BIOSYNTHESIS OF Au, Ag AND BIMETALLIC Au-Ag NANOPARTICLES USING AQUEOUS LEAF EXTRACT OF *COSMOS CAUDATUS*

RAPIDAH BINTI MOHAMAD

UNIVERSITI TEKNOLOGI MALAYSIA

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RAPIDAH BINTI MOHAMAD

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Thank you Allah and Rasulullah....

For my supervisor, beloved parents and friends, thanks a lot for everything

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ABSTRACT

There is great interest in synthesizing metal nanoparticles due to their remarkable application in medicinal, catalysis and electronic. Single step biosynthetic approaches utilising plant extracts have emerged as a simple, eco-friendly and viable alternative to chemical synthetic procedures and physical methods. In the present study, we have synthesized mono- and bimetallic gold and silver nanoparticles by reduction of Au^{3+} and Ag^{+} ions using aqueous leaf extract of *Cosmos caudatus* (ulam raja). The formation of the Au and Ag nanoparticles was monitored using UV-Vis spectroscopy and was visually confirmed by colour change from yellowish to violet and from colourless to dark brown, respectively. The surface plasmon resonance (SPR) band appeared at 536 nm and 439 nm for Au and Ag, respectively. Meanwhile, competitive simultaneous reduction of Au^{3+} and Ag^{+} ions present in same solution led to the production of bimetallic Au-Ag in which the SPR band appeared at 533 nm. The nanoparticles were also characterised by using TEM, FESEM-EDX, XRD and FTIR analyses. FESEM micrographic images show the spherical shape for both gold and silver nanoparticles with average particle size 22.79 ± 6.81 and 21.49 ± 7.43, respectively. TEM images of the bimetallic Au-Ag nanoparticles showed the spherical shape of nanoparticles with average particles size of 13.98 ± 6.21 nm. Profile EDX spectra show the signals for both Au and Ag in the sample of AuNPs and AgNPs, respectively. Both signals appear for bimetallic sample, indicating that the bimetallic Au-Ag was in alloy form. X-ray diffraction (XRD) analysis revealed that the mono- and bimetallic nanoparticles were face centered cubic (fcc) in structure. FTIR spectra of the nanoparticles showed main absorptions at 3364.81 cm^{-1}(-OH stretching vibrations), 2925.49 cm^{-1} (asymmetric stretching of C-H groups), 1650.59 cm^{-1}(stretching vibrations C=O (amide and aldehyde), 1384.67 cm^{-1} (C-N stretching vibrations of amines) and 1067.62 cm^{-1}(C-OH stretching of secondary alcohols) indicating the nanoparticles are capped with the bioactive compounds present in the plant extract.

ABSTRAK

Sintesis logam nanopartikel mendapat perhatian menyeluruh berikutan aplikasi logam tersebut dalam bidang perubatan, pemangkinan dan elektronik. Biosintesis menggunakan ekstrak tumbuhan telah membawa kepada suatu alternatif yang ringkas, mesra alam dan praktikal bagi menggantikan kaedah kimia atau fizikal. Dalam kajian ini, kami telah menghasilkan logam aurum dan argentum dalam bentuk mono- dan dwilogam melalui penurunan ion aurum, Au^{3+} dan ion argentum, Ag^{+} menggunakan akueus ekstrak daun *Cosmos caudatus* (ulam raja). Pembentukan logam Au dan Ag diperhatikan menggunakan Spektroskopi Nampak Ultralembayung (UV-Vis) dan dibuktikan secara visual melalui perubahan warna larutan daripada kekuningan kepada ungu dan daripada larutan tidak berwarna kepada perang gelap, bagi kedua-dua Au dan Ag. Jalur serapan (SPR) muncul pada panjang gelombang 536 dan 439 nm, masing-masing untuk Au dan Ag. Pembentukan dwilogam Au-Ag terhasil apabila kedua-dua ion Au^{3+} dan Ag^{+} mengalami tindak balas penurunan secara serentak dan jalur serapan pada 533 nm muncul dalam spektrum UV. Imej mikrografik bagi Mikroskop Medan Pancaran Imbasan Elektron (FESEM) telah menunjukkan bahawa kedua-dua logam Au dan Ag berbentuk sfera dengan purata saiz 22.79 ± 6.81 dan 21.49 ± 7.43 nm. Imej Mikroskop Pemancaran Elektron (TEM) bagi dwilogam Au-Ag menunjukkan nanopartikel tersebut berbentuk sfera dengan purata saiz partikel adalah 13.98 ± 6.21 nm. Profil X-Ray Serakan Tenaga (EDX) masing-masing menunjukkan kehadiran logam aurum dan argentum dalam sampel nanopartikel Au dan Ag, manakala kedua-dua logam hadir bersama dalam sampel dwilogam dan membuktikan dwilogam tersebut adalah dalam bentuk aloi. Pembelauan sinar X-ray (XRD) menunjukkan bahawa struktur nanopartikel mono- dan dwilogam yang terhasil kiub muka berpusat (fcc). Spektrum inframerah (FTIR) menunjukan puncak penting pada 3364.81 cm^{-1}(getaran regangan bagi -OH), 2925.49 cm^{-1} (regangan tidak simetri bagi kumpulan C-H), 1650.59 cm^{-1}(getaran regangan bagi C=O (amida and aldehida), 1384.67 cm^{-1} (getaran regangan C-N amina) dan 1067.62 cm^{-1}(regangan bagi C-OH alcohol sekunder) yang menandakan bahawa nanopartikel telah diselaputi dengan molekul bioaktif yang terdapat dalam ekstrak tumbuhan.

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LIST OF ABBREVIATIONS

AgNPs	-	silver nanoparticles
AuNPs	-	gold nanoparticles
EDX	-	Energy Dispersive X-Ray
fcc	-	face centered cubic
FESEM	-	Field Emission Scanning Electron Microscopy
FTIR	-	Fourier Transform Infrared
HPLC/MS	-	High Performance Liquid Chromatogram and Mass Spectrometry
NPs	-	nanoparticles
PVP	-	poly (vinyl pyrrolidone)
ROS	-	reactive oxygen species
scMeOH	-	supercritical methanol
SPR	-	surface plasmon resonance
TAE	-	tannic acid equivalent
TEM	-	Transmission Electron Microscopy
UV-Vis	-	Ultraviolet Visible
XPS	-	X-ray Photoelectron Spectroscopy
XRD	-	Powder X-ray Diffraction

LIST OF SYMBOLS

µm	-	micrometre
ºC	-	degree Celsius
Å	-	armstrong
cm^{-1}	-	per centimetre
g	-	gram
kV	-	kilo volt
mA	-	milli Ampere
mM	-	millimetre
MPa	-	Mega Pascal
nm	-	nanometre
rpm	-	revolution per minute
λ	-	wavelength
λ_{max}	-	maximum wavelength

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nanoparticles can be defined as particulate materials with at least one dimension less than 100 nanometers (nm) (Prasad and Elumalai, 2011). Nowadays, nanoparticles are being viewed as fundamental building blocks of nanotechnology. They exhibit larger surface area to volume ratio in which it is the most important and distinct property of nanoparticles (Annamalai *et al.*, 2011). Nanoparticles are of great scientific interest as they connect the gap between bulk materials and atomic or molecular structures. A bulk material has constant physical properties regardless of its size, but at the nanoscale this is often not the case. Several well characterized bulk materials have been found to possess most exciting characteristics when studied in nanoscale (Thakkar *et al.*, 2010). Nanoparticles are expected to show totally new or improved properties based on specific characteristic such as size, distribution and morphology from which they are made (Song and Kim, 2008).

For the past two decades, there has been a rapid advancement in various technologies for the fabrication of metal nanoparticles due to their application in many fields of science and technology (Singh *et al.*, 2012). Metal nanoparticles have tremendous applications in the area of catalysis, optoelectronics, diagnostic biological probes and display devices (Annamalai *et al.*, 2011). The most effectively studied metal nanoparticles are those made from noble metals like silver, gold, platinum and palladium. In particular, gold and silver nanoparticles are getting much more attention due to their unique and tunable surface plasmon resonance (SPR), shape and size-dependent properties (Sheny *et al.*, 2011). Furthermore, both of the gold and silver nanoparticles are excellent nanomaterials providing a powerful platform in biomedical applications of biomolecular recognition, biosensing, drug delivery and molecular imaging (Khalil *et al.*, 2012).

In addition to pure metallic nanoparticles, bimetallic nanoparticles are also being increasingly investigated either in the form of alloys or core-shell nanostructure. This is because they offer the synergetic effect resulting from the combination of two metals which are highly reactive. Thus they are believed to promise better catalytic properties than their monometallic counterparts and hence, name these bimetallic nanoparticles particularly important in the field of catalysis (Devarajan *et al.*, 2005).

Several methods have been employed to produce gold and silver nanoparticles, with chemical approaches being the most popular. Unfortunately, these methods cannot avoid the use of toxic chemicals in the synthesis protocols (Song and Kim, 2008). However, there is a growing concern towards use of these chemicals as they are reported to be very toxic to the environment (Singh *et al.*, 2012). In addition, gold and silver are applied widely in human contact areas such as shampoos, soaps, detergents, as well as in medical and pharmaceutical applications. Thus, it increases the demand towards the development of environmentally benign process in synthesizing these metal nanoparticles.

Recently, biosynthetic routes have emerged as simple and viable alternative to chemical synthetic procedures as these methods can provide nanoparticles of better defined size and morphology. Among the various environment friendly biological processes, using plants for the synthesis could be more advantageous by eliminating the elaborate process of maintaining cell culture besides can also be suitably scale up for large-scale synthesis of nanoparticles (Shankar *et al.*, 2004). The nanoparticles produced by plants are also more stable and the rate of synthesis is faster.

Plants are potential sources of natural antioxidants, in which the antioxidative compounds are used to counteract reactive oxygen species (ROS) in order to survive. *Cosmos caudatus* is one of the locally grown herbs that have been investigated and identified to have high antioxidative activities (Huda-Faujan *et al.,* 2007). These antioxidative activities are contributed by the presence of flavonoid compounds such as proanthocyanidins. These biomolecules give *Cosmos caudatus* the great potential as antioxidant and thus making them suitable to be used in biosynthesis of metal nanoparticles.

1.2 Problem Statement

Conventionally, metal nanoparticles are synthesized through seed-mediated growth, template synthesis, chemical reduction and laser ablation (Sehayek *et al.*, 2006). These methods usually utilise hazardous and toxic chemicals and require specialized and expensive equipment. In addition handling of toxic chemicals and specialised instrument require skilled personnel.

Increasing environmental concerns demand the development of environmental friendly metal nanoparticles synthesis protocol. Biogenic synthesis is beneficial and advantageous not only because it reduces environmental impact compared with some of the physiochemical methods, but it can also be used to produce large quantities of nanoparticles that are free of contamination and have a well-defined size and morphology. Accordingly, many biological systems such as microbes, fungi and plant extracts have been investigated as metal reducing agents in the formation of metal nanoparticles. However, using microorganisms as reducing agents require high maintenance in cell cultures and suffer from various problems like availability and cost effectiveness during the scale up process. Thus, plant extracts are nowadays getting much more attention as bio-reducing agent due to its simple procedure in the preparation of extract.

However, there are not many attempts reported on the biosynthesis of bimetallic nanoparticles. Most of the previous works have focused solely on the biosynthesis of monometallic nanoparticles. Bimetallic metal nanoparticles are of greater interest than monometallic in enhancing interesting size-dependent electrical, chemical and optical properties due to the expected synergistic effect between the two elements. Hence, in this work, we proposed the biosynthesis protocols in producing gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) and bimetallic Au-Ag nanoparticles at ambient conditions using the aqueous leaf extract of *Cosmos caudatus*.

Hypothetically, plants can also be potential reducing agent to substitute chemical reducing agents because the presence of many biomolecules such as flavanoids, terpenoids and other polyphenolic compounds that may act as potential reducing and capping agents in the nanoparticles synthesis. Due to its high antioxidant properties among the local herbs, *Cosmos caudatus* is also considered as a strong reducing agent.

1.3 Objectives of Study

The objectives of this study are:

i. To synthesize gold nanoparticles (AuNPs), silver nanoparticles (AgNPs) and bimetallic Au-Ag nanoparticles using the aqueous leaf extract of *Cosmos caudatus*.
ii. To study the effects of different volume of leaf extract and metal salt concentration on the shape and size of nanoparticles.
iii. To characterize the nanoparticles formed by using Ultraviolet-Visible (UV-Vis) spectroscopy, Transmission Electron Microscopy (TEM), Field Emission Scanning Electron Microscope with Energy Dispersive X-Ray (FESEM-EDX), Powder X-Ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy.

1.4 Significance of Study

This study has provided a simple and green method in the synthesis of monometallic and bimetallic Au-Ag nanoparticles using the aqueous leaf extract of *Cosmos caudatus* as a response to fulfil the demand towards green process in the synthesis of metal nanoparticles. *Cosmos caudatus* used as a reducing and capping agent in the biosynthesis procedure is an important feature of this work. On the other hand, the biosynthesis of nanoparticles using plant extract is highly advantageous due to its easy availability, simple laboratory set-up for the synthesis process which be carried out at room temperature and pressure. In addition, no toxic reducing agents like lithium aluminium hydrate ($LiAlH_4$), sodium borohydrate ($NaBH_4$) and hydrazine are used in this process and thus completely eliminate any hazardous residue and environmental contamination that may arise from this synthesis. The implementation of green chemistry principles in this work is significant in view of fact that the development of this technology can be applied globally using any plant that has antioxidant property and potentially can acts as reducing agent .

1.5 Scope of Study

This study involved the synthesis of AuNps, AgNPs and bimetallic Au-Ag nanoparticles using aqueous leaf extract of *Cosmos caudatus*. Two synthesis parameters were studied, namely the effect of different volume of leaf extract and the effect of metal ions concentration towards the shape and size of AuNPs, AgNPs and bimetallic Au-Ag nanoparticles. The formation of the nanoparticles was monitored by UV-Vis spectroscopy and was characterized by FESEM-EDX, TEM, XRD and FTIR spectroscopes.

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