthesis of Silver Nanoparticles

The chemical synthesis route is the standard method used by researchers and academia to fabricate silver nanoparticles. This is due to its process flexibility, cost-effectiveness, and efficient process mechanisms. Chemical synthesis route requires three essential factors, which are metal-based precursors, capping oxidants, and reducing agents. Spherical-shaped silver nanoparticles can be obtained using this approach, where the yield is influenced by pH, temperature, precursors, reducing substance, and capping material (Haider & Kang, 2015). Various reduction agents can be utilized, such as glucose ($C_6H_{12}O_6$), hydrazine (N_2H_4), hydrazine hydrate, ascorbate ($C_6H_7NaO_6$), ethylene glycol ($C_2H_6O_2$), N-dimethylformamide (DMF), hydrogen, dextrose, ascorbate, citrate, and sodium borohydride (BSS method) (Yaqoob et al., 2020). The Ag^+ ions receive an electron from the reducing agent and switch into a zero-valence state with further nucleation and growth. The existence of surfactants such as thiols, amines, acids, and alcohols in the reducing agents act as a stabilizing agent with interaction with silver nanoparticles. One of the advantages of the chemical synthesis route is the size controlling of silver nanoparticles synthesized. Research conducted by Chen et al. (2007) using oleylamine-liquid paraffin system resulted in the formation of monodispersed nanoparticles. Paraffin with a higher boiling point makes the process able to be conducted at wide range of temperatures. It makes them easy to regulate the size of nanoparticles formed. The major drawback of the chemical synthesis method is the toxicity associated with reducing agents, which make it undesirable to healthcare application.

2.4.3 Green Synthesis of Silver Nanoparticles

The green synthesis method of synthesizing silver nanoparticles usually conducted using various biological mediums as a reducing agent to reduce Ag^+ ions. The common biological mediums utilized are plant extract, algae, fungi, and bacteria. This technique is cost-effective, simple, sustainable, non-toxic, and well suited to medical field applications. One of the drawbacks of the method is, unable to produce nanoparticles or nanowires at a large scale, where this method is still under lab-scale production (Haider & Kang, 2015). Green synthesis method is suitable and environmentally benign alternative to chemical synthesis route due to its eco-friendly and economical way of production of silver nanoparticles.

Algae Mediated Green Synthesis of Silver Nanoparticles

A wide range of aquatic algae is utilized to synthesize silver nanoparticles, from microscopic (picoplankton) to macroscopic (Rhodophyta). *Chaetoceros calcitrans, Cuscuta salina, Isochrysis galbana,* and *Cystophora moniliformis* are the examples of algae usually used to produce silver nanoparticles (Ahmad et al., 2019). Exposure time and pH are the important controlling factors when synthesizing silver nanoparticles using algae extract. Rajeshkumar et al. (2014) successfully synthesized finer nanoparticles using widely available algae *Sargassum longifolium* with a maximum UV-Vis absorption band at a wavelength of 460 nm. The algae-mediated synthesized silver nanoparticles exhibit more significant antifungal activity against fungal strains. However, algae-mediated synthesis of silver nanoparticles cannot be scaled up to commercial way due to its slow reaction kinetics, low yield of nanoparticles, poor morphological characteristics of synthesized nanoparticles, and choice of algal strain (Chaudhary et al., 2020). Table 2.2 tabulates the characteristics, synthesis