ANTIBACTERIAL ACTIVITY OF BIOSYNTHESIZED SILVER NANOPARTICLES USING *Clinacanthus nutans* EXTRACT

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ABSTRACT

Several techniques were used to produce nanoparticles due to various advantages and disadvantages. Hence, the suggested method to overcome this problem was a ‘green’ method. This study employed *Clinacanthus nutans* (*C. nutans*) extract in the synthesis of silver nanoparticles (Ag NPs) and evaluated its antibacterial potential. In this study, Ultraviolet-visible spectroscopy (UV-Vis) result revealed that the formation of the Ag NPs with an absorption peak at approximately 380 to 420 nm is an optimized condition. The synthesized Ag NPs was then characterized based on the particle size, shape and morphology using X-Ray Powder Diffraction Analysis (XRD), Energy Dispersive X-ray spectroscopy (EDX), Transmission Electron Microscopy (TEM), and Field Emission Scanning Electron Microscopy (FESEM). The XRD confirmed the crystal nature of the Ag NPs while the FESEM showed the spherical shape of the Ag NPs. FESEM and TEM indicated the range of Ag NPs size is in between 20 – 30 nm with a spherical shape. Subsequently, the antibacterial activity of the synthesized Ag NPs through Disk Diffusion Technique (DDT) indicated effective inhibition against a few bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In contrast, there was a nominal antibacterial activity against *Enterococcus faecalis* and *Methicillin-resistant Staphylococcus aureus.* In general, this study focuses on the potential of *C. nutans* in the reduction, stabilization, capping, and synthesizing the Ag NPs as an environment economic, and easy technique.

ABSTRAK

Beberapa teknik digunakan untuk menghasilkan nanopartikel kerana wujud pelbagai kelebihan dan kekurangan. Oleh itu, kaedah yang dicadangkan untuk mengatasi masalah ini adalah kaedah ‘hijau’. Kajian ini menggunakan ekstrak Clinacanthus nutans dalam sintesis Ag NPs dan menilai potensi antibakteria dari Ag NP yang disintesis. Dalam kajian ini, spektroskopi yang dapat dilihat oleh ultraviolet (UV-Vis) mendedahkan pembentukan Ag NP dengan puncak penyerapan sekitar 380 hingga 420 nm dalam keadaan yang dioptimumkan. Ag NP yang disintesis kemudian dicirikan berdasarkan ukuran, bentuk, dan morfologi partikel mereka menggunakan analisis belauan serbuk sinar-X (*X-Ray Powder Diffraction Analysis*) (XRD), Spektroskopi sinar-X Kuasa Sebaran (*Energy Dispersive X-ray spectroscopy)* (EDX), Mikroskop Elektron Penghantaran (*Transmission Electron Microscopy*) (TEM), dan Mikroskopi Elektron Penskanan Pancaran Medan (*Field Emission Scanning Electron Microscopy)* (FESEM). XRD mengesahkan sifat kristal Ag NPs manakala FESEM menunjukkan bentuk dan morfologi Ag NP. FESEM dan TEM menunjukkan pelbagai ukuran Ag NP berbentuk sfera di antara 20 - 30 nm. Kemudian, aktiviti antibakteria Ag NPS yang disintesis melalui Teknik Disk Diffusion (DDT) menunjukkan perencatan yang berkesan terhadap beberapa bakteria seperti *Escherichia coli*, *Pseudomonas aeruginosa* dan *Staphylococcus aureus*. Namun, terdapat aktiviti antibakteria yang nominal terhadap *Enterococcus faecalis* dan *Staphylococcus aureus yang tahan akan Methicillin*. Secara umum, kajian ini memberi tumpuan pada potensi *C. nutans* dalam pengurangan, penstabilan, pembatasan dan sintesis Ag NP kerana ia mesra alam, menjimatkan, mudah untuk disintesis, tidak beracun dan bahan mudah dicapai/diperoleh.

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LIST OF ABBREVIATIONS

$AgNO_3$	-	Silver nitrate
AgNPs	-	Silver nanoparticles
Cu Kα		Copper K-alpha
DDT	-	Disk Diffusion Technique
DNA	-	Deoxyribonucleic Acid
EDX	-	Energy Dispersive X-ray spectroscopy
FESEM	-	Field Emission Scanning Electron Microscopy
MRSA	-	Methicilin-resistant Staphylococcus aureus
NPs	-	Nanoparticles
TEM	-	Transmission Electron Microscopy
UV-Vis	-	Ultraviolet-visible spectroscopy
XRD	-	X-Ray Powder Diffraction Analysis

LIST OF SYMBOLS

ml	-	Milliliter
3D		Three-dimensional
C˚	-	Degree Celsius
g	-	Gram
g/L	-	Gram per liter
h	-	hour
kV		Kilovolt
mg		Milligram
min	-	minute
nm	-	Nanometer
rpm		Revolutions per minute
T		Time
μL	-	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background of the Research

According to Nayak *et al.* (2011), metal NPs are widely studied for their optical, electrical, and catalytic abilities. Several studies have been performed to control the size and shape of NPs, which is significant in controlling and regulating the physical, chemical, and optical properties that are related to the different activity of these particles such as antibacterial, fungicidal, and catalytical (Coe *et al*., 2002; Ferry, 2018). Nayak *et al.* (2011) also reported NPs preparation using different methods such as heat evaporation, chemical reduction, electrochemical reduction, and photochemical reduction.

Nonetheless, the NPs tend to aggregate. Therefore, it is crucial that the reagents used in the synthesis of NPs to prevent agglomeration. However, these reagents are noxious, and utilizing it in a considerable amount will pollute the environment. Toxic reagents such as thiophenol, thiourea, and mercapto acetate have been widely used for NPs synthesis (Nayak *et al.*, 2011; Singh *et al.*, 2016). In contrast, biosynthesis of NPs using natural products offer an environmentally friendly method in the process of synthesizing.

Noroozi *et al.* (2014) stated that Ag NPs are very important due to their optical, electrical, and thermal properties. Furthermore, due to the chemical, physical, and biological properties of Ag NPs, it was recommended to be the highest level of interest than other metal of NPs as they hold the catalytic activities, bactericidal influences, and fungicidal effects (Rana & Kalaichelvan, 2011; Firdhouse & Lalitha, 2015). Currently, the amount of antibiotics consumption is widely increasing due to the bacteria resistance to the antibiotics (Li *et al*., 2011). Therefore, the use of Ag in medicine has great advantages due to the antimicrobial potential of over 650 microorganisms including fungi, viruses, bacteria, and eukaryotic microorganisms. According to Gong *et al*. (2007), the antimicrobial ability of Ag particles increased in a nanoparticle form as it displays an ample surface space to volume ratio. Furthermore, the Ag NPs can be used as an antibacterial agent in the purifying water (Marambio & Hoek, 2010).

The antibacterial mechanism of Ag (Ag ions and Ag NPs) is linked to its interaction with thiol group compounds in the respiratory enzymes of the bacterial cells. The Ag sticks to the cell wall and cell membrane of the bacteria to prevent respiration stream. The action of Ag includes preventing the acceptance and release of phosphate, mannitol, succinate, proline, and glutamine from E. coli cells (Rai, Yadav, & Gade, 2009). Ag in an NPs form is able to attack the respiratory chain and cell division leading to the death of the cell. Furthermore, the NPs release Ag ions inside the cell thus, improving the antibacterial activities. However, injecting Ag inside the bacterial cell turned the DNA molecule into a condensed form in which this action caused the cell loses its replication ability hence leads to death (Al-Sharqi *et al*., 2020).

There are many disadvantages of using chemical and physical methods to synthesize Ag NPs. In order to overcome these disadvantages, green synthesizing methods were suggested in which this method also has the advantage in increasing the biological function of Ag NPs. According to Chintawar (2016), the extracts of medicinal plants *Andrographis paniculate* and *Nordostachys jatamansi* were used as the reducing, stabilizing, and capping agents in the synthesis and to increase the antibacterial applications of the Ag NPs. Another ingredient that was used in medicinal history is *C. nutans*, which has been used for the synthesis of Ag NPs. However, many studies and research with various methods and parameters to synthesize NPs are necessary to improve the characteristics of the NPs such as the mono-dispersity, crystallinity, and shape control (Nayak *et al*., 2011). A natural resource such as plant extracts can be used to synthesize NPs and this method is inexpensive, environmentally friendly, and simple to be utilized. In this study, *C. nutans* was used to synthesize Ag NPs. *C. nutans* is a small native shrub found in the tropical countries such as Indonesia, Malaysia, and Thailand. *C. nutans* is commonly found and cheap in these countries, particularly the leaves extracts that have been utilized for cancer treatments (P’ng *et al*., 2012).

1.2 Problem Statement

The chemical and physical methods to produce NPs have some disadvantages such as high cost, poisonous, heterogeneous particle size, flammable, nonenvironmental, and unhealthy (Tran & Le, 2013; Keat *et al*., 2015). In addition, the common drawbacks of these methods are the deformation of the synthesized particles and inhibition of the particle growth. Therefore, it is crucial to identify and find alternative methods for the synthesis of NPs to meet global demand (Gebear-Eigzabher, 2018). Green synthesis is one of the reliable methods to synthesize NPs (Ahmad *et al*., 2003). Green chemistry/green method was introduced in the field of science and industry to overcome some of the drawbacks with the conventional methods in synthesizing NPs such as reducing the production of the unsafe waste products that could damage the environment (Sharma *et al*., 2009). Utilizing plant extract is a preferred approach for the synthesis of NPs. It is a good example of green NPs synthesis method. It is relatively cheap, environmentally friendly, and the most important is the starting materials can be easily found (Sharma, Yngard, & Lin, 2009). The important point in the use of plant extract to synthesize Ag NPs is that the plant biomolecules and the secondary metabolites could reduce and stabilize the silver ions during the biosynthesis route (Hamid & Mutazah, 2019).

Hamid and Mutazah (2019) reported that the leaves extracts of *C. nutans* are widely used in the treatment of cancer patients. The pharmacological analysis of *C. nutans* by Alam *et al*. (2016) demonstrated that the plant exhibits the properties that are suitable to be used for cancer patients such as antimicrobial activity, anti-inflammatory, antiviral, antioxidant, and anti-diabetic actions. Apart for cancer treatment, the plant has been used for different applications such as treatment for skin rashes, bites from snake and insects, diabetes, and gout management. This study highlighted the potential of *C. nutans* in the synthesis of Ag NPs as it is environmentally friendly, cheap, easy to synthesize, and easy to obtain. The antibacterial potential of biosynthesized Ag NPs using *C. nutans* extracts were also examined.

1.3 Objectives of the Study

1. To synthesize Ag NPs using *C. nutans* extract.
2. To characterize the biosynthesized Ag NPs.
3. To determine the antibacterial activity of the biosynthesized Ag NPs.

1.4 Scope of the Research

This study presented a simple and effective method to synthesize Ag NPs using *C. nutans* extracts. The first scope of this research is to extract the leaves of *C. nutans* and drying the leaves at room temperature. After the drying process, 1 mM solution of silver nitrate (AgNO3) was added to the dried leaves for extraction purposes in an optimum condition by monitoring these four important parameters namely temperature, pH, volume, and time. The biosynthesized of Ag NPs were monitored and characterized using UV-Visible spectrophotometer, Field Emission Scanning Electron Microscopy (FESEM), Transmission Electron Microscopy (TEM), and X-ray Diffraction (XRD). Finally, the synthesized Ag NPs were analyzed based on the morphology, structure, dimension, and shape.

The next scope of this study is to assess the antibacterial efficiency of the biosynthesized Ag NPs. Antibacterial assays were conducted using the disc diffusion technique (DDT). DDT was carried out to examine the inhibition properties of synthesized Ag NPs using *C. nutans* against common bacterial strains such as Gram-negative bacteria (*Escherichia coli and Pseudomonas aeruginosa*) and Gram-positive bacteria (*Enterococcus faecalis, Staphylococcus aureus, and Methicilin-resistant Staphylococcus aureus*).

1.5 Significance of the Research

The Ag NPs have a wide range of applications including cleaning the drinking water, degrading insecticides, and to kill human pathogenic bacteria (Marambio-Jones & Hoek, 2010). According to a research conducted by Nayak *et al*. (2011), the biosynthesized silver NPs with were discovered with variations in surface and shape to prove the antibacterial activity against pathogenic bacteria such as *E. coli*, *P. aeruginosa*, and *S. aureus*.

This study was conducted using a green synthesis method since it is easy to use and to synthesize. The materials used were derived from natural-waste products that can produce high quality products. The green synthesis method is also considered cheap since it does not require high energy, pressure, and temperature and the outcome is non-toxic compared to the chemically based synthesis method (López *et al*., 2016). Looking at these factors, the Ag NPs in this study was synthesized using the cost-effective green method. *C. nutans* extract was used as reducing agents to change Ag^+ to Ag^0 as it has the potential of capping to increase the antibacterial activity of Ag NPs against *E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*, and *MRSA*.

There are different antibacterial applications of Ag NPs, however, this green method can be an added value for Ag NPs as an antibacterial agent, which should be industrialized. It is hoped that this study will provide knowledge regarding the potential use of biosynthesized Ag NPs from *C. nutans* as an antibacterial agent. Besides that, this study could provide the framework for the synthesis of Ag NPs with a safe and cheap process that could improve the antibacterial properties and capacity of Ag NPs for a safer and healthier setting.

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