PRODUCTION OF SILVER NANOPARTICLES USING SPENT COFFEE GROUND FOR ANTIBACTERIAL EFFECT

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DEDICATION

I dedicate my dissertation work to my family and many friends. Deep gratitude to my loving parents, whose words of encouragement and push for tenacity ring in my ears. Thank you to my special sister and brothers who have never left my side.

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ABSTRACT

In the past few years, many efforts were put into developing new greener and cheaper methods for the synthesis of silver nanoparticles (AgNPs). This study opted two different types of spent coffee grounds (SCGs) extracts, obtained from pressed spent coffee grounds (Pr-SCGs) and simple boiled spent coffee grounds (SB-SCGs) for the biosynthesis of AgNPs. Several parameters affecting silver reduction, such as the volume ratio of SCG extracts, the concentration of silver nitrate, pH, temperature and reaction time were investigated to obtained optimal reaction conditions for the preparation of AgNPs. Successful synthesis of Pr-SCGs-AgNPs and SB-SCGs-AgNPs were confirmed via characterization analysis using UV-Vis spectrophotometer, FESEM (Field Emission Scanning Electron Microscopy), EDX (Energy Dispersive X-ray), FTIR (Fourier-transform infrared spectroscopy) and XRD (X-ray diffraction). Finally, Pr-SCGs-AgNPs and SB-SCGs-AgNPs were tested for their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* Pr-SCGs-AgNPs and SB-SCG-AgNPs reduced the silver ions into AgNP within 90 min of heating the reaction mixture (90°C) as indicated by the developed dark brown and reddish-brown colours, respectively. The UV-Vis spectrum of AgNPs revealed a characteristic surface plasmon resonance (SPR) peak at 407 nm and 404 nm for Pr-SCGs-AgNPs and SB-SCGs-AgNPs, respectively. The morphology of both samples was identified in spherical shape with an average size of 19±6 nm and 16±1 nm. The FTIR spectroscopy confirmed the role of SCG as a reducing and capping agent of silver ions by revealing the presence of functional groups, such as carboxyl, hydroxyl, and amine groups, and the crystallinity of the nanoparticles confirmed by XRD is as face-centered cubic (FCC). Pr-SCGs-AgNPs and SB-SCGs-AgNPs showed effective antibacterial activity against the representative bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) results of Pr-SCGs-AgNPs and SB-SCGs-AgNPs for both *E. coli* and *S. aureus* were (5-18) mg/mL, and (3.4-16) mg/mL, respectively. The difference in the coffee brewing process contributed to the variation in the properties of the AgNPs. Overall, Pr-SCGs-AgNPs and SB-SCGs-AgNPs have proven their antimicrobial properties, showing their potential as antimicrobial products in the future.

ABSTRAK

Di kebelakangan tahun ini, pelbagai usaha telah dijalankan bagi membangunkan kaedah hijau yang terkini dan lebih murah bagi penghasilan nanopartikel perak. Kajian ini memilih dua jenis ekstrak serbuk kopi terpakai (SCGs), iaitu serbuk kopi tertekan yang terpakai (Pr-SCGs) dan serbuk kopi dididihkan yang terpakai (SB-SCGs) bagi biosintesis nanopartikel perak (AgNPs). Beberapa parameter yang mempengaruhi penurunan perak, seperti nisbah isipadu ekstrak SCGs, kepekatan perak nitrat, pH, suhu dan masa tindak balas telah diselidik bagi memperolehi keadaan reaksi optima bagi penyediaan AgNPs. Sintesis Pr-SCGs-AgNPs dan SB-SCGs-AgNPs yang berjaya terhasil disahkan melalui analisis pencirian spektrofotometer UV-Vis, mikroskop elektron pengimbasan medan (FESEM), spektroskopi tenaga serakan sinar X (EDX), infra merah transformasi Fourier (FTIR) dan penyerakan sinar X (XRD). Akhir sekali, aktiviti antibakteria Pr-SCGs-AgNPs dan SB-SCGs-AgNPs ke atas *Escherichia coli* dan *Staphylococcus aureus* diuji. Pr-SCGs-AgNPs dan SB-SCGs-AgNPs masing-masing didapati telah menurunkan ion perak kepada nanopartikel perak dalam tempoh pemanasan campuran tindak balas (90°C) selama 90 min, yang ditunjukkan oleh warna coklat gelap dan warna coklat kemerah-merahan yang terhasil. Spektrum UV-VIS AgNPs menunjukkan ciri puncak resonans plasmon permukaan (SPR) di 407 nm dan 404 nm untuk kedua-dua Pr-SCGs-AgNPs dan SB-SCGs-AgNPs. Identifikasi morfologi mendapati kedua-dua Pr-SCGs-AgNPs dan SB-SCGs-AgNPs berbentuk sfera dengan saiz purata masing-masing pada 19±6 nm dan 16±1 nm. Spektroskopi FTIR mengesahkan peranan SCG sebagai agen penurun dan agen pencakupann ion perak, yang ditunjukkan melalui kehadiran kumpulan berfungsi seperti kumpulan karboksil, hidroksil dan amina, serta kehabluran nanopartikel yang terhasil telah dikenalpasti sebagai kubus berpusat muka (FCC) menggunakan XRD. Kedua-dua Pr-SCGs-AgNPs dan SB-SCGs-AgNPs menunjukkan aktiviti antibakteria yang efektif terhadap kedua-dua wakilan bakteria. Keputusan kepekatan perencat minima (MIC) dan kepekatan bakterisidal minima (MBC) Pr-SCGs-AgNPs untuk *E. coli* dan *S. aureus* adalah 5-18 mg/ml, manakala bagi sampel SB-SCGs-AgNPs s pula adalah 3.4-16 mg/mL. Perbezaan dalam proses pembruan kopi didapati menyumbang kepada kepelbagaian sifat AgNPs yang dihasilkan, Secara keseluruhannya, Pr-SCGs-AgNPs dan SB-SCGs-AgNPs telah menunjukkan potensi sebagai bahan antimikrob yang boleh digunakan untuk pembangunan produk antimikrob pada masa hadapan.

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LIST OF ABBREVIATIONS

AgNPs	-	Silver Nanoparticles
DDT	-	Disc Diffusion Technique
DNA	-	Deoxyribonucleic Acid
EDXS	-	Energy Dispersive X-Ray Spectroscopy
XRD	-	X-Ray Diffraction
FT-IR	-	Fourier-Transform Infrared Spectroscopy
ICO	-	International Coffee Organization
MBC	-	Minimum Bactericidal Concentration
MIC	-	Minimum Inhibitory Concentrations
OD	-	Optical Density
pH	-	Potential of Hydrogen
Pr-SCG-AgNPs	-	Pressed Spent Coffee Ground-Silver Nanoparticles
ROS	-	Reactive Oxygen Species
rpm	-	Round Per Minute
S.B-SCG-AgNPs	-	Simple Boiled Spent Coffee Ground-Silver Nanoparticles
SCG	-	Spent Coffee Ground
SCG-AgNPs	-	Spent Coffee Ground-Silver Nanoparticles
FCC	-	Face-centered-cubic
JCPDS	-	Joint Committee for Powder Diffraction Standards
NADH	-	Nicotinamide Adenine Dinucleotide
CLSI	-	Clinical Laboratory Standard Institute
TEM	-	Transmission Electron Microscopy

LIST OF SYMBOLS

Ag	-	Silver
Au	-	Gold
Pb	-	Lead
NaBH4	-	Sodium borohydride
PVP	-	Polyvinylpyrrolidone
PEG	-	Polyethylene Glycol
PMAA	-	Poly (methacrylic acid)
PMMA	-	Poly (methyl methacrylate)
CO2	-	Carbon dioxide
Mt	-	Metric Ton
nm	-	Nanometre
Ag+	-	Silver Ions
$AgNO_3$	-	Silver nitrate
ml	-	Millilitres
cm	-	Centimetre
mg	-	Milligram
h	-	Hour
min	-	Minute
µg	-	Microgram
CFU	-	Colony Forming Unit
(% W/V)	-	Weight/Volume per cent
µL	-	Microlitre
mm	-	Millimetre
D	-	Crystallite size diameter
λ	-	X-ray wavelength
°C	-	Celsius
θ	-	Braggs' angle
K	-	Scherrer constant
β	-	Half-maximum full width
mM	-	Millimolar

A	-	Absorbance of solution at a particular wavelength
ε	-	Molar Absorptivity/ Molar Absorption coefficient
L	-	Path length of the sample/ width of cuvette (cm)
c	-	Concentration of Solution (mol/L)

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

In the past few years, silver nanoparticles (AgNPs) are widely considered in biomedical applications. Most current nanoparticle synthesis technologies depend on the use of dangerous chemicals as reducing and stabilizing agents. While they are extremely effective at producing nanoparticles, they have a significant environmental effect. This has prompted attempts to develop more environmentally friendly AgNPs synthesis methods, such as those based on non-toxic chemicals or biological resources, like bacteria, fungi, and plants (Rolim et al., 2019).

Attempts to replace plant parts with agro-industrial waste have been made to advance greener and more sustainable processes. As a next step, coffee waste is being disposed of as solid waste because it has no economic value. It has also been confirmed that coffee waste extract produces methane, a greenhouse gas that has 20 times the global warming potential compared to carbon dioxide (Chien et al., 2019). However; it can be used for a variety of applications, including composting, gardening, metal ion adsorbent, antioxidant biomaterials, and many others (McNutt & He, 2019).

The extract from spent coffee grounds, like many other wastes such as fruit peels, may have the potential as a source of reducing and capping agents in the AgNPs synthesis (Rónavári et al., 2017). Coffee extract from roasted dry Coffea Arabica seed for instance, was employed for AgNPs synthesis using a green method and was shown to have strong microbial activity (Panzella et al., 2020). This approach reduces the unwanted consequences of waste since it produces less residues and dissolves in water with no side effect.

The biological synthesis of AgNPs appears to solve the problems of conventional methods that use toxic and volatile chemical compounds. In the conventional method, for instance, volatile solvents such as aromatic amines and thiols cause air pollution due to high vapour pressure, including reducing agents such as sodium borohydride and its derivatives. Evaporation-condensation and chemical reduction by organic and inorganic reducing agents are known to be used in the green method, which is deemed as a safe and environmentally friendly process. This process transforms inorganic metal ions into metal nanoparticles via the reductive capacities of the proteins and metabolites present in the biological component (Siddiqi et al., 2018). Sugar, proteins, alkaloids, and phenolic acids are common extract metabolites that play a vital role in the bio-reduction of metal ions into well-defined sizes and morphologies of AgNPs under optimal conditions (Chien et al., 2019).

In this study, the suitability of spent coffee ground (SCG) to produce anti-microbial agents has been assessed as a source of reduction and stabilization. SCG contains substantial quantities of phenolic compounds, such as chlorogenic acid including caffeoylquinic acid, feruloyquinic acid, p-coumaroylquinic acid, and mixed caffeic and ferulic diesters with quinic acid and other metal-reducing substances, although their levels and profiles may differ (Kourmentza et al., 2018; Okur et al., 2021; Seo & Park, 2019). The AgNPs production using coffee seed extracts has been documented by many studies, but very limited information has been reported on coffee extract residuals. By using the pressed and simple boiled spent coffee ground to compare their AgNPs production, it was confirmed that brewed coffee grounds (BCG) could lose some of their bioactive components (Cruz et al., 2012). The SCG extract and SCG-AgNPs properties were then confirmed and characterized before subjected to antibacterial analysis.

1.2 Problem Statement

In general, nanoparticle synthesis is very cost-effective using physical and chemical processes. Generally, such chemicals are highly toxic, flammable and difficult to be disposed (Ahmed et al., 2016). The green synthesis of nanoparticles has recently been favoured as a new method to synthesize AgNPs using microorganisms and plant extracts since they are relatively economical, non-toxic, and environmentally friendly. Apart from that, attempts have been made to replace plants and microbiological cells with home/industrial waste SCG as a step towards greener and more sustainable processes in the biosynthesis of AgNPs. As for the use of plant cells to synthesise AgNPs, more stable nanoparticles are generated compared to other physical and chemical methods, and it is very easy to scale up with a low risk of contamination. In addition to being cost-effective and environmentally sustainable, using biological waste plant products, such as fruit peel and SCG has an enormous potential to synthesize nanoparticle due to the ease of scaling up for larger production (Sharma et al., 2016). The high phenolic content in the SCG extract obtained from the different brewing coffee methods was evaluated for its feasibility as reductant and synthesizing small sized and stable AgNPs.

1.3 Research Objectives

The research objectives are:

a. To prepare spent coffee ground (SCG) extract using the press and simple boiled coffee ground via solid-liquid extraction method.
b. To synthesize silver nanoparticles using SCG extract and characterize them.
c. To evaluate the antibacterial efficacy of Pr-SCG-AgNPs and SB-SCG-AgNPs against *Escherichia coli* and *Staphylococcus aureus*.

1.4 Significant of the study

The most important achievement in this research is to synthesize AgNPs using biological method from SCG. This approach will enable us to improve this waste with a value-added potential to produce metal-based nanomaterial. The antibacterial efficiency of the SCG-AgNPs against *E. coli* and *S. aureus* is important to promote reusing coffee ground for bioreduction and antibacterial application.

1.5 Scope of the study

This work has examined the characteristics of extracting two spent coffee ground samples, pressed SCG (Pr-SCG) and simple boiled SCG (SB-SCG), for AgNPs biosynthesis, and investigated its antimicrobial activity against *S. aureus* and *E. coli* using minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. The waste from coffee seeds was extracted using distilled water as a solvent. AgNPs biosynthesis was executed through a reaction of the coffee extract from the coffee seeds waste with $AgNO_3$. In addition, several different parameters have been adjusted to the optimum condition for AgNPs biosynthesis. The volume ratio of coffee ground extract, temperature, pH, $AgNO_3$ concentration and reaction time were determined afterwards. The AgNPs produced under its optimum conditions were characterized using ultraviolet visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR), field emission scanning electron microscopy (FESEM), energy dispersive X-ray spectroscopy (EDXS) and X-ray diffraction (XRD) to determine the presence of the functional group responsible for reducing Ag+ to AgNPs, size, shape, structure, intense signal of Ag and crystallinity nature of AgNPs. Furthermore, the pressed SCG-AgNPs (Pr-SCG-AgNPs) and simple boiled SCG-AgNPs (SB-SCG-AgNPs) were tested for antibacterial activities.

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