IN SITU SYNTHESIS OF BIO-GREEN SILVER NANOPARTICLES-INCORPORATED ZEOLITE A USING *Orthosiphon aristatus* LEAVES EXTRACT FOR ANTIBACTERIAL WOUND HEALING

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DEDICATION

This thesis is fully dedicated to my late grandmother, supervisors, families, friends and colleagues for all the knowledge and sharing given to me along this challenging path. It is also dedicated to myself for the persistence and perseverance, which I was able to put upon myself from the starting line until the end of this meaningful journey.

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ABSTRACT

Silver in its various forms is well known to have a potent antibacterial property. Despite the high antibacterial activity and efficacy of silver nanoparticles (AgNP), its frequent use could lead to bacterial resistance. Without a proper release mechanism, the efficacy of AgNP is often questioned. Additionally, chemical and physical methods to synthesize AgNP pose threats to the environment and health. Thus, alternative approach using biological resources are desired. However, AgNP produced through this method still needs preclinical evaluation on toxicity and biocompatibility. Thus, a novel *in situ* biosynthesis of AgNP-incorporated synthesized zeolite A (AgNP-SZ) was developed. The AgNP-SZ was then assessed for their antibacterial activity, *in vitro* cytotoxicity and wound healing potency. Zeolite A (SZ) was synthesized from kaolinite through hydrothermal method whereas AgNP was produced from $AgNO_3$ using *Orthosiphon aristatus* leaves extract as the green reducing and capping agent. The AgNP-SZ was synthesized using 0.4 mL 5 % *O. aristatus* leaf extract solution and mixed physically with Ag-SZ. The synthesized materials SZ, Ag-SZ and AgNP-SZ were characterized for their morphological and physicochemical properties. In the present study, the characterization results validated that the synthesized product was zeolite A. Characterization by Transmission Electron Microscope (TEM) showed AgNP with particle size of 20.01 nm in diameter and area of 381.61 nm^2 was incorporated in the zeolite A. TEM analysis, surface and pore analysis (BET/BJH), thermogravimetric and differential temperature analysis (TGA-DTA), and inductively coupled plasma-optical emission spectrometry (ICP OES) were used to assess the synthesized products. These characterizations validated the *O. aristatus* leaves extract acted as natural reducing and capping agents with a timely release mechanism of AgNP from zeolite A. SZ, Ag-SZ and AgNP-SZ were assessed for antibacterial activity against *E. coli* and *S. aureus* using disc diffusion technique (DDT) and minimum inhibitory/bactericidal concentration (MIC/MBC), biofilm inhibition against *P. aeruginosa*, *in vitro* cytotoxicity against human skin fibroblast (HSF 1184) cells and wound healing potency through *in vitro* scratch assay. The powder form of the samples was pressed into pellets for DDT, whereas MIC/MBC and biofilm study utilized the powder form in both water and saline solution. Inhibition zones and bacterial growth inhibition were observed. The DDT showed clear zone of inhibitions for Ag-loaded materials on both bacteria, with *E. coli* was more susceptible than *S. aureus* in both water and saline solutions based on the MIC/MBC values. The AgNP-SZ also showed potential biofilm inhibition action against *S. aureus* compared to *P. aeruginosa*. SZ, Ag-SZ and AgNP-SZ at 0.5, 1.0, 1.5, and 2.0 mg/mL were tested for cytotoxicity. *In vitro* scratch assay determined the HSF 1184 cell migration rate after treatment with the synthesized products. The absence of cytotoxicity in all concentrations of AgNP-SZ proved that the material is biocompatible. Although cell migration rate by AgNP-SZ was slower compared to the SZ and control in *in vitro* scratch assay, the material did not hinder cell migration and proliferation. These findings show the potential of green synthesized AgNP-incorporated zeolite A using plant extract to substitute conventional methods, with good antibacterial application and sustainable production.

ABSTRAK

Argentum dalam pelbagai bentuk adalah sangat terkenal dengan ciri antibakteria yang kuat. Meskipun mempunyai aktiviti antibakteria yang tinggi, penggunaan nanozarah Argentum (AgNP) yang kerap menyebabkan kerintangan bakteria. Tanpa mekanisme pelepasan yang terkawal, keberkesanan AgNP sering dipertikaikan. Tambahan pula, kaedah sintesis secara kimia dan fizikal menjadi ancaman kepada alam sekitar dan kesihatan. Justeru, kaedah alternatif menggunakan sumber biologi adalah diperlukan. Namun, AgNP yang terhasil menggunakan sumber biologi masih memerlukan ujian pra-klinikal toksisiti dan bioserasi. Oleh itu, kaedah baharu biosintesis *in situ* AgNP sebatian sintesis zeolit A (AgNP-SZ) telah dibangunkan. AgNP-SZ telah dinilai berdasarkan aktiviti antibakteria, kadar ketoksikan sel, dan keupayaan menyembuh luka *in vitro*. Zeolit A (SZ) dihasilkan daripada kaolinit melalui proses hidroterma, manakala AgNP dihasilkan menggunakan ekstrak daun *Orthosiphon aristatus* sebagai agen penurun dan penukup. Penghasilan *in situ* ini telah dijalankan dengan menggunakan 0.4 mL larutan ekstrak daun *O. aristatus* (5%). Dalam kajian ini, kesemua sampel SZ, Ag-SZ dan AgNP-SZ telah dicirikan sifat morfologi dan fizikokimia mereka. Pencirian ini telah mengesahkan penghasilan zeolit A. Pencirian mikroskopi elektron transmisi (TEM) menunjukkan penyebatian AgNP dengan saiz zarah berdiameter 20.01 nm dan luas 381.61 nm^2 dalam struktur rangka zeolit A. Analisis TEM, kaedah BET/BJH, analisis termogravimetri (TGA-DTA), dan spektrometri pancaran optik-plasma terganding induktif (ICP-OES) telah digunakan untuk menilai produk yang disintesis. Pencirian tersebut mengesahkan bahawa ekstrak daun *O. aristatus* bertindak sebagai agen penukupan dan perlepasan terkawal AgNP daripada zeolit A. SZ, Ag-SZ dan AgNP-SZ telah dinilai menggunakan kaedah peresapan cakera (DDT) dan kepekatan merencat/bakteriasid minimum (MIC/MBC) terhadap *Escherichia coli* dan *Staphylococcus aureus*, perencatan biofilem terhadap *Pseudomonas aeruginosa*, serta analisis ketoksikan dan potensi menyembuh luka melalui ujian cakar *in vitro* ke atas sel fibroblaskulit manusia (HSF 1184). Sampel berbentuk serbuk diproses menjadi palet untuk digunakan dalam ujian DDT, manakala sampel berbentuk serbuk digunakan dalam ujian MIC/MBC dan biofilem di dalam larutan air dan garam. Zon perencatan dan pertumbuhan bakteria telah direkodkan. Hasil DDT telah menunjukkan zon perencatan bagi bahan terkandung Ag, dengan *E. coli* didapati lebih rentan berbanding *S. aureus* melalui ujian MIC/MBC. Ujian perencatan biofilem menggunakan AgNP-SZ juga menunjukkan potensi yang baik terhadap *S. aureus* berbanding *P. aeruginosa*. SZ, Ag-SZ dan AgNP-SZ pada kepekatan 0.5, 1.0, 1.5, dan 2.0 mg/mL telah dinilai bagi ujian ketoksikan sel. Ujian cakaran *in vitro* telah dilakukan untuk menentukan kadar penghijarahan sel HSF 1184. Ketiadaan aktiviti ketoksikan sel pada kesemua kepekatan AgNP-SZ telah membuktikan bahan tersebut adalah bersifat bioserasi. Walaupun kadar migrasi sel dilaporkan lebih perlahan bagi sampel AgNP-SZ berbanding zeolit A dan sampel kawalan dalam ujian cakaran *in vitro*, bahan tersebut tidak merencatkan migrasi sel. Penemuan ini menunjukkan kebolehupayaan sintesis hijau AgNP sebatian zeolit A daripada ekstrak tumbuhan berbanding teknologi lazim dengan sifat antibakteria dan bioserasi yang baik serta penghasilan produk yang lebih mampan.

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LIST OF ABBREVIATIONS

Kaol	-	Kaolinite
Meta-Kaol	-	Metakaolin
SZ	-	Synthesized zeolite A
Ag	-	Silver
AgNP	-	Silver nanoparticles
Ag-SZ	-	Silver-zeolite A
AgNP-SZ	-	Bio-synthesized AgNP incorporated zeolite A
XRD	-	X-ray diffraction
FTIR	-	Fourier transform infrared
FESEM	-	Field emission scanning electron microscope
TEM	-	Transmission electron microscope
EDX	-	Energy dispersive X-ray
FRIM	-	Forest Research Institute Malaysia
BET/ BJH	-	Brunauer-Emmett-Teller/ Barrett-Joyner-Halenda
LCMS	-	Liquid chromatography-mass spectrometry
TGA	-	Thermogravimetric analysis
DTA	-	Differential thermal analysis
NA	-	Nutrient agar
MHA	-	Mueller-Hinton agar
LB	-	Luria-Bertani
DDT	-	Disc diffusion technique
MIC	-	Minimum inhibition concentration
MBC	-	Minimum bactericidal concentration
ATCC	-	American-Type Cell Culture
HSF	-	Human skin fibroblast
MTT	-	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)
PBS	-	Phosphate buffer saline
FBS	-	Fetal bovine serum

LIST OF SYMBOLS

°C	-	Degree Celsius
%	-	Percent
cm	-	Centimetre
g	-	Gram
mg	-	Milligram
µg	-	Microgram
h	-	Hour
M	-	Molar
L	-	Litre
mL	-	Millilitre
mm	-	Millimetre
µm	-	Micrometre
nm	-	Nanometre
µL	-	Microliter
v/v	-	Volume/volume
w/v	-	Weight/volume
kV	-	Kilovolt
kPa	-	Kilopascal
ϴ	-	Theta
Å	-	Angstrom
a.u.	-	Astronomical unit
mV	-	Millivolt
µV	-	Microvolt

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Various types and compositions of clays and minerals have been frequently studied and utilized for their multipurpose applications. Studies have focused on zeolite type-A out of several types of zeolites that include A, X, and Y (common types). Zeolite A is widely used for various purposes, and it can be used as a carrier for silver ions and silver nanoparticles (AgNP) (Jiraroj, Tungasmita, and Tungasmita, 2014), biogas purification (Abdullah et al., 2018), and wastewater treatment (Mahmoodi and Saffar-Dastgerdi, 2019). It is a porous material with various molecular size channels, enabling specialized functions in catalysis, separation, and ion exchange (Kazemimoghadam, 2016). Zeolite A can be obtained through synthesis from kaolin for research purposes (Kwakye-Awuah et al., 2014; Loiola et al., 2012; Mgbemere et al., 2018). Kaolin, a type of clay, was used as the precursor for the synthesis of zeolite due to its abundant composition of silicon (Si), oxygen (O) and aluminium (Al). These elements are important in determining the outcome of the desired zeolite type. Therefore, the synthesized zeolite A was modified by the incorporation or ion-exchanged with Ag and AgNP in advancing the development of composite material of zeolite A. This synergy has created potentials for advanced materials in biological and medical applications. Zeolite A composite is known for its antibacterial function after being modified with several appropriate and compatible antibacterial agents. Independently, zeolite A has no special feature to inhibit the growth of bacteria on its own. Antibacterial agents are compounds that can inhibit or kill bacteria. One of the antibacterial compounds such as silver, whether in the form of ion (Salim and Malek, 2016) or nanoparticle (Hu et al., 2016), has become a significant benchmark for incorporating onto these zeolites. Zeolite can promote wound healing with the addition of antibacterial agent (Neidrauer et al., 2014; Purnomo et al., 2018).

Malaysia is a tropical country which houses abundance of local herbs and plants. These plants contain a rich amount of phytochemical components beneficial for many uses. In this work, a local herb known scientifically as *Orthosiphon aristatus* or *misai kucing* in Malay is highlighted for its use as a green reducing agent. On the other hand, kaolinite, a type of clay, is also abundantly exist in Malaysia and is frequently used in many advanced construction technologies. These two resources are the main materials used in this work. Hence, this study is feasible and attainable to develop an impactful research finding. With reference to previous studies, the green reducing agent has become an alternative method to reduce silver (Ag) ions to silver nanoparticles (AgNP) (Helmy et al., 2020; Sytu & Camacho, 2018; Thi Lan Huong & Nguyen, 2019). Furthermore, zeolite A is also capable of being synthesized from the kaolinite (Cundy & Cox, 2005; Gougazeh & Buhl, 2014). AgNP is a worldwide nanomaterial known as an antibacterial agent and is conventionally synthesized using chemical and physical methods. This conventional method usually involves a risk and hazard to the user and requires a lot of energy input and a greener approach has been introduced over the decades using plant extract (Mittal et al., 2013). Plant extract contains a large number of phenolic compounds and alkaloids responsible for reducing Ag ions into AgNP (Mittal et al., 2013). Other than reducing capability, compounds found in plants are also able to cap the AgNP for the nanoparticles stability (Sytu & Camacho, 2018). Its nano-sized particle is also an interesting feature that is used for antibacterial application in which the very high surface area enables effective interaction with the targeted bacteria (Kim et al., 2007). AgNP is also reported as the most effective antibacterial agent against a wide range of pathogenic microorganisms (Sharma et al., 2009). The biocompatibility of the materials are often tested to assess their suitability for pre-clinical and clinical testing against human skin cells (HSF 1184) (Asraf et al., 2019), mice (Akkol et al., 2011), and zebrafish (Ramachandran et al., 2018). Following this trend, any wound healing research needs to adopt a biocompatibility test. Additionally, it can further enhance the value of the modified materials.

Therefore, this work is gearing up for green technology in the biosynthesis of AgNP and zeolite A from their sources and precursors, respectively. Primarily, this work introduced the functional term of *in situ* synthesis method of AgNP-incorporated zeolite A (AgNP-SZ) using the green reducing agent of *O. aristatus* which is the novelty of this study. So far, Thus, the modification of synthesized zeolite A (SZ) with the biosynthesized AgNP promotes an opportunity and interesting feature in biomedical application. The research gap in this project includes the innovation of synthesizing a material using green technology and assessing the material in both applications on the antibacterial property and *in vitro* skin wound healing.

1.2 Problem Background

Synthetic zeolite has become a mainstream process due to its relevant features for the industrial application. Zeolite can be synthesized using chemical or aluminosilicate precursors which made them comparable materials to the one synthesized with biological sources. Each of the synthesis method or route defines its own strength and weakness. A chemical precursor affects a high reproducibility and purity of the end material (zeolite A) (Huang et al., 2012). However, chemically-synthesized zeolite requires chemicals which are not readily available and may cause a health hazard. The chemical precursors in this study refer to chemical sources other than biological sources such as sodium borohydride and sodium citrate. This study focused on the abundance of kaolinite in Malaysia. Therefore, it is wise to utilize this country's abundant natural resource through the use of kaolinite as the aluminosilicate precursor to synthesize zeolite A. Besides, an important question is posed on the use of chemical and physical methods to synthesize AgNP. Although these chemicals such as sodium borohydride ($NaBH_4$) and trisodium citrate are good reducing agents, they are risks to the environment if not properly disposed of (Banfi et al., 2014). Other than that, a physical method such as laser ablation requires a vast amount of energy to project the beam (Wei et al., 2015). Thus, this has led to the alternative use of biological organisms, including plant, microbes, and yeast, to synthesize AgNP. This study has demonstrated the use of local herb named *O. aristatus* which the reducing potential rivals to that chemical process.

The frequent use of antibacterial agents such as silver, copper, and zinc has promoted bacterial resistance towards antibacterial agents. Although at a slow pace, bacteria can develop a resistive mechanism to counter the antibacterial materials

(Ferreira et al., 2016). These resistances of gram-positive and gram-negative bacteria are dependent on the difference in their membrane structure such as the peptidoglycan and membrane thickness (Durán et al., 2016). In addition, available antibacterial agents such as antibiotic and antiseptic cream, gel or solution are not as effective as they were in certain cases. The reduced effectiveness is due to the allergic reaction of the skin to certain compounds and a low-dose ingredient. Thus, the development of a new material that can deliver antibacterial agents using suitable mechanisms such as controlled release, high surface area of contact and low to null toxicity is needed. The exploration of new antibacterial products also helps consumers to choose based on their preferences in the market.

There is rising concern of environmental factor when utilizing Ag in salts or nanoparticles although Ag is the most potent antibacterial agent to date. When the Ag is not properly disposed of, the environment will suffer a bioaccumulation phenomenon of heavy metals. A research was conducted to determine the level of bioaccumulation of $AgNO_3$ and AgNP in *daphnia magna* showing a higher amount of AgNP in the diet and water exposure (Ribeiro et al., 2017). A similar study also stated that Ag bio-accumulative index are higher in the form of $AgNO_3$ than AgNP in rainbow trout (Clark et al., 2019) The uncontrolled release of AgNP will accumulate in the host of the first consumer and later snowball to the end of the food chain. Humans as the end consumer will suffer the most contributing health hazard and illness. Current innovations are still lacking in utilizing natural materials such as plants and microbes in the production of AgNP. Therefore, green technology ranging from production to packaging has been the utmost concern for most industries and suppliers.

The toxicological property of the synthesized AgNP using a greener approach must also be assessed, although many claim that the green synthesis poses less threat to human health. Bio-synthesized AgNP has never been claimed to not cause any environmental issue at all. However, the use of biological synthesis would simply reduce the significant adverse effect on the environment by removing harmful by-products of chemical synthesis. A review stated that there are many possible mechanisms that lead to an increase or decrease in the toxicity level of AgNP in general, depending on the factors such as interaction with organic matters, cations, pH, and oxygen (Clark et al., 2019). A high dose of Ag as in ions and nanoparticles will inhibit bacterial growth. However, a proper guideline is needed to assess the toxicity of these materials towards human health in general. Other than that, scientific data and results have to be produced to rectify the claim of toxicity level, thus creating a proper experimental setup on cytotoxicity testing of AgNP in the laboratory. On the other hand, wound infection is a frequent case that arises from the area of the wound being infected with bacteria. Wounded skin can be properly and quickly healed if personal hygiene is well-taken care of. However, in some cases, a wound can be severe due to this bacterial infection. According to a study done by swabbing infected wounds of patients in a hospital, common bacteria found on the swabs include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, and *Corynebacterium* spp (Bessa et al., 2015). Undeniably, bacteria are the most common cause of wound persistence, according to the mentioned study. The wound can heal within a certain timeframe, but it can be impaired by bacterial infection. When skin is injured, bacteria can quickly obtain access to the underlying tissue making it difficult to heal (Bucknall, 1989). This will further give rise to inflammatory reaction on the skin since bacteria have the ability to form biofilm which act as their protective layer against antibacterial agent. The development of composite material with added value mainly the antibacterial property is sought. A good antibacterial activity shows decreases or kills a wide range of bacteria completely. Thus, these gaps in the research problem need to be explored and studied to produce a functional material with good antibacterial activity and biocompatible to the human skin cells.

1.3 Research Objectives

The objectives of this work are as follows:

1. To synthesize zeolite A from kaolinite and bio-green silver nanoparticles from *Orthosiphon aristatus* extract and characterize them.
2. To incorporate bio-synthesized silver nanoparticles onto synthesized zeolite A using *in situ* method and characterize them.

3. To assess the antibacterial property of bio-synthesized silver nanoparticles immobilized on zeolite A towards gram positive and gram negative bacteria.

4. To evaluate the cytotoxicity and skin wound healing capability of the bio-synthesized silver nanoparticles incorporated on zeolite A on human dermal fibroblast cells HSF 1184.

1.4 Research Scope

There are several important steps in producing the material for this research. Based on the objectives, the research scopes are divided into (1) the syntheses of zeolite A from kaolinite and the bio-synthesis of AgNP using *O. aristatus* leaf extract as the reducing and capping agent, (2) the *in situ* preparation method of biosynthesized AgNP-incorporated zeolite A, (3) antibacterial assay, and (4) *in vitro* biocompatibility and wound healing assessments on HSF 1184 cells.

The first scope included the synthesis of zeolite A from raw kaolinite and the green biosynthesis of AgNP using *O. aristatus* leaves extract as the reducing and capping agents. Zeolite A is well-known for its hydrothermal synthesis with various potential applications (Collins et al., 2020). This hydrothermal method transformed kaolinite into zeolite A. On the other hand, AgNP was biosynthesized using *O. aristatus* leaves extract as the reducing and capping agents. The synthesized materials (AgNP and SZ) were optimized and characterized before proceeding for *in situ* synthesis method.

In the next scope, both syntheses methods were combined into an *in situ* synthesis method through the synthesis, capping and incorporation of AgNP into the zeolite A. Similarly, the synthesized materials (Ag-SZ and AgNP-SZ) were characterized to confirm the incorporation of Ag and AgNP on or in the zeolite A framework. Figure 1.1 shows the schematic diagram of the synthesis pathway of *in situ* biosynthesized AgNP-incorporated zeolite A.

The characterization instruments used include Fourier transform infrared (FTIR) spectroscopy and X-ray powder diffraction (XRD). Furthermore, the surface morphology and properties were examined using field emission scanning electron microscopy (FESEM), energy dispersive X-ray (EDX) spectroscopy, transmission electron microscope (TEM), inductively coupled plasma-optical emission spectrometry (ICP-OES) and dispersion behaviour. Additional characterizations to complement the results were also carried out using thermogravimetric analysis (TGA), zeta potential, Brunauer-Emmett-Teller (BET) Surface Area Analysis and Barrett-Joyner-Halenda (BJH) Pore Size and Volume Analysis (BET-BJH), and inductively-coupled plasma optical emission spectroscopy (ICP-OES). Additionally, the *O. aristatus* was also identified and characterized for its phytochemical constituents. The plant extract was characterized using liquid chromatography-mass spectrometry (LCMS), and the synthesis of AgNP using the extract was optimized based on specific parameters.

The third scope emphasized the application and evaluation of the synthesized materials for antibacterial application. Disc diffusion technique (DDT) and minimum inhibitory/bactericidal concentration (MIC/MBC) were carried out against gram negative and gram positive of *Escherichia coli* ATCC 11229 and *Staphylococcus aureus* ATCC 6538. In addition, biofilm inhibition study was also conducted against *S. aureus* ATCC 6538 and *Pseudomonas aeruginosa* ATCC 15442. The synthesized materials were applied to the bacteria and assessed for the capability of Ag- and AgNP-associated materials to inhibit the bacterial growth or kill the bacteria completely.

The last scope involved the *in vitro* study of the synthesized materials towards human dermal fibroblast (HSF 1184) cells. The cells were grown and maintained until stable. Afterwards, the prepared samples were tested on the cells using cytotoxicity assay (direct method) and scratch assay (indirect method). The direct method involved a direct placement of the synthesized samples into the media containing cells. On the other hand, the indirect method required the sample extracts to be incorporated with the media for cell proliferation. Concentration of samples with no toxicity were selected and used for the scratch assay. The wound healing capability between the samples (SZ, Ag-SZ and AgNP-SZ) were evaluated and compared.

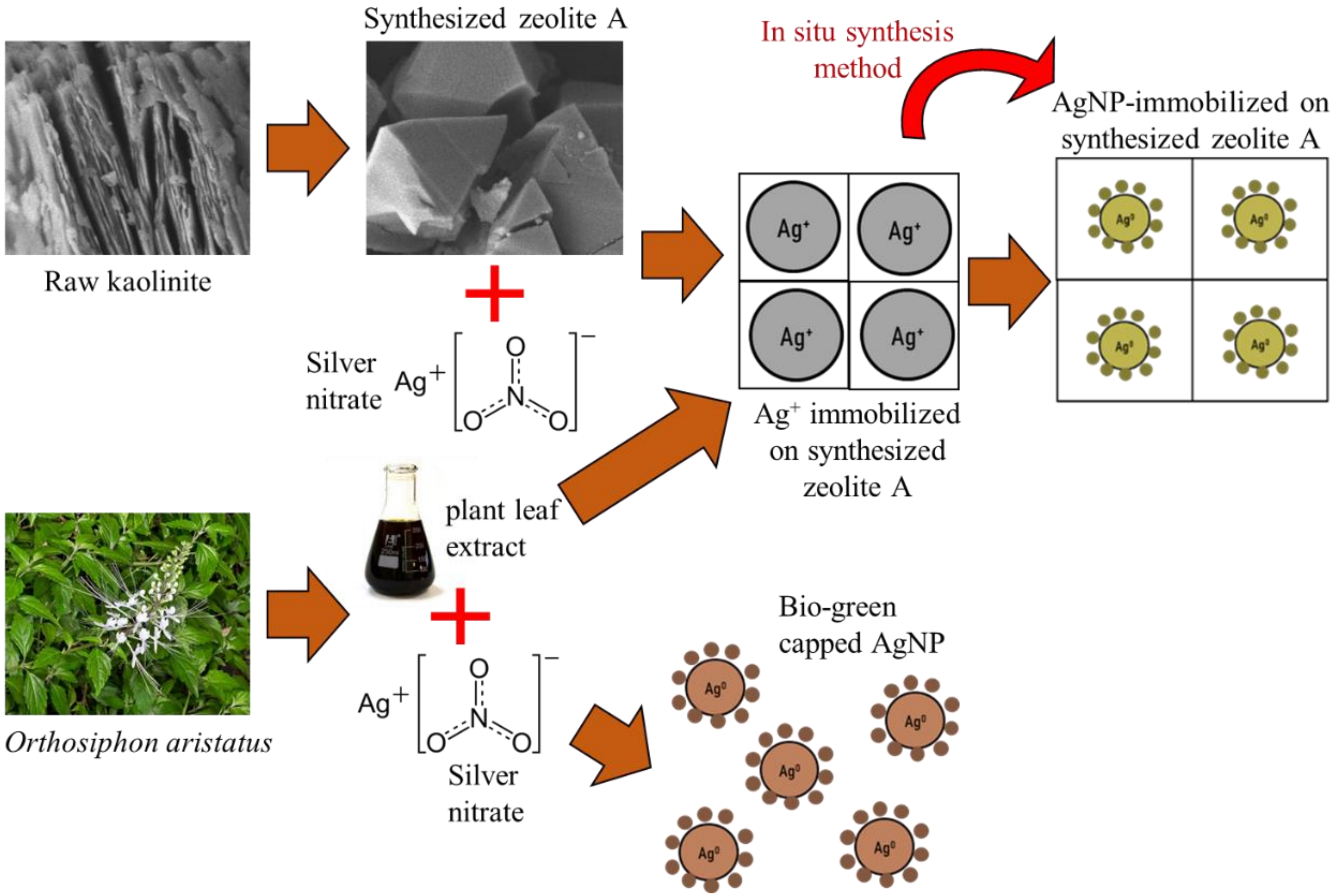

Figure 1.1 Schematic diagram of the *in situ* biosynthesized AgNP-incorporated zeolite A synthesis pathway

1.5 Research Outline

The outline of the research is shown schematically in Figure 1.2. The outline comprises of several stages divided into the synthesis of materials including zeolite A from kaolinite and AgNP using *O. aristatus*, the *in situ* synthesis antibacterial assessments and biofilm inhibition study, and biocompatibility and wound healing evaluation of the materials.

Stage 1 and 2 describes the preliminary synthesis of the respective zeolite A and AgNP. These syntheses were carried out firstly to determine the success of both synthesized materials (SZ and AgNP). SZ was synthesized from Kaol whereas AgNP was bio-synthesized from the *O. aristatus* leaves extract.

After the confirmation of the success of both synthesized materials, *in situ* method was carried out in Stage 3. In this stage, Ag-SZ and AgNP-SZ were synthesized. The difference between these two materials were the method of synthesis and the state of Ag. Ag was in the state of ions whereas AgNP was in the state of nanoparticles. In addition, Ag-SZ was used as a comparison to AgNP-SZ to assess the effect of reducing agent and capping agent derived from the phyto-compounds extracted from *O. aristatus* leaves.

Next stage was the application of the synthesized materials. Kaol, SZ, Ag-SZ and AgNP-SZ were the studied materials for antibacterial activity whereas SZ, Ag-SZ, and AgNP-SZ were tested for the *in vitro* cytotoxicity and wound healing assessments. Antibacterial assays were inclusive of DDT and MIC/MBC with the addition of biofilm inhibition study. Other than that, *in vitro* studies involved the use of cytotoxicity assay and scratch assay. In-depth methods are described in a later Chapter 3.

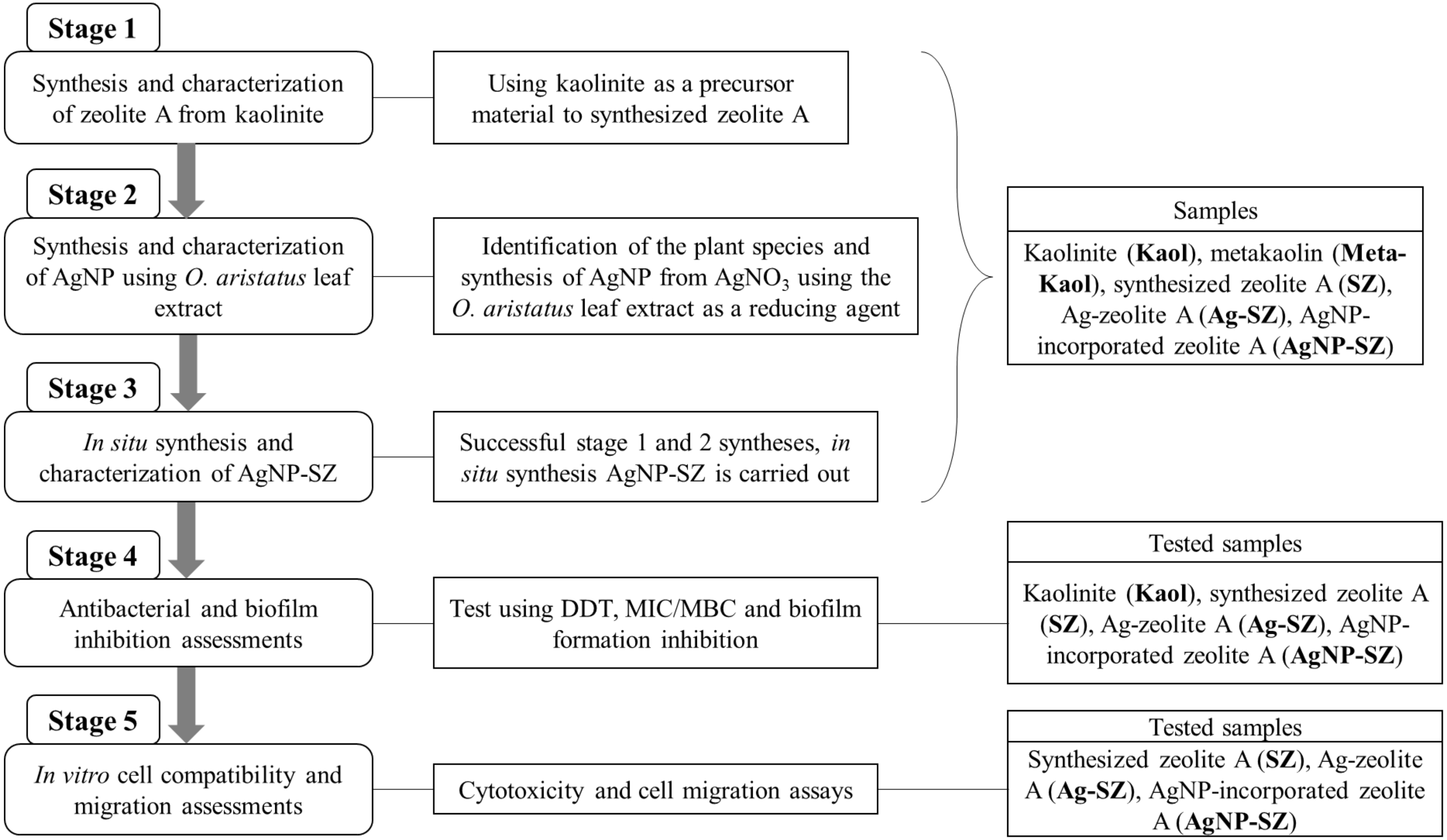

Figure 1.2 Outline of research stage-wise for the syntheses and applications of the materials.

1.6 Research Significance

The development of inorganic materials is important to enhance their attributes by giving them additional properties such as antibacterial, antifungal, promoting wound healing, and increasing livestock. It is important to show that synthetic zeolite can be synthesized abundantly because the source of raw kaolinite is available locally. Since Malaysia does not produce zeolite minerals, so our researchers can synthesize zeolites from abundantly available kaolinite sources with high aluminosilicate content (Abdullahi et al., 2019). Furthermore, the rising trend for green synthesis of nanoparticles using microbes and plants has provided vast opportunities for our researchers to utilize natural products and green approach. Our tropical region is rich with plants and herbs which are waiting to be discovered. There are many types of research in synthesizing nanoparticles using plants such as *Dodonaea visco*sa (Anandan et al., 2019), *Polygonum minus* (Ullah, Wilfred, and Shaharun, 2019) and *Sapindus mukorossi* (Thi Lan Huong & Nguyen, 2021) These green syntheses resulted in more biocompatibility, scalability, and applicability of the materials (Rajan et al., 2015).

Since there is no research conducted on the development of AgNP incorporated onto zeolite A using *O. aristatus*, this project is considered novel in its field. The intended use of this medicinal herb is due to its abundance and locality in the tropical climatic forest of the southeast of Asia (Febjislami et al. 2019). Additionally, Malaysia has many herbs for application in green technology. *O. aristatus* contains rich amounts of plant metabolites (Samidurai et al., 2020). These plant metabolites are responsible for reducing and capping the synthesized AgNP. The property of these natural metabolites is very much comparable to the conventional chemicals used to synthesize AgNP. Therefore, instead of using chemicals, plant metabolites are the potential substitutes. Potentially, all compatible materials or compounds can be adhered to or immobilized on the zeolite framework through ionic interaction (Nik, Chen, and Kaliaguine, 2011) or simply through the deposition of nanoparticles in the zeolitic pores (Jiraroj, Tungasmita, and Tungasmita, 2014). Ag ions from silver nitrate can be ion-exchanged with other cations such as sodium and magnesium ions (Ebadi Amooghin et al., 2016). On the other hand, AgNP can be deposited or adsorbed into the zeolite A framework due to its nano-size (Shameli et al., 2011). The development of AgNP incorporated onto synthesized zeolite A using a greener approach is expected to set a benchmark for future researchers to continue discovering the potential of tropical plants for the biosynthesis of AgNP.

There is a limited finding related to the use of zeolite in wound healing. A review summarized the use of zeolite as a scaffold to enhance dermal tissue regeneration (Ninan et al., 2015). This report provided insight into the possible mode of actions of the synthesized materials and their antibacterial wound healing assessment. Thus, the outcome of this project has established a reproducible method and protocol in conducting antibacterial assay and *in vitro* study, which is acceptable and more scientific. Other than that, the scientific exploration of using local plants and herbs to synthesize AgNP will promote a greener technology application.

Wounded skin is prone to bacterial infection due to the exposed inner skin layer. Simply being said, wounded skin and bacterial infection are inseparable. Bacteria exist on the outermost layer of skin or dead skin layer. Minor wound opens up the skin which makes it easier for the bacteria to penetrate (Tomic-Canic et al., 2020). There are more than a type of bacterium resides on the skin each with different roles. Some are the contributing factors in developing chronic wound while the others are believed to promote wound healing on the skin. Therefore, antibacterial and wound healing assessments are suggested as a complementary for this type of work.

In a summary, this project is significant to promote an innovative and systematic approach in developing applied inorganic material with a greener method to solve wound infection on the skin. The method applied a biological synthesis method to replace the conventional chemical and physical methods. The systematic approach was reflected on the step-wise experimentation on antibacterial wound healing. The steps started with comprehensive antibacterial testing and followed by cytotoxicity and cell migration assessments.

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LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Asraf, M. H.,** Malek, N. A. N. N., Jemon, K., Sani, N. S., Muhammad, M. S. (2019). Cytotoxicity and Wound Healing Assessments of Amine-Functionalized Zeolite Y, *Particuology*, 45, 116 – 123. https://doi.org/10.1016/j.partic.2018.09.006. (Q2, IF: 3.067)

2. **Asraf, M. H.,** Sani, N. S., Williams, C. D., Jemon, K., Malek, N. A. N. N. (2021). In Situ Biosynthesized Silver Nanoparticle-Incorporated Synthesized Zeolite A Using Orthosiphon Aristatus Extract for In Vitro Antibacterial Wound Healing, *Particuology*, In Press, https://doi.org/10.1016/j.partic.2021.09.007. (Q2, IF: 3.067)

3. Isah, M., **Asraf, M. H.,** Malek, N. A. N. N., Jemon, K., Sani, N. S., Muhammad, M. S., Wahab, M. F. A., Saidin, M. A. R. (2020). Preparation and Characterization of Chlorhexidine Modified Zinc-Kaolinite and Its Antibacterial Activity Against Bacteria Isolated from Water Vending Machine, *Journal of Environmental Chemical Engineering*, 8, 103545. https://doi.org/10.1016/j.jece.2019.103545 (Q1, IF: 4.3)

Indexed Conference Proceedings

1. **Asraf, M. H.,** Malek, N. A. N. N. (2020). Effect of Different HDTMA Loading on Silver Modified Kaolinite on Its Antibacterial Activity, *AIP Conference Proceeding*, 2231, 020003. https://doi.org/10.1063/5.0002423. (Indexed in WOS & Scopus)