SYNTHESIS OF SILVER NANOPARTICLE SYNTHESIS FROM TEA LEAF (CAMELLIA SINENSIS) EXTRACT AND STUDY ON ITS ANTIMICROBIAL PROPERTIES

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ABSTRACT

Current nanotechnology research uses a lot of chemicals, which are potential threat to both environment and public health. Therefore the need to find more environmental friendly procedures for the production of nanoparticles arises. This research is done in with the purpose of synthesizing silver nanoparticles from tea leaves while studying its antimicrobial properties on Gram positive and Gram positive bacteria. Tea leaves (*Camellia sinensis*) has been used because of its rich source of polyphenolic compounds used for the reduction and capping of silver nanoparticles, making it a complete green chemical route. The reduction process of Ag^{+} to Ag^{0} was observed by the change of colour from clear brown to milky grey. The reaction was followed by the characterization of the silver nanoparticles using UV-Vis, FTIR, TEM and XRD. An average particle size of 26 nm silver nanoparticles could be obtained using TEM. The antimicrobial activity was tested using agar well method and it is found that the antimicrobial activity is observed to be higher against Gram negative bacteria. As a result of the research, it is determined that local plants such as tea leaves is an alternative to a safer, more eco-friendly alternative of synthesizing silver nanoparticles.

ABSTRAK

Kajian nanoteknologi terkini menggunakan terlalu banyak bahan kima yang menjadi ancaman terhadap alam sekitar dan kesihatan awam. Oleh itu, timbulnya keperluan untuk mencari alternatif bagi prosedur menghasilkan nanozarah. Kajian ini dijalankan bertujuan untuk mengsintesis nanozarah argentum (Ag) dari daun teh serta mengkaji kesan ciri-ciri antimikrob nanozarah tersebut ke atas bakteria Gram positif dan juga Gram negatif. Daun teh (*Camellia sinensis*) telah digunakan berdasarkan sifatnya yang kaya dengan kandungan polifenol yang boleh menjadi agen pengurangan lantas menjadikan proses ini suatu proses yang mesra alam. Proses pengurangan dari Ag^+ ke Ag^0 telah diperhatikan melalui perubahan warna dari coklat jernih kepada kelabu keruh. Tindak balas tersebut diikuti dengan pencirian nanozarah argentum menggunakan UV-Vis, FTIR, TEM dan XRD. Purata saiz zarah yang telah diukur menggunakan TEM adalah 26 nm. Aktiviti antimikrob yang telah diperhatikan menerusi kaedah kolam agar menunjukkan bahawa ciri-ciri antimikrob pada nanozarah argentum bertindak lebih baik ke atas bakteria Gram negatif. Secara keseluruhannya, telah terbukti bahawa tumbuhan tempatan seperti daun teh boleh menjadi alternatif kepada kaedah mengsistesis nanozarah argentum yang lebih selamat dan mesra alam.

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LIST OF ABBREVIATIONS

TEM	-	Transmission Electron Microscopy
XRD		X-ray Diffusion
FTIR	-	Fourier Traonsform Infra-red Spectroscopy
UV-Vis	-	Ultra-violet Visible Spectrophotometer

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF RESEARCH

Nanoparticles possess a very high surface to volume ratio. This can be utilized in areas where high surface areas are critical for success. This could for example be in the catalytic industry; some nanoparticles actually have proven to be good catalysts. Some nanoparticles also show bactericidal effects and here, a high surface to volume ratio is important.

In biology and biochemistry, nanoparticles have attracted much attention. Nanoparticles are often in the range 10-100 nm and this is the size as that of human proteins. In the production of anti-reflective optical coatings, nanoparticles have also proven valuable. Using metal oxides to coat polymeric films, anti-reflective surfaces have been created. Nanoparticle does exhibit many interesting properties, and it is just a matter of time until more of these properties will be exploited.

Recent agriculture and medicine have been applying nanoparticles in multiple ways. Hence, attempts have been made to synthesize nanoparticles using both chemical and biological methods. Nanomaterials such as Ag, Au, Pt and Pd have been synthesized by different methods, including hard template, using bacteria, fungi and plants (Krishnaraj et. al., 2009). Among the noble metals, silver nanoparticles play a significant role in the field of biology and medicine due to its attractive physiochemical properties such as increased optical, electromagnetic and catalytic properties from the bulk materials (Wenseelers et al, 2002; Kelly et al, 2003).

Silver nanoparticles can be synthesized through many different routes. The routes can be divided into three broad categories, which are physical vapor disposition, ion implantation and wet chemistry. The silver ion in silver nitrate undergoes reduction to become silver molecule. The formula is shown in Equation 1.

$$Ag^{+} \rightarrow Ag^{0} \tag{1}$$

Since the first nanoparticles were synthesized, their applications have found their ways into many different areas of science. It has been applied as a catalyst. In the article *Catalytic Properties of Silver Nanoparticles Supported on Silica Spheres* (Jiang et al., 2004) this catalytic effect has been proven. Another area where silver nanoparticles have proven to be effective is in controlling and suppressing bacterial growth. Several applications which use the bactericidal effect of silver nanoparticles have already been developed.

Various techniques such as chemical and physical mean were developed to prepare metal nanoparticles such as chemical reduction (Vorobyova et al., 1999), electrochemical reduction (Sandmann et al., 2000), photochemical reduction (Keki et al., 2000), heat evaporation (Smetana et al., 2005) and so on. However, green process for the synthesis of silver nanoparticles has been developed and it is an important aspect of current nanotechnology research. Biosynthesis of nanoparticles has received considerable attention due to the growing need to develop environmentally benign technologies in material synthesis. Synthesis of nanoparticles using microorganism or plants can potentially eliminate this problem by making the nanoparticles more bio-compatible.

The possible mechanism of biosynthesis of nanoparticles by biological system was reductases and any other equivalent reductants. Polyphenol was reported as one of the catalysts in the synthesis of nanoparticles. It is known to induce particle formation in infusions of black decaffeinated tea (Groning et al., 2001). Polyphenol is, in general, a moderately water-soluble compound with more than 12 phenolic hydroxyl groups and 5-7 aromatic rings per 1000 Da. It is found in virtually all families of plants, and comprising up to 50% of the dry weight of leaves. It is most commonly found in the leaves of the tea plant. Therefore, the leaves of *Camellia sinensis* are chosen to be used as a catalyst in the synthesis of silver nanoparticles in this experiment.

This research is carried out to synthesis silver nanoparticles from tea leaves extract. The silver nanoparticles were synthesized by mixing the tea leaves extract with silver nitrate. The amount of silver nanoparticles is measured by using the Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), ultraviolet-visible spectrophotometer (UV-Vis) and x-ray diffusion (XRD), and testing of the nanoparticles for any anti-microbial properties on gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Micrococcus luteus* using agar well method.

1.2 PROBLEM STATEMENT

Antibiotic resistance developed by bacteria has been a global concern due to their capability causing community-acquired infection (Alanis, 2005). Many discussions have been made on the approaches to deal with antibiotic resistant problem. Beside the appropriate antibiotic use in the therapeutic and non-therapeutic purpose, searching for safe and effective antibacterial agents also has been encouraged (Rai et al., 2009). Silver has been known to possess antimicrobial effects with distinctive properties of conductivity, stability, and activity (Das et al., 2011). Previous studies have indicated that silver nanoparticles are effective against a wide spectrum of bacteria, fungi and viruses. In fact, Quang et al. stated that the antibacterial effect of silver and its compounds have been well known for a long time as an antibiotic to treat some infectious diseases and burn wounds. With the emergence and increase of microbial organisms resistant to multiple antibiotics, and the continuing emphasis on health-care cost, researchers are urged to develop new and effective antimicrobial reagents free of resistance and cost. Such problems have led to the resurgence in the use of Ag-based antiseptics that may be linked to broad-spectrum activity and far lower propensity to induce microbial resistance than antibiotics.

1.3 RESEARCH OBJECTIVE

The purpose of doing this research is to synthesis silver nanoparticles from tea leaves while studying their antimicrobial properties on Gram positive and Gram negative bacteria.

1.4 SCOPE OF RESEARCH

To achieve the objective of this research, there are a few main scopes that the study focuses on. Firstly, tea leaves are used in order to synthesize the silver nanoparticles. Secondly, the characteristic of the silver nanoparticles are determined using FTIR, TEM, UV-Vis and X-ray diffusion. Thirdly, the antimicrobial properties of silver nanoparticles synthesized are studied on a gram-negative bacteria *Escherichia coli* and gram-positive bacteria *Micrococcus luteus*.

1.5 SIGNIFICANCE OF RESEARCH

The silver nanoparticles were synthesized using a local plant (tea leaves) because it has a high content of polyphenol. It is also easier to obtain and more cost effective. Furthermore, the antimicrobial properties of the silver nanoparticles serve well as an alternate antiseptic against both gram-negative and gram-positive bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 SILVER NANOPARTICLES

Silver is a metallic chemical element with the chemical symbol Ag and atomic number 47. It is a soft, white, lustrous transition metal with the highest electrical conductivity of any element and the highest thermal conductivity of any metal. It occurs naturally in its pure, free form (native silver), as an alloy with gold and other metals, and in minerals such as argentite and chlorargyrite. Most silver is produced as a byproduct of copper, gold, lead, and zinc refining.

Recently, the development of nanotechnology has resulted in the synthesis of nano scale silver particles which have particular properties that are superior to the bulk silver metal. (Quang et al., 2011). Silver nanoparticles are silver particles of between 1 nm and 100 nm in size. It can be synthesized through different methods which included physical vapor deposition, ion implantation or wet chemistry. Silver nanoparticles have found applications in catalysis, optics, electronics and other areas due to their unique sizedependent optical, electrical and magnetic properties. Compared to the bulk silver metal, nano scale silver particles have larger surface areas and unique physical, chemical and biological properties (Sharma et al., 2009). Moreover, silver nanoparticles can be easily embedded within porous substrates like mesoporous silica and zeolite (Matsumura, 2003). Figure 2.1 shows the chemical structure of silver.

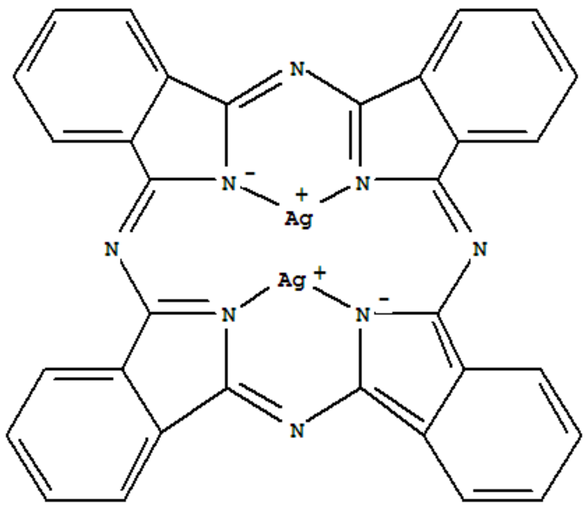

Figure 2.1: Chemical structure of silver

2.2 SILVER AS ANTIMICROBIAL AGENT

An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial agents either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Antimicrobials have long been developed since the observations of Pasteur and Joubert. However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. Old antimicrobial technology was mostly based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become resistant to them. Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms, replacing heavy metals and toxins.

Free silver ion (Ag^{+}) has been well known for its strong toxicity against some bacteria, viruses, algae and fungi, typical for heavy metals like lead, or mercury, but without the high toxicity to humans. It is recently shown that silver nanoparticles can be a promising antimicrobial material. Song et al. reported that silver nanoparticles showed higher antibacterial ability compared to that of silver compounds like silver nitrate, silver sulfadiazine, etc. It has been demonstrated that the highly reactive metal oxide nanoparticles exhibit excellent bactericidal action against Gram positive and Gram negative bacteria (Klabunde, 2002). Silver nanoparticles also have a high surface area to volume ratio along with high fraction of surface atoms that elicits elevated antimicrobial activity compared to the silver metal as a whole (Shahverdi, 2007). Silver supported on various suitable materials, such as carbon, polyurethane foam, polymers, and sepiolite have also been effectively used for bactericidal applications (Siva et al., 2004 & Jain et al., 2005).

Various hypotheses have been proposed to explain the mechanism of antimicrobial activity of silver nanoparticles. It is widely believed that silver nanoparticles are incorporated in the cell membrane, which causes leakage of intracellular substances and eventually causes cell death (Sondi et al., 2004). Some of the silver nanoparticles also penetrate into the cells. It is reported that the bactericidal effect of silver nanoparticles decreases as the size increases and is also affected by the shape of the particles (Pal et al., 2007). Although most studies have utilized spherical particles, truncated triangular shaped particles are reported to have greater bactericidal effect compared to that of spherical and rod shaped particles. The bactericidal efficiency of silver nanoparticles is also reported to be affected by the type of microorganisms. Kim et al. reported in studies with Gram-negative, *Escherichia coli* and Gram-positive, Staphylococcus aureus, that greater bactericidal efficiency of silver nanoparticle for Escherichia coli is greater and this is attributed to the difference in cell wall structure between Gram negative and Gram positive microorganisms.

2.3 SYNTHESIS OF SILVER NANOPARTICLES FROM PLANT

Nanoparticles have been synthesized without aggregation using chemical methods for its simplicity and the added advantage of high yield for large scale production. However, hazardous reducing agents used for chemical procedures mounts a bias for an eco-friendly and feasible approach for the synthesis of nanoparticles. Hence, plants are used as an alternative trigger for the green synthesis of nanoparticles (Medina-Ramirez et al. 2011). Biological means of synthesizing nanoparticles provides an edge over chemical means as it is cost effective, does not involve physical barriers with regard to reducing agents and eliminates the toxic effects of chemicals used for the synthesis (Saxena et al., 2010 & Song et al., 2009). Figure 2.2 shows the chemical structure of silver nitrate which can be used to synthesis silver nanoparticles.

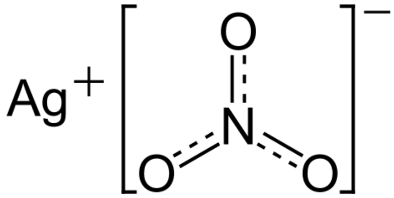

Figure 2.2: Chemical structure of silver nitrate

Synthesis of nanoparticles using plants can potentially eliminate the problem of the presence of some toxic chemical species absorbed on the surface by making the nanoparticle more bio-compatible. Bar et al. stated that the use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes by eliminating the elaborate process of maintaining cell cultures. Exploration of the plant systems as the potential nanofactories, has increased interest in the biological synthesis of nanoparticles. Sastry et al. reported the biosynthesis of nanoparticles using plant leaf extracts and their potential. They studied bioreduction of chloraurate ions and silver ions by extracts of germanium and neem leaf.

Tea leaf extract (*Camellia sinensis*) has also been used to synthesize silver nanoparticles (Vilchis-Nestor et al., 2008). Tea leaf contains polyphenols and terpenoids, such as β-cariophyllene, linalool, cis-jasmone, α-terpineol, δ-cadiene, indole, geraniol, among the major bio-components, which have bactericidal and antioxidant activity, and several other useful properties (Kaufman et al., 1999). Caffeine and theophylline present in tea extracts were also reported to catalyze the synthesis of nanoparticles (Groning et al., 2001). Figure 2.3 shows the chemical structure shows the chemical structure of polyphenol found in tea.

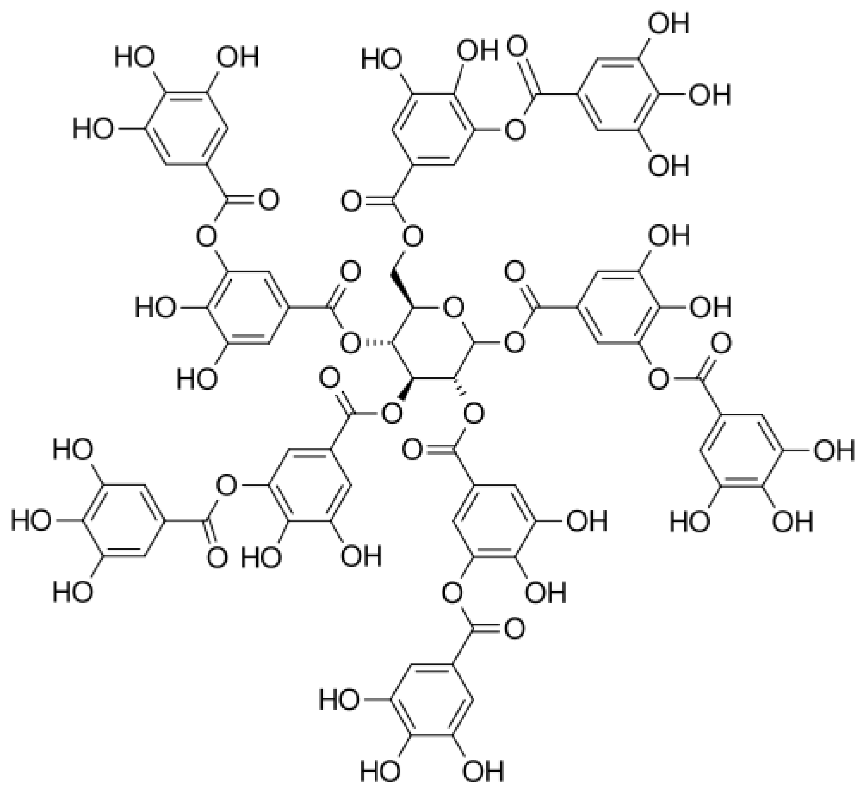

Figure 2.3: Chemical structure of polyphenol.

However, Kamal et al. reported that among the major setbacks of chemically synthesized silver nanoparticles is that the synthesized nanoparticles are covered by capping agents. This not only reduces their activity in the semiconductor nanoparticles but also some of the organic layers cannot be used for bio applications as they hinder the biocompatibility of the nanoparticles. Therefore, pretreatment such as washing of the surfactant or stabilizer becomes a prime requisite.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

This chapter includes the methodology applied in order to carry out the experiments for the synthesis of silver nanoparticles from tea leaves. The experiments will be carried out in the Chemical Engineering Analytical Laboratory, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang. Before the experiments are carried out, all the chemicals, raw material and apparatus needed to be set up and ready to run.

The methodology for this research is divided into three main steps; the synthesis of silver nanoparticles from tea leaf (*Camellia sinensis*) extract, washing and characterization of silver nanoparticles, and testing of the nanoparticles for any anti-microbial properties using agar well method.

3.2 SYNTHESIS OF SILVER NANOPARTICLES

3.2.1 Raw Material and Reagents

Silver nitrate ($AgNO_3$, 99.9% pure) was obtained from Sigma-Aldrich, Malaysia. Commercially available dry tea leaves, Boh Plantations Sdn. Bhd, Malaysia, Was used for preparation of tea extract and ultra-pure water with a resistivity of 18.2 MΩcm was used as a medium to dissolve the silver salt and for making the tea extracts.

3.2.2 Procedure

5 g of ground tea leaves were weighed and transferred into a 500ml beaker and was filled up to the mark with water. The contents of the beaker were thoroughly agitated for 1 hour using a heated stirrer and left to settle. The extract is then filtered through a 0.2 µm membrane filter and used for further experiments. A dilute solution of silver (0.1 M) was prepared by dissolving 0.017 g $AgNO_3$ in 1 ml water.

To carry out the $Ag^+ \rightarrow Ag^0$ reaction, 10 ml of the extract is added with 2 ml of 0.1 M solution of $AgNO_3$. The mixture is gently mixed while being left overnight for the reaction to occur.

Figure 3.1: Filtration of tea extract using membrane

Figure 3.2: Silver nitrate and tea extract mixture stirred overnight

3.3 WASHING AND CHARACTERIZATION OF SILVER NANOPARTICLES

3.3.1 Instruments

A desk-top centrifuge is used in the washing process of silver nanoparticles synthesized from the tea extract. This is to ensure that the polyphenol content is removed from the nanoparticles and further purify the silver nanoparticles. After the washing process, the silver nanoparticles are characterized using Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), ultraviolet-visible spectrophotometer and x-ray diffusion (XRD).

3.3.2 Procedure

12 ml of tea extract was put into a 15 ml centrifuge bottle and centrifuged at 10,000 rpm and 4°C for 10 minutes. After centrifugation, aliquot containing the polyphenols was separated from the sediment using a dropper. Ultra-pure water was then added to fill up to 12 ml once again. The sample was washed at least three times before it can be tested for silver nanoparticles characteristics.

The obtained emulsions were characterized using UV-Vis spectrometer. Fourier transform infra-red spectroscopy was used to study the interactions between the tea extract and silver nanoparticles for which, a FTIR is used to record the spectra. Silver nanoparticles were separated from the mother solution by centrifugation at very high temperature of 20,000 rpm, and the powders thus obtained were placed on silicone (Si) substrate to obtain the crystal structure using X-ray diffractometer (XRD). The powders were redispersed in water using a rod ultrasonicator and a drop of the redispersed solution was dropped onto a carbon coated copper grid to carry out transmission electron microscopy (TEM) studies.

3.4 DETERMINATION OF ANTIBACTERIAL PROPERTIES

The antibacterial activity of silver nanoparticles formed is tested on a gram-negative strain *Escherichia coli* and gram-positive strain *Micrococcus luteus*. The bacteria are used for inhibitory zone tests to investigate the antibacterial properties of silver nanoparticles using agar well method. A nutrient broth is used as the growing medium. Bacteria are grown aerobically in nutrient broth at 37°C for 24 h.

3.4.1 Materials

Escherichia coli and Micrococcus luteus strains were obtained from Chemical Engineering Laboratory, Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang. An agar well method was adopted to assay the nanoparticles for bactericidal activity against the test strains on Mueller-Hinton agar plates. A cork borer used to punch the agar wells was from Industial Science and Technology Faculty laboratory. Sterilized cotton swabs are used to spread the bacterial strain.

3.4.2 Procedure

Using aseptic techniques, petri plates containing 20 ml Mueller-Hinton agar medium were seeded with 24 hours culture of bacterial strains. A sterilized cork borer was used to punch through the agar and 20 µl of the silver nanoparticles were added into the