BIOSYNTHESIS OF ANTIBACTERIAL SILVER NANOPARTICLES USING PLANT ORGANS OF *Ficus deltoidea* Jack var. *kunstleri* (King) Corner

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ABSTRACT

The wide range of silver nanoparticle (AgNP) applications in industrial and biomedical disciplines has elevated their demand. Physical and chemical methods of AgNP synthesis are ecological harmful due to the generation of toxic by-products and high energy consumption. However, this drawback can be solved by using plant extract to synthesize AgNP because it is environmentally friendly and cost-effective. Hence, this study explored and evaluated the ability of extracts from different plant organs (leaf, stem, fig and root) of *Ficus deltoidea* Jack var. *kunstleri* (King) Corner (Mas Cotek) on AgNP production. The AgNP was characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), energy dispersive X-ray (EDX), and thermogravimetric-differential thermal analysis (TGA-DTA). The result showed that liquid chromatography mass spectroscopy (LCMS) detected more than 100 phytochemical compounds present in the extracts and approximately 50% of them belong to phenolic and flavonoid compounds. Vitexin and isovitexin were discovered only in leaf, while fig had the highest total phenolic content (TPC) and total flavonoid content (TFC). Approximately, 92% of 107 mg/L Ag^{+} precursor was successfully collected as AgNP when stem extract was used while 71%, 48% and 39% for AgNP-Root, AgNP-Fig and AgNP-Leaf, respectively. Outcome from the optimization of synthesizing condition, the highest peak of the localized surface plasmon resonance (LSPR) from AgNP-Fig was observed which was 5.7 (a.u.), which aligned with the high TPC and TFC values. The optimal synthesizing conditions for each organ extract in synthesizing AgNP were different; leaf (1.0 mL, 30 hours, 60°C, pH 10), stem (1.0 mL, 21 hours, 90°C, pH 12), fig (0.8 mL, 33 hours, 100°C, pH 10) and root (3.0 mL, 21 hours, 90°C, pH 12). All AgNP was found to be stable for 26 days. It was observed that the AgNP particles were spherical, and the size was in the order of AgNP-Root (15.4 ± 3.4 nm) < AgNP-Stem < AgNP-Fig < AgNP-Leaf. Meanwhile, AgNP-Root and AgNP-Stem showed antibacterial activity when tested using disc diffusion technique (DDT) and minimum inhibitory/bactericidal concentration (MIC/MBC) against *Escherichia coli* and *Staphylococcus aureus* bacteria due to its small particle size. In addition, visible damage on the bacterial wall of *S. aureus* was observed when treated with AgNP-Stem. Finally, human skin fibroblast (HSF 1184) cells exhibited lower viability when treated with AgNP-Root and AgNP-Stem compared to AgNP-Fig and AgNP-Leaf. In conclusion, this research showed the diversity and complexity of phenolic and flavonoid compounds in each plant organs of *F. deltoidea* were able to synthesize distinct characteristics of AgNP, and subsequently small-sized of AgNP-Root and AgNP-Stem significantly impacted their antibacterial property.

ABSTRAK

Pelbagai aplikasi nanozarah argentum (AgNP) dalam bidang perindustrian dan bioperubatan telah meningkatkan permintaan pasaran mereka. Penggunaan kaedah fizikal dan kimia dalam menghasilkan AgNP yang memudaratkan ekologi kerana kaedah ini menjana keluaran sampingan yang toksik serta pengguna tenaga yang tinggi. Walau bagaimanapun, kelemahan ini boleh diatasi dengan menggunakan ekstrak organ tumbuhan untuk mensisntesis AgNP kerana ia merupakan teknik yang kos efektif dan hijau. Justeru, ujikaji dan penerokaan potensi mengenai ekstrak organ tumbuhan (daun, batang, ara dan akar) pokok herba *Ficus deltoidea* Jack var. *kunstleri* (King) Corner (Mas Cotek) dalam mensintesis antibakteria AgNP dijalankan. AgNP yang terhasil telah dicirikan menggunakan pembelauan sinar-X (XRD), spektroskopi inframerah transformasi Fourier (FTIR), mikroskopi elektron transmisi (TEM), serakan tenaga sinar-X (EDX) dan termogravimetri-terma kebezaan (TGA-DTA. Hasil daripada analisis spektrometri jisim kromatografi (LCMS) telah menemui lebih dari 100 sebatian fitokimia pada setiap organ dan dianggarkan 50% merupakan sebatian fenolik dan flavonoid. Viteksin dan isoviteksin pula hanya dijumpai di dalam daun, manakala kandungan jumlah fenolik (TPC) serta kandungan jumlah flavonoid (TFC) didapati tertinggi pada bahagian ara. Dianggarkan 92% AgNP dari 107 mg/L pelopor Ag^+ berjaya diperoleh semula apabila estrak batang digunakan (AgNP-Stem), manakala pada bahagian akar (AgNP-Root), ara (AgNP-Fig) dan daun (AgNP-Leaf) adalah masing-masing pada 71%, 48% dan 39%. Hasil daripada pengoptimuman keadaan sintesis, puncak tertinggi resonans plasmon permukaan setempat (LSPR) diperhatikan dari AgNP-Fig iaitu 5.7 (a.u.), yang mana keputusan ini sejajar dengan nilai TPC dan TFC. Sementara itu, keadaan sinthesis yang ideal bagi ekstrak tumbuhan mensintesis AgNP telah ditentukan; daun (1.0 mL, 30 hours, 60°C, pH 10), batang (1.0 mL, 21 hours, 90°C, pH 12), ara (0.8 mL, 33 hours, 100°C, pH 10) dan akar (3.0 mL, 21 hours, 90°C, pH 12). Kesemua AgNP didapati stabil selama 26 hari. perincian mendapati AgNP adalah berbentuk sfera dan saiz mengikut urutan AgNP-Root (15.4 ± 3.4 nm) < AgNP-Stem < AgNP-Fig < AgNP-Leaf. Seterusnya, AgNP-Root dan AgNP-Stem menunjukkan sifat antibakteria apabila dinilai menggunakan kaedah peresapan disk (DDT) dan kepekatan perencatan/ bakteriasid minimum (MIC/MBC) terhadap bakteria *Escherichia coli* dan *Staphylococcus aureus*. Akhir sekali, sel fibroblas kulit manusia (HSF 1184) menunjukkan kebolehhidupan yang kurang apabila dirawat dengan AgNP-Stem dan AgNP-Root berbanding AgNP-Fig dan AgNP-Leaf. Secara keseluruhannya, kajian ini menunjukkan kepelbagaian sebatian fenolik dan flavonoid dalam setiap organ *F. deltoidea* menghasilkan AgNP dengan ciri yang tersendiri dan seterusnya menghasilkan AgNP-Root dan AgNP-Stem yang bersaiz kecil dan mempunyai sifat antibakteria.

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LIST OF ABBREVIATIONS

AgNP	-	Silver nanoparticle
AgNP-Leaf	-	Silver nanoparticle that had been biosynthesized using leaf
AgNP-Stem	-	Silver nanoparticle that had been biosynthesized using stem
AgNP-Fig	-	Silver nanoparticle that had been biosynthesized using fig
AgNP-Root	-	Silver nanoparticle that had been biosynthesized using root
Ag^+	-	Silver ions
ATCC	-	American Type Culture Collection
ATP	-	Adenosine triphosphate
AFM	-	Atomic force microscopy
cDMEM	-	Complete Dulbecco Modified Eagle Media
CE	-	Catechin equivalent
CPD	-	Critical point drying
CV	-	Coefficient of variance
DDT	-	Disc diffusion technique
DI	-	Deionized
DMSO	-	Dimethyl sulfoxide
DNA	-	Deoxyribonucleic acid
DPPH	-	2,2-diphenyl-1-picryl-hydrazyl-hydrate
DLS	-	Dynamic Light Scattering
EDX	-	Energy dispersive X-ray spectrometer
Eurachem	-	The Fitness for Purpose of Analytical Methods
ECACC	-	European Cell Culture Association
FTIR	-	Fourier transform infrared spectrometer
FESEM	-	Field emission scanning electron microscopy
FRIM	-	Forest Research Institute Malaysia
FRAP	-	Ferric reducing ability of plasma
GAE	-	Gallic acid equivalent
HSF	-	Human skin fibroblast
HPLC	-	High performance liquid chromatography
ICPOES	-	Inductively coupled plasma – optical emission spectrometer
LCMS	-	Liquid chromatography mass spectrometer

LSPR	-	Localized surface plasmon resonance
MIC	-	Minimum inhibitory concentration
MBC	-	Minimum bactericidal concentration
MHA	-	Mueller-Hinton agar
MHB	-	Mueller-Hinton broth
NCL	-	Nanotechnology Characterization Laboratory
NMR	-	Nuclear magnetic resonance
NATA	-	National Association of Testing Authorities
OFAT	-	One-factor-at-a-time
OD	-	Optical density
PBS	-	Phosphate buffer saline
QE	-	Quercetin equivalent
QC	-	Quality control
ROS	-	Reactive oxygen species
RSD	-	Relative standard deviation
RT	-	Retention time
SEM	-	Scanning electron microscope
SPR	-	Surface plasmon resonance
SDG	-	Sustainable Development Goals
TPC	-	Total phenolic content
TFC	-	Total flavonoid content
TEM	-	Transmission electron microscope
TGA-DTA	-	Thermogravimetric - differential thermal analysis
TIC	-	Total ion chromatography
UV	-	Ultraviolet
WHO	-	World Health Organization
XRD	-	X-ray diffraction
XPS	-	X-ray photoelectron spectroscopy

LIST OF SYMBOLS

°C	-	Degree Celsius
°	-	Degree
%	-	Percent
µm	-	Micrometre
nm	-	Nanometre
mm	-	Millimetre
cm	-	Centimetre
m	-	Metre
µg	-	Microgram
mg	-	Milligram
g	-	Gram
kg	-	Kilogram
µL	-	Microlitre
mL	-	Millilitre
L	-	Litre
w/w	-	Weight over weight
kV	-	Kilovolt
θ	-	Theta
r^2	-	Correlation coefficient
λ_{max}	-	Maximum wavelength
Å	-	Angstrom
min	-	Minute
M	-	Molar
mM	-	Millimolar
ppm	-	Part per million
CFU	-	Colony forming unit
m/z	-	Mass to ion ratio
rpm	-	Rotation per minute

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Among nanoparticles, silver nanoparticle (AgNP) is considered the most dynamic and exciting nanomaterial because of its toxicity to bacteria and extensive application in research and industrial sectors such as for drug delivery and cell imaging in the biomedical field, as fuel cell catalyst, food packaging films and in membrane filter in water treatments plant (Zhang et al., 2016; Keshari et al., 2020; Liao et al., 2019). The physical and chemical properties of AgNP are crucial factors in determining its usage in related fields (Lee & Jun, 2019). In addition, synthesizing is critical to get the best quality of AgNP (Zhang et al., 2016). Currently, chemical and physical methods are commonly used to synthesize AgNP (Paula et al., 2020). The physical synthesis of AgNP includes the evaporation-condensation method and the laser ablation technique, which are capable of producing huge amounts of AgNP; nevertheless, agglomeration is sometimes a significant difficulty because capping agents are not used (Guzel et al., 2014; Lee et al., 2019). Furthermore, both processes consume more electricity, have a longer synthesis time, and require more complex equipment, expanding operational costs (Lee et al., 2019). Meanwhile, the chemical reduction method entails using harmful chemicals that generate toxic and non-ecological compounds (Alves et al., 2019). For example, diborane is produced when sodium borohydride is utilized as a reducing agent (Hu et al., 2021). According to the safety data sheet, diborane can cause skin and eye irritation and cause damage to organs such as the lung, kidney and central nervous system. Furthermore, both approaches are often associated with problems such as low stability of AgNP due to the lack of capping agent, uncontrolled crystal growth and aggregation of particles with a slight change in the temperature and/or pH (Nezhad et al., 2020). Therefore, the trend of synthesizing AgNP has shifted to biosynthesis methods, which are considered cost-effective and safe (Ovais et al., 2018). Biosynthesis methods are able to overcome the limitations and challenges faced when synthesizing AgNP using conventional methods and avoid harmful by-products (Kandiah et al., 2021; Zhang et al., 2016).

The differences in the physicochemical properties of AgNP significantly impact antibacterial and toxicity. Specifically, the size of AgNP affects antibacterial (Slavin et al., 2017) and cytotoxicity activities (He et al., 2017). Smaller AgNP have higher antibacterial activity and greater stability. This is because smaller nanoparticles have a larger surface area, which allows for more significant contact and increased intracellular penetration (Roy et al., 2019). Whereas smaller-sized AgNP exhibited a more significant cytotoxicity effect since it could enter the mitochondria of a human fibroblast cell by multiple mechanisms, causing oxidative stress and eventually cell death (Avalos et al., 2016). For this reason, this study attempted to evaluate the antibacterial activity of biosynthesized AgNP using *Ficus deltoidea* Jack var. *kunstleri* (King) Corner, which has not been reported yet. This research focused on synthesizing AgNP using different plant organs: leaf, stem, fig, and root. This study was the first attempt to analyze the antibacterial activity of AgNP that were biosynthesized using leaf (AgNP-Leaf), stem (AgNP-Stem), fig (AgNP-Fig), and root (AgNP-Root). The antibacterial property of the biosynthesized AgNP was tested against *Staphylococcus aureus* and *Escherichia coli*, representing Gram-positive and Gram-negative bacteria. Furthermore, these two bacteria are the most prevalent pathogens in humans, causing healthcare-associated illnesses (Poolman et al., 2018). In addition, the potential cytotoxicity assay of the biosynthesized AgNP against human skin fibroblast (HSF 1184) cells was also evaluated.

1.2 Problem Statement

Silver and silver-based ions have been used in medicinal and antibacterial applications since the nineteenth century (Chopra, 2007). The suitability of applying AgNP in healthcare, the food industry, and other large-scale industries is attributed to its unique chemical and physical properties (Kalia et al., 2020). Nowadays, nanoparticles are manufactured using physical and chemical methods, producing low stable AgNP and by-products that are harmful to the environment (Akbari et al., 2017). These methods rely on the site equipment capability and involve using harmful reduction chemicals such as sodium borohydride and toxic solvents, such as sodium dodecyl benzyl sulfonate (Roy et al., 2019). To overcome these problems, biosynthesized method for producing AgNP is preferable. The biosynthesis of AgNP involved reduction reactions using bacteria and phytochemical compounds in plant extracts and fungi (Gudikandula et al., 2016). The bacteria, plants, and fungi contain peptides, phytochemical compounds, and modified cell walls that attached and reacted to metal ions and finally formed stable nanoparticles (Pantidos et al., 2015). Additionally, the biosynthesized method for producing AgNP is simple, safe, and reliable.

F. deltoidea is a shrub that has broadly spoon-shaped to obovate (ovate with the narrower end at the base) leaves. It has bright green (above) and rust-red to olive (beneath) coloured leaves, as well as a whitish-grey stem or bark, and produces figs that are spherical to round (Bunawan et al., 2014). Furthermore, the shrub possesses fibrous brown roots (Din et al., 2021). Several investigations have been conducted on the phytochemical compounds in the leaf of *F. deltoidea*. However, comparative research on the different plant organs is still lacking. Thus, this study aimed to assess the total phenolic and flavonoid contents of the aqueous extract of *F. deltoidea* leaf, stem, fig, and root. Findings regarding the relationship between phenolic and flavonoid contents and AgNP synthesis could pave the way for more research into the production of biosynthesized AgNP.

Age, climate, plant organ, environment, stress, season, and time of harvest significantly affected phytochemical compound detection in plants (Manurung et al., 2017). Phytochemical compounds composition differences can be found between plant organs and those between populations (Lavola et al., 2017). Depending on their physiological functions, these phytochemical compounds are diverse in plant organs (Korkina et al., 2017). Phytochemical compounds in plants play crucial roles relating to survival, protection, defence propagation, growth and development (Huang et al., 2016). These phytochemical compounds have been used in recent studies to stimulate the synthesis of AgNP from silver nitrate precursors as reducing agents

(Carson et al., 2020; Vo et al., 2021). Vo et al. (2021) confirmed that phytochemical compounds present during synthesis act as a capping and stabilizing agent for the AgNP. In line with that, this study aimed to evaluate the effects of different plant organs of *F. deltoidea* on the biosynthesis of AgNP by comparing the synthesizing results using different parameters. The parameters include the pH of plant extract, reaction time, reaction temperature, and volume of plant extracts required to yield AgNP consistently and productively. The information obtained from these parameters was used to enhance the control of the morphology, size, and optimal production rate of AgNP. It also paves the way for an up-scalable process and highly stable AgNP. Based on a previous study, the highest intensity in the visible peak was considered for the optimization of the AgNP (Kalyani et al., 2019). This study utilizes the available resources in the laboratory to the maximum benefit.

Phytochemical compounds serve as a capping and stabilizing agent for AgNP, making it safer since it reduces the dissolution rate of AgNP. *Eisenia fetida* and *Eisenia andrei* worm showed higher survival rates when exposed to AgNP compared to Ag^{+} (Baccaro et al., 2021; Brami et al., 2017). Meanwhile, the dissolution rate is affected by several reactions coinciding and depends on the morphology of AgNP, such as size, shape, and capping phytochemical compounds (Gilbertson et al., 2018). The phytochemical compounds that capped the biosynthesized AgNP will help the continuous controlled release of the silver ions. Hence, the particle characterization of the biosynthesized AgNP is essential. This is because the physicochemical properties of AgNP could significantly affect its biological properties. The characteristic features of the biosynthesized AgNP, such as size, shape, and capping phytochemicals were evaluated, followed by the antibacterial activity against Gram-positive and Gram-negative bacteria.

According to Al-Talib et al. (2019), about 10% of admitted patients would contract the nosocomial infection after a prolonged stay in hospitals in Malaysia. Nosocomial infections are referred to as infections occurring after 48 hours of hospital admission or three days of discharge. Gram-positive bacteria, *S aureus* and Gram-negative bacteria, *E. coli,* have become endemic in healthcare facilities as they are associated with nosocomial infections (Poolman et al., 2018). *E. coli* and *S.*

aureus are commonly known as foodborne pathogens, and they cause illness at infectious doses via the consumption of contaminated food (Carson et al., 2020). More importantly, the infections caused by foodborne pathogens have become a common public health issue worldwide (Carson et al., 2020). This study was therefore conducted to evaluate the capability of biosynthesized AgNP using plant organs of *F. deltoidea*, which acted as an antibacterial agent for these two bacteria. The antibacterial activity and effect of the biosynthesized AgNP were evaluated against Gram-positive and Gram-negative bacteria that possess different types of cell membranes; which are a thick layer of cell wall which are composed of peptidoglycan in *S. aureus* and thin three layers of the cell membrane (outer, peptidoglycan and inner) of *E. coli* (Slavin et al., 2017).

The usage of Ag compounds such as silver nitrate ($AgNO_3$) and silver sulfadiazine ($C_{10}H_9AgN_4O_2S$) can cause cosmetic abnormalities after prolonged treatment. This disorder is known as argyria, which results from the formation of blue-grey colouration. Furthermore, it can also antagonise the healing process of fibroblast and epithelial cell toxicity (Galandakova et al., 2015). Avalos et al. (2016) stated that the toxicity of AgNP depended on the size, whereby the smaller size of AgNP exhibited higher cytotoxicity compared to the large type. Smaller AgNP is more likely to enter the membrane wall and cause cytotoxicity to the cell because they tend to accumulate in the mitochondria, causing mitochondrial dysfunction and promoting reactive oxygen species (ROS) production, eventually leading to the damage of intracellular proteins and nucleic acids (Liao et al., 2019). Thus, this study was designed to evaluate the cytotoxicity of the biosynthesized AgNP from *F. deltoidea* of different plant organs with distinct characteristics against normal human skin fibroblast cells.

1.3 Research Objectives

The objectives of the research are:

(a) To analyse the phytochemical compounds in *Ficus deltoidea* Jack var. *kunstleri* (King) Corner aqueous extract from different organs (leaf, stem, fig and root).

(b) To evaluate the effect of different synthesis parameters on the AgNP using the plant organs of *F. deltoidea*.

(c) To characterize the biosynthesized AgNP for its physicochemical properties.

(d) To analyse the antibacterial activity of biosynthesized AgNP against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and evaluate the *in vitro* cytotoxicity against normal human fibroblast cells.

1.4 Scope of Research

At the early stage of the research, the focus was on plant analysis, where the phytochemical compounds in each organ were identified, followed by the determination of phenolic and flavonoid contents. Then the focus shifted to synthesizing AgNP using different plant organs of *F. deltoidea*. In this particular process, the optimum synthesizing conditions were determined, and as a result, the characteristic of the biosynthesized AgNP was evaluated using various scientific instruments. Finally, the antibacterial activity of AgNP was assessed against Gram-positive and Gram-negative bacteria and *in vitro* cytotoxicity against normal human fibroblast cells.

This study aimed to synthesize AgNP from different plant organs (leaf, stem, fig and root) of the *F. deltoidea* plant and evaluated their antibacterial capability. To date, *F. deltoidea* has not been used as a reducing agent for AgNP. Furthermore, this study utilized the whole plant organs. This research approach provides an overview of the diversity of phytochemical compounds in each organ affecting the characteristics of AgNP and eventually affecting its antibacterial property. *F. deltoidea* was chosen because this herb had been reported to contain several phytochemical compounds based on previous studies (Abrahim et al., 2018; Bunawan et al., 2014). Moreover, those phytochemical compounds were found in other plants that were used to reduce other nanomaterials (Kalia et al., 2020; Pradeep et al., 2022). The phytochemical compounds of *F. deltoidea* were screened using liquid chromatography mass spectrometry (LCMS). Each peak in LCMS represents a different phytochemical compound based on the library installed. The high peak area indicated the rich components in the extract. LCMS results were used to identify the phenolics and flavonoid constituents present in the samples (Bakari et al., 2018). Fourier transform infrared spectrometry (FTIR) was employed to identify the functional groups present in the phytochemical compounds. Total phenolic content (TPC) and total flavonoid content (TFC) were analyzed for all plant organs, which provided an overview of the sources of antioxidants in the plant. While in this study, high performance liquid chromatography (HPLC) was used to determine the concentrations of vitexin and isovitexin in all plant organs as a continuation of the previous study that had found these two substances in the leaf of *F. deltoidea*.

In order to obtain productive and consistent biosynthesized AgNP, different conditions or parameters were investigated. Specific conditions of nanoparticle synthesis have a decisive influence on the more controlled morphology and production of nanoparticles. A study by Smiechowicz et al. (2021) showed that the volume of plant extract, reaction time, reaction temperature and pH are the most critical factors influencing the synthesis rate. These parameters were optimized to ensure adequate reducing and capping agents, and time was provided for the synthesizing. While small-sized AgNP was observed to be produced with the effect of pH of plant extract and reaction temperature (Marciniak et al., 2020). The synthesize parameters involved are; volume of plant extracts (0 – 10 mL), reaction time (0 – 36 hours) reaction temperature (30 – 100°C), and pH of plant extracts (pH 2 – pH 12). The production and stability of AgNP were observed using a visible spectrophotometer at 400 – 500 nm. Figure 1.1 shows the overall method used in synthesizing the AgNP.

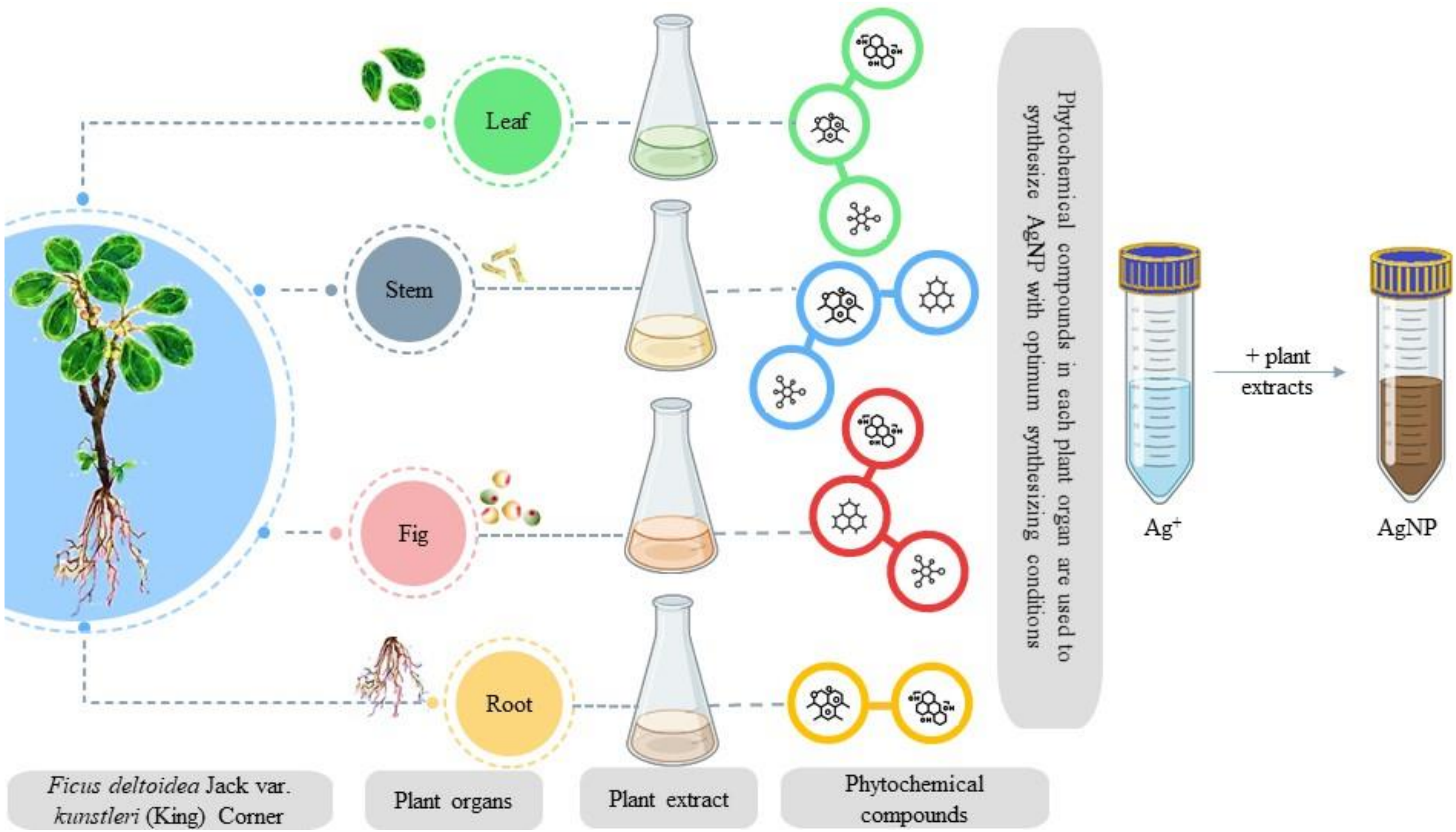

Figure 1.1 The schematic diagram on the synthesizing of AgNP using different plant organs of *F. deltoidea*.

The product obtained from the synthesis was characterized to gain information about the size, shape, and capping phytochemical compounds of the AgNP. The phase and capping agent analysis were performed using FTIR, *X-ray diffraction analysis* (XRD), and thermogravimetric-differential thermal analysis (TGA-DTA). Meanwhile, the morphology of the synthesized AgNP was examined using a transmission electron microscope (TEM). Besides, the elemental analysis was carried out using energy dispersive X-ray spectrometry (EDX). The percentage of AgNP recovery after centrifugal was analyzed using inductively coupled plasma – optical emission spectrometry (ICPOES).

Antibacterial activity and *in vitro* cytotoxicity against normal human fibroblast cells were conducted for the biosynthesized AgNP. The antibacterial assay was performed based on the disc diffusion technique (DDT) against Gram-positive bacteria, *S. aureus* (ATCC 6538) and Gram-negative bacteria, *E. coli* (ATCC 11229). The formation of the inhibition zone was evaluated. Subsequently, the minimum inhibitory concentration (MIC) analysis was performed to determine the lowest concentration (mg/mL) of AgNP synthesized from all plant organs that inhibits the growth of *S. aureus* and *E. coli*. Besides that, the minimum bactericidal concentration (MBC) was evaluated to confirm the absence of bacterial growth relative to the MIC value. The results provided an overview of the characteristics of AgNP, which has good antibacterial activity. Next, cytotoxicity of the biosynthesized AgNP of different plant organs of *F. deltoidea* against human fibroblast (HSF 1184) cells was evaluated using a cell viability assay. The scope of the study encompasses plant analysis, synthesis, characterization, antibacterial assay, and cytotoxicity evaluation. The outline of the research stage-wise for the synthesis, characterization, and application of the material is shown in Figure 1.2.

1.5 Significance of Research

In recent years, the biological method to synthesize AgNP has drawn a great deal of attention due to the low-cost, non-toxic, and environmentally friendly features of the technique. The biological method of synthesizing AgNP utilizes the biological properties of plant extracts or microorganisms cultures, such as bacteria, fungi, and yeast (Singhal et al., 2017). AgNP that is synthesized using plant extract is advantageous as the phytochemical compounds can act as a capping agent. This capping agent will stabilize the AgNP and maintain the colloidal property, thereby avoiding agglomeration and preventing uncontrolled growth (Gulati et al., 2018). Not only do the conventional physical and chemical methods used to synthesize AgNP generate unstable AgNP, but they also harm the environment because they produce harmful by-products that are toxic to the environment (Vorobyova et al., 2020). Furthermore, the AgNP yielded by those methods cannot be employed in clinical fields due to a lack of knowledge regarding health-related issues and toxicity (Kandiah et al., 2021).

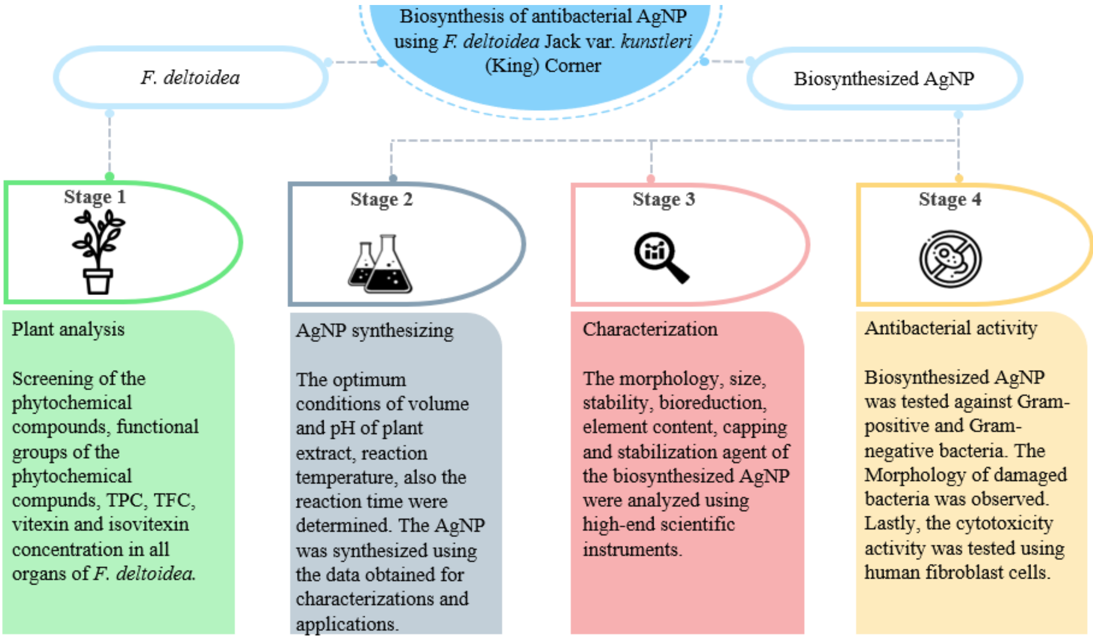

Figure 1.2 The outline of this research in stage-wise diagram.

The AgNP is known to have a wide range of applications in agronomy, beauty products, defence, environmental safety, food, health care, and medicine (Nahar et al., 2020). Inshakova et al. (2017) stated that Global Market Ins. Analysis indicated that AgNP is the most commercialized nanoparticle, with over 50% of consumer products worldwide in 2015. It is estimated to grow by 13% between 2016 and 2024, which expected improvement in the applications of AgNP in healthcare and life sciences, food and beverages packaging industries (packaging and active packaging), electronics, and information technology. Hence, this research has significant competitiveness and holds a high-value market demand.

As a result, this research uses all the plant organs of *F. deltoidea*, which possess competitiveness and commercial value in its marketed fields. Each plant organ contains various phytochemical compounds that affect the AgNP properties. This condition facilitates the manipulation of the AgNP according to the desired requirements. The usage of the whole plant will benefit a large-scale company known to use all resources optimally.

This study uses safe resources and green methods while refraining from applying harmful chemicals. Hence, these procedures lead to the production of a non-toxic by-product that is environmental friendly and meets the criteria of Sustainable Development Goals (SDG) 12: Ensure sustainable consumption and production patterns in the United Nations SDG (Abdullah et al., 2021). The objective of the framework is to create prosperity while safeguarding the environment. The member of SDG acknowledges that eradicating poverty requires strategies that promote economic growth and fulfil various social necessities, such as education, health, social protection, and job prospects while tackling climate change and protecting the environment. Since 2014, Malaysia has pledged its commitment, and members of the United Nations has dedicated themselves to working toward this goal by 2030.

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LIST OF PUBLICATIONS

1. **Din, S. M**., Malek, N. A. N. N., Shamsuddin, M., & Matmin, J. (2021). Effect of Plant Organs of *Ficus deltoidea* in the Synthesize of Silver Nanoparticles. *Malaysian Journal of Analytical Sciences*, *25*(5), 716–727.
2. **Din, S. M**., Malek, N. A. N. N., Shamsuddin, M., Matmin, J., Hadi, A. A., & Asraf, M. H. (2022). Antibacterial Silver Nanoparticles using Different Organs of *Ficus deltoidea* Jack var. *kunstleri* (King) Corner. *Biocatalysis and Agricultural Biotechnology*, *44*, 102473.