GREEN-SYNTHESIZED ZINC OXIDE NANOPARTICLES FOR DEVELOPMENT OF ELECTROSPUN ANTIBACTERIAL WOUND DRESSINGS

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by

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LIST OF SYMBOLS

cm	Centimeter
cm^2	Centimeter square
°C	Degree Celsius
g	gram
L	Litter
MPa	Megapascal
μm	Micrometer
mg	Milligram
mL	Milliliter
mm	Millimeter
mV	Millivolt
M	Molarity
nm	Nanometer
mol	Number of moles
%	Percentage
RH	Relative humidity
rpm	Round per minute
R^2	The coefficient of determination
wt.	Weight
w/w	Weight/ weight
w/v	Weight/volume

LIST OF ABBREVIATION

ANOVA	Analysis of variance
ATR-FTIR	Attenuated total reflectance-fourier transform infrared
BPE	Banana peel extract
CA	Cellulose acetate
DLS	Dynamic light scattering
UHPLC	Ultra-High performance liquid chromatography
LC	Liquid chromatography
MS	Mass spectroscopy
MW	Molecular weight
NPs	Nanoparticles
PBS	Phosphate buffer saline
PDI	Polydispersity index
ROS	Reactive oxygen species
SD	Standard deviation
SEM	Scanning electron microscopy
T_g	Glass transition
TGA	Thermogravimetric analysis
Tm onset	Melting point
UV-Vis	Ultra violet visible spectroscopy
XRD	Powder X-ray diffraction
ZAD	Zinc acetate dihydrate
ZnO	Zinc Oxide

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NANOPARTIKEL ZINK OKSIDA TERSINTESIS HIJAU UNTUK PEMBANGUNAN PEMBALUT LUKA ANTIBAKTERIA ELEKTROPUTARAN

ABSTRAK

Nanopartikal logam seperti nanopartikal zink oksida (ZnO NPs) telah dikaji secara meluas kerana aktiviti antibakteria yang kuat dan biokompatibilitinya. Sifat-sifat ini menjadikan ZnO NPs sesuai untuk digabungkan dengan bahan pembalut luka. Dalam kajian ini, sintesis NP ZnO dengan kaedah hijau mesra alam dalam penggunaan ekstrak tumbuhan, yang kemudiannya digabungkan ke dalam gentian polimer dengan teknik elektrospinning untuk membuat pembalut luka fabrik telah dilakukan. Di sini, dua cabaran untuk meningkatkan sifat mekanikal sambil menyediakan aktiviti antibakteria yang kuat dalam bahan penjagaan luka telah disasarkan. Kulit pisang dengan spesies *Musa acuminata* (*pisang Cavendish*) digunakan untuk ekstrakan. Hasil ekstrak kulit pisang (BPE) ialah 11.89 ± 0.45% w/w. Kemudian, unsur-unsur fitokimia ekstrak dianalisis, untuk mengesahkan kehadiran fitokimia bioaktif yang terlibat dalam proses sintesis NP, yang termasuk flavonoid, saponin, dan sebatian fenolik, yang penting untuk pengurangan dan penstabilan ZnO NPs. Proses sintesis ini telah dioptimumkan pada pH 12 pada 70 °C dengan suhu penyepuhlindapan 400 °C. NP yang dihasilkan memaparkan struktur dan saiz hablur heksagon wurtzite antara 21 hingga 33 nm dan spektrum UV antara 344 hingga 369 nm, seperti yang disahkan oleh pembelauan sinar-X (XRD) dan mikroskopi elektron pengimbasan (SEM), ATR-FTIR, spektroskopi boleh dilihat ultraviolet (UV-Vis), dan penyerakan cahaya dinamik (DLS). Proses sintesis menghasilkan ZnO NP antara 31.89% dan 38.72%w/w.

ZnO NPs yang diperoleh telah dimasukkan ke dalam gentian polimer menggunakan selulosa asetat (CA) sebagai polimer untuk proses pemutaran elektro. Gentian yang terhasil mempunyai diameter 1 hingga 3 µm dengan sifat mekanikal gentian yang telah ditambah baik, dengan kekuatan tegangan meningkat daripada 0.106 MPa kepada 0.169 MPa dan kestabilan haba telah ditambah baik daripada 303°C kepada 309.1°C. Analisis XRD mengesahkan kehabluran gentian CA telah dipertingkatkan kerana kehadiran ZnO NPs. Aktiviti antibakteria ZnO NPs yang disintesis dan polimer CA electrospun yang digabungkan dengan ZnO NPs (CA-ZnO) telah dikaji. Berdasarkan kajian antimikrob ke atas *Escherichia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus), dan Staphylococcus epidermidis (S. epidermidis)*, yang diketahui menyebabkan jangkitan pada luka, ZnO NP mempamerkan aktiviti antibakteria yang ketara, dengan nilai kepekatan perencatan minimum (MIC) 500 µg/mL untuk *S. aureus, S. epidermidis,* dan *E. coli*, dan 600 µg/mL untuk *P. aeruginosa*. Kepekatan bakteria minimum (MBC) untuk *S. aureus dan S. epidermidis* ialah 500 µg/mL. Keputusan menunjukkan kesan antibakteria yang kuat, dengan kadar perencatan pertumbuhan mencapai sehingga 97.61% untuk *S. aureus*, 98.67% untuk *S. epidermidis*, 95.21% untuk *E. coli*, dan 88.39% untuk *P. aeruginosa* pada kepekatan tertinggi (600 µg/ mL). Menggabungkan ZnO NPs ke dalam gentian electrospun CA telah meningkatkan aktiviti antibakterianya dengan ketara, terutamanya terhadap *E. coli* dan *P. aeruginosa*. Terutamanya, gentian electrospun menunjukkan keberkesanan antibakteria yang lebih tinggi berbanding serbuk ZnO NPs sahaja, di mana perencatan pertumbuhan *E. coli* meningkat daripada 79.11% (serbuk ZnO NPs) kepada 89.33% (gentian CA-ZnO), dan *P. aeruginosa* meningkat daripada 51.91 % kepada 72.94%. Prestasi yang dipertingkatkan ini disebabkan oleh proses pemutaran elektro, yang menyediakan kawasan permukaan

yang lebih besar dan penyebaran NP ZnO yang lebih baik dalam matriks CA, memudahkan sentuhan bakteria yang lebih berkesan dan kadar perencatan yang lebih tinggi. Kesimpulannya, kajian ini menunjukkan potensi antibakteria ZnO NP yang disintesis melalui kaedah hijau dan keberkesanan antimikrob yang dipertingkatkan apabila dimasukkan ke dalam gentian CA. Gentian electrospun CA-ZnO yang dibangunkan dalam kajian ini mempunyai potensi yang sangat baik untuk digunakan sebagai bahan pembalut luka termaju, menawarkan aktiviti antibakteria berspektrum luas.

GREEN-SYNTHESIZED ZINC OXIDE NANOPARTICLES FOR DEVELOPMENT OF ELECTROSPUN ANTIBACTERIAL WOUND DRESSINGS

ABSTRACT

Metallic nanoparticles, such as Zinc oxide nanoparticles (ZnO NPs), are widely recognized and studied due to their strong antibacterial activity and biocompatibility. These properties make ZnO NPs suitable for incorporation with wound dressing materials. This study delineated the synthesis of ZnO NPs by an eco-friendly green method using plant extract, which is then incorporated into polymer fibers by electrospinning technique to make a fabric wound dressing. Herein, the dual challenge of enhancing mechanical properties while providing strong antibacterial activity in wound care materials was targeted. Banana peel with the species of *Musa acuminata* (*Cavendish banana*) was used for extraction. The extracted banana peel (BPE) yield was 11.89 ± 0.45% by weight. Then, the extract's phytochemicals were analyzed, confirming the presence of bioactive phytochemicals involved in the NPs synthesis process, including flavonoids, saponins, and phenolic compounds, which are essential for reducing and stabilizing ZnO NPs. The synthesis process was optimized at a pH of 12 at 70 °C with an annealing temperature of 400 °C. The resulting NPs displayed Wurtzite hexagonal crystalline structure. The particle size ranges from 21 to 33 nm, while UV spectra range from 344 to 369 nm, as confirmed by Powder X-ray diffraction (XRD) and scanning electron microscopy (SEM), attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), ultraviolet, visible spectroscopy (UV-Vis), and dynamic light scattering (DLS). The synthesis process yields ZnO NPs

ranging from 31.89% to 38.72%. The obtained ZnO NPs were incorporated into a polymer fiber using cellulose acetate (CA) as a polymer for the electrospinning process. The resulting fibers had diameters of 1 to 3 μm with improved mechanical properties of the fibers, with tensile strength increasing from 0.106 MPa to 0.169 MPa and thermal stability improving from 303°C to 309.1°C. XRD analysis confirmed the enhanced crystallinity of the CA fibers due to the presence of ZnO NPs. The antibacterial activity of the synthesized ZnO NPs and electrospun CA polymer incorporated with ZnO NPs (CA-ZnO) was studied. Based on the antimicrobial study on *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus)*, and *Staphylococcus epidermidis (S. epidermidis)*, which are known to cause infections in wounds, the ZnO NPs exhibited significant antibacterial activity, with minimum inhibitory concentration (MIC) values of 500 μg/mL for *S. aureus, S. epidermidis, and E. coli*, and 600 μg/mL for *P. aeruginosa*. The minimum bactericidal concentration (MBC) was 500 μg/mL for *S. aureus and S. epidermidis*. The results demonstrated strong antibacterial effects, with growth inhibition rates reaching up to 97.61% for *S. aureus*, 98.67% for *S. epidermidis*, 95.21% for *E. coli*, and 88.39% for *P. aeruginosa* at the highest concentration (600 μg/mL). Incorporating ZnO NPs into CA electrospun fibers significantly increased their antibacterial activity, particularly against *E. coli and P. aeruginosa*. Notably, the electrospun fibers exhibited higher antibacterial efficacy compared to ZnO NPs powder alone, where the growth inhibition of *E. coli* increased from 79.11% (ZnO NPs powder) to 89.33% (CA-ZnO fibers) and *P. aeruginosa* increased from 51.91% to 72.94%. This enhanced performance is attributed to the electrospinning process, which provides a larger surface area and better dispersion of ZnO NPs within the CA matrix, facilitating more effective

bacterial contact and higher inhibition rates. In conclusion, the study confirmed the antibacterial potential of ZnO NPs synthesized via the green method and the enhanced antimicrobial effectiveness when incorporated into CA fibres. The developed CA-ZnO electrospun fibers in this study have excellent potential for use as an advanced wound dressing material, offering broad-spectrum antibacterial activity.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Indeed, wound dressing microbial barriers are important in preventing bacterial infection and enhancing wound healing. A physical pathogen blockage can be provided using designed microbial barriers incorporating antibacterial agents for future infection risk reduction (Augustine et al., 2014a). On top of the wound dressing microbial barriers, the antibacterial effect can reduce pathogen invasion and colonization in wounds (Augustine et al., 2014b). This can provide wound dressing with dual functionality, making them useful in various medicinal and biological applications. Wound healing can be enhanced by using a well-designed wound dressing formula that can maintain a moist environment and accelerate the healing process by reducing dehydration, facilitating cell migration, and providing external contaminants protection (Liang et al., 2022).

Moreover, many bacteria strains can infect skin wounds and colonize, presenting treatment challenges. The common bacterium types triggering skin wounds are gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidemidis, and streptococcus pyogenes*) and gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, and Acinetobacter baumannii*) (Percival et al., 2012). Among the most frequent species that infect wounds is *S. aureus*; this gram-positive bacterium is a part of normal skin flora, but if it enters the body through a contaminated wound, it can cause serious infections (Purohit & Solanki, 2013). *S. epidemidis* is normally found on human skin and is harmless. However, it also can cause severe infections if it enters through a break in the skin, especially with immunocompromised patients or

patients using medical devices like catheters or implants. It can create a biofilm to defend itself against the human immune system and antibacterial agents such as antibiotics (Sabaté Brescó et al., 2017). *E. Coli* is another bacterium species which found in the digestive system normally. However, it can cause a series of infections in surgical incisions (Russo and Johnson, 2003). Furthermore, one of the most frequent species associated with burn wounds and ulcers is *P. aeruginosa*. This bacterium type generates a biofilm, increasing its resistance and protecting itself (Mulcahy et al., 2014).

Topical and systematic antibiotics such as amoxicillin-clavulanate, cephalexin, ciprofloxacin, mupirocin, and bacitracin are administered as treatments for bacterial wound infection based on the bacterial type and infection severity (Guay, 2003). The use of antimicrobial dressings can significantly reduce and avoid wound infections can be achieved, particularly in cases of burns and chronic wounds. The prevention of wound infection complications will eliminate the use of the mentioned antibiotics, leading to a reduction in the use of antibiotics and their side effects (Punjataewakupt et al., 2019).

On the other hand, Nanomaterial applications, especially nanoparticles (NPs), in wound dressings for wound infection prevention and wound healing acceleration have remarkably increased recently. These NPs can be incorporated in suitable polymers such as cellulose, liposomes, and silica that are used as carriers of the antimicrobial in wound dressings formulas, representing the targeted approach of advanced dressings (Pormohammad et al., 2021).

The current chapter will provide a general overview of the eco-friendly methods used for ZnO NPs by plant extract, particularly banana peel extract (BPE), as

well as cellulose acetate (CA) electrospun fibres' importance as a promising antibacterial formula for wound dressing applications. ZnO NPs applications, CA medicinal uses, and the electrospinning technique were also covered.

1.2 Nanoparticles

1.2.1 Types of nanoparticles and their usage

Nanoparticles (NPs) are ultra-small particles that have a size measured in nanometres (nm), ranging from 1 – 100 nm (Khan et al., 2019). It can present unique chemical and physical properties due to their small size, such as the ability to penetrate biological membranes and a high surface area to volume ratio, making them valuable in a wide range of applications such as electronics, medicine, and environmental science (Khan et al., 2019). The treatment methods using NPs are based on targeting specific cells and tissues to avoid side effects and increase efficacy (Raj et al., 2021). In addition, many types of NPs are used in pollution control and water purification due to their catalytic properties (Vasantharaj et al., 2021).

1.2.2 Metallic nanoparticles

In general, nanomaterials' large surface area and high particle number per mass unit make them unique and preferred over bulk materials since these properties increase their reactivity due to the larger surface reaction (Buzea et al., 2007). These properties make NPs exhibit enhanced and novel medicinal, catalytic, and optical activity (Gade et al., 2010). Therefore, they can be used in different applications such as medicine, pharmaceutical industries, water treatment, and agriculture (Gajanan & Tijare, 2018; Khot et al., 2012).

There are various types of NPs, including polymer NPs, nanotubes, solid lipid NPs, nanocrystals, dendrimers, and inorganic NPs (Figure 1.1).

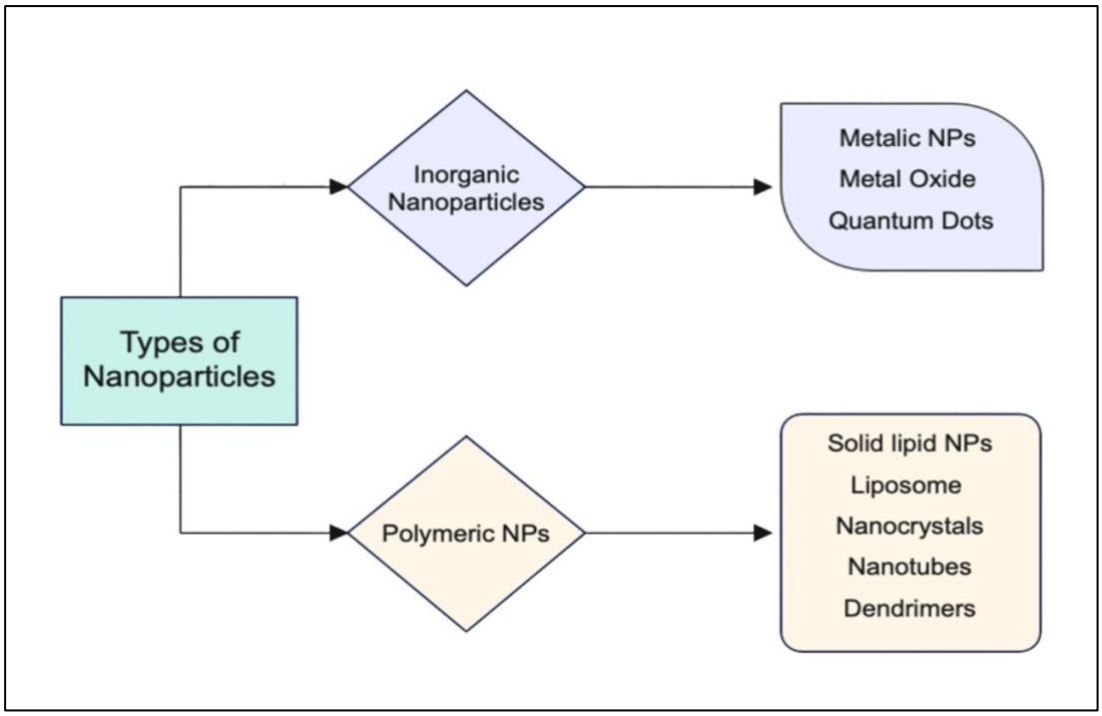

Figure 1.1 General types of nanoparticles

Based on Figure 1.1, Inorganic NPs include metal and metal oxide NPs, which are mean NPs that are formed based on metallic sources, in addition to quantum dots NPs, which are made from a complex of several metals. Metal NPs include gold, silver, copper, and platinum, while metal oxide NPs include zinc oxide. These NPs have general NPs properties in addition to their exceptional, unique properties such as large surface area-to-volume ratio (Walia, 2014), quantum effects (Campos et al., 2019), antibacterial activity (Makvandi et al., 2020), and many optical properties that have garnered significant interest in various scientific, industrial and medical fields (Nikzamir et al., 2021). Metallic NPs (ZnO NPs and Ag NPs) are used in different applications, such as cosmetics, sunscreens and as antimicrobial agents in food packaging. It can also be used for water purification purposes and wound dressing materials (Kumar et al., 2023).

Metallic NPs work as antibacterial agents in various applications by their' mechanisms for killing bacteria, as explained briefly in the following section.

- Liberation of ions: releasing ions from NPs according to their type, such as Titanium oxide (TiO_2 NPs), Copper (Cu NPs), Iron oxide (Fe_2O_3 NPs), zinc oxide (ZnO NPs), and silver (Ag NPs). This mechanism includes the diffusion of ions inside the targeted bacterial cell wall, cell membrane, nucleic acids and proteins (Godoy-Gallardo et al., 2021).

- Physical disruption: bacterial cell membrane damaged and disrupted by NPs through attaching to the bacterial cell membrane, leading to increased permeability and leakage of cellular contents (Rice & Bayles, 2008).

- Interaction with bacterial Proteins and DNA: metallic NPs can interact directly with the cell's proteins and DNA by penetrating bacterial cells, leading to DNA replication inhibition, loss of enzyme activity, and protein synthesis inhibition (A. Qiu et al., 2020; Wigginton et al., 2010).

- Generation of Reactive Oxygen Species (ROS): ZnO NPs and Ag NPs can catalyze ROS and damage cellular components, including DNA, lipids, and proteins, leading to oxidative stress and bacterial death (Ivask et al., 2014; Sirelkhatim et al., 2015).

In the case of ZnO NPs, the most important antibacterial mechanism was the ion diffusion mechanism. The release of Zn^{+2} ions from ZnO NPs depends on several factors, including temperature, media pH, and complexation (Hazeem, 2022). The released ions are responsible for NP's antibacterial activity in different media (Li et

al., 2011). It inhibits bacterial cell viability by increasing the leakage of intracellular fluid and osmotic stress (Kumar et al., 2017; Li et al., 2011).

1.2.3 Synthesis of nanoparticle

In general, NPs synthesis methods can be classified into two approaches (bottom-up and top-down). Each approach includes its own methods, as stated in Figure 1.2 (Rajput, 2015).

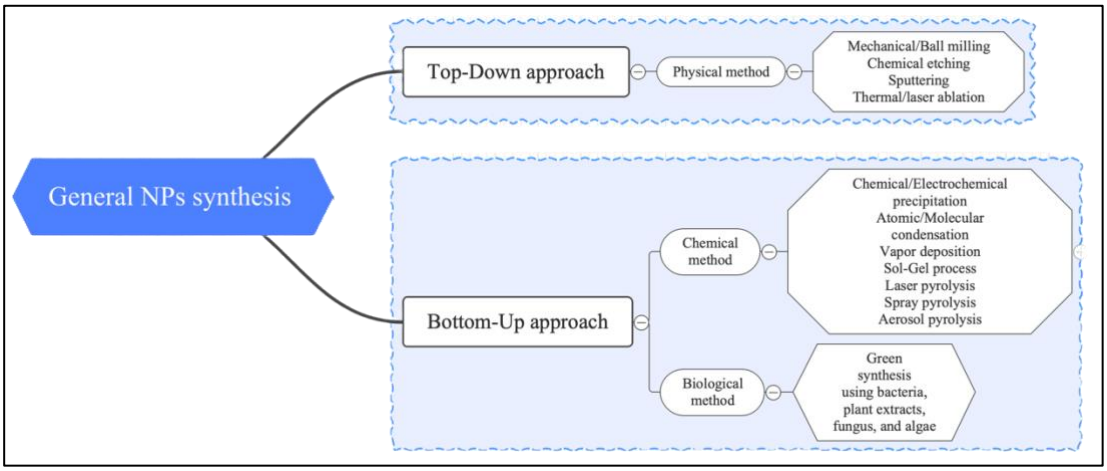

Figure 1.2 Nanoparticles synthesis methods (Habibullah et al., 2021; Rajput, 2015)

According to Rajput (2015) and Habibullah et al. (2021), this is a self-constructed diagram (Figure 1.2) that outlines two major strategies for NP synthesis. The top-down approach means NPs produced from bulk materials by physical methods, as stated in the diagram below. On the other hand, the bottom-up approach means NPs produced through building NPs from molecular precursors by either chemical or biological methods (Rajput, 2015). Among NP synthesis methods, bottom-up methods stand out for their ability to produce pure NPs with precise size and shape (Habibullah et al., 2021).

Significant environmental consequences can occur when NPs are created using hazardous solvents and severe reaction conditions (Iravani et al., 2014). Hazardous solvents, stabilizers, and reducing agents such as sodium borohydride, hydrazine, and organic solvents like toluene might be used within the chemical method, generating harmful byproducts. These solvents will lead to soil and water contamination due to their accumulation in the environment and degradation difficulties (Ramanathan et al., 2021). Moreover, high energy is consumed during NPs synthesis by physical methods (laser ablation and thermal decomposition methods) (Krishnia et al., 2022; Semaltianos, 2010). The resultant by-products from the physical and chemical synthesis methods cannot be fully recovered or treated, resulting in long-term environmental damage (Abid et al., 2022).

Many physical methods, like milling and sputtering, are prone to producing non-uniform particles, necessitating additional processing steps that consume energy and resources leading to increased environmental pollution (Iravani et al., 2014; Dikshit et al., 2021).

1.2.4 Green synthesis of nanoparticles

The production of NPs by green methods, also known as biogenic synthesis, is a synthesis technique based on plant extracts or biological organisms like algae, fungus, bacteria, and yeasts. This method can produce metal NPs through chemical reduction into their stable form (Deplanche et al., 2010). The NPs synthesis reaction can be initiated by mixing the plant extract with metal precursor salts to allow the interaction of plant phytochemicals with the salt solutions to form metallic NPs (Mittal et al., 2013).

These methods are considered eco-friendly, inexpensive and safer to the environment compared to other methods (Bhardwaj et al., 2020).

Figure 1.3 classifies different green synthesis methods for NPs according to the biological source used.

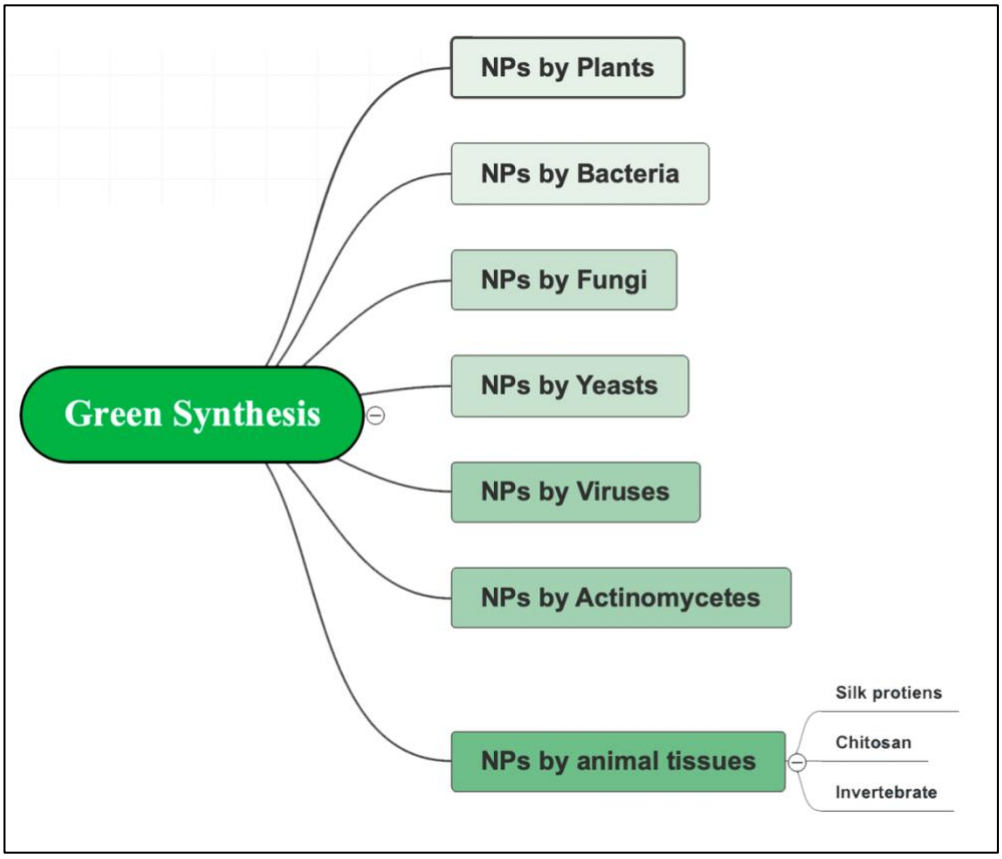

Figure 1.3 The classification of green methods for the synthesis of NPs

The synthesis of ZnO NPs by green methods can be considered as a viable approach that can solve physical and chemical issues due to green method advantages, including environmental friendliness and low cost, which gives unique properties to the produced NPs (Akintelu & Folorunso, 2020). Plant extracts, algae, fungi, and bacteria were the most viable options for synthesizing ZnO NPs. Using plant extract phytochemicals, which have capping and reducing effects in the green synthesis process, to stabilize the synthesized NPs (Ahmed et al., 2017).

Besides, biosynthesis processes or green synthesis are considered as environmentally friendly since there is less waste produced during the process, no hazardous chemicals are used, and are operated at standard pressure and temperature (Jiang et al., 2018). The ZnO NPs can be produced greenly by various plant species with special properties, such as uniform shape and size, due to the plant phytochemical profile diversity (Parveen et al., 2016; Sharma et al., 2019). The production of NPs by the green method on a large scale can face various challenges in the manufacturing process to be commercially viable, including scalable production and synthesis conditions control; thus, further researches have to be conducted to address these concerns (Fakhari et al., 2019). The principle of production for most of the metallic NPs involves the reaction of a precursor with plant extract phytochemicals; for example, the synthesis of ZnO NPs can be conducted using zinc acetate di-hydrate solution (Zn (CH3COO)$_2$, 2H2O) (ZAD) as a precursor to interact with plant extract phytochemicals such as flavonoids and phenolic acids, which are suggested to be the main constituents responsible for the occurrence of the reaction of NPs formation (Alamdari et al., 2020).

Plant phytochemicals have a role in the green synthesis by functioning as reducing, capping, and stabilizing agents, assisting in the stabilization of NPs throughout the synthesis process and controlling the formation of crystals. These phytochemicals include phenol, alkaloids, tannins, flavonoids, terpenes, saponins, and protein, which undergo the redox reaction and enhance electron transfer to the metal ions, resulting in the formation of NPs (Abdullah et al., 2020).

Indeed, ZnO is a semiconductor with a high band gap width (3.37 ev) and an excitation binding energy of 60 meV (Ong, C. B. et al., 2018). It has strong pyroelectric and piezoelectric characteristics (Hameed et al., 2019). The US FDA considers ZnO

materials GRAS (generally recognized as safe), hence, they are commonly utilized in food packaging as antibacterial agents (Kim et al., 2022). Green synthesis can generally be characterized as a process that uses biological entities or natural products, such as plants and their extracts, microorganisms, and biopolymers, to produce ZnO NPs (Bandeira et al., 2020). The formation of ZnO NPs by the green synthesis process from the extract's phytochemicals is illustrated in Figure 1.4. Figure 1.4 below is a schematic presentation explaining the role of flavonoids and phenolic compounds in the donation of electrons to reduce Zn^{+2} rather than the electron transfer, as presented by the curved arrow shown below, suggesting Zn^{+2} undergoes oxidation. Meanwhile, the reduction process occurs from the Zn^{+2} source (Zn acetate dihydrate) (Alamdari et al., 2020).

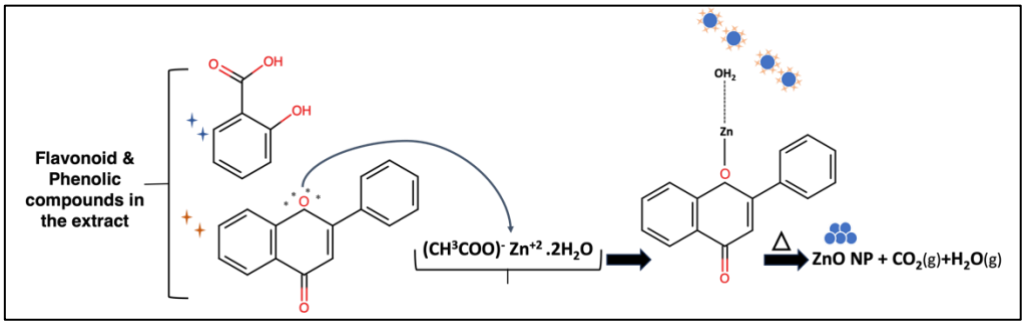

Figure 1.4 The Redox reaction between Flavonoids & phenolics compounds with Zinc acetate dihydrate (Alamdari et al., 2020).

1.3 Zinc oxide nanoparticles pharmaceutical applications

ZnO NPs are well-known for their unique properties, which include superior UV-blocking capabilities, high electron mobility, a broad bandgap, and a high exciton binding energy, making them suitable for a wide range of applications in industries such as cosmetics, electronics, textiles, and pharmaceuticals. Green-synthesized ZnO NPs exhibit stronger antibacterial, antifungal, and antioxidant activity than other NPs. ZnO NPs have demonstrated potential in drug delivery, wound healing, and biosensors

(Ogunyemi et al., 2019). Because of its features, ZnO NPs are being applied widely in the pharmaceutical industry. These NPs have gotten much interest because of their good antibacterial activity, UV-blocking capabilities, ability to use them in cancer therapy, gene transfer, and bioimaging. They are strongly effective as antibacterial and antifungal agents due to their high surface area-to-volume ratio and unique physicochemical characteristics. Several studies have reported their effectiveness against various infections caused by *E. coli, S. aureus, and P. aeruginosa* (Sirelkhatim et al., 2015). It is widely used in sunscreen products due to its ability and great effectiveness in blocking UV light. As a result, they are a safer option than organic UV filters, which may enter the skin and induce bad responses (Mohammed et al., 2019).

Using photodynamic therapy, ZnO NPs can be used to deliver anticancer drugs and treat cancer. When exposed to light, the particles can generate reactive oxygen species, which trigger cell death in malignant cells (Ali et al., 2016). ZnO NPs can be used as drug delivery carriers. They may be made to deliver medications directly to the intended areas, minimizing systemic adverse effects and enhancing therapeutic effectiveness. ZnO NPs have shown promise in studies as drug delivery methods for medications like doxorubicin (Rasmussen et al., 2010). ZnO NPs' luminous characteristics have been exploited in bioimaging. They can potentially be used in imaging and diagnostic instruments since they can be altered to emit light at various wavelengths (Misra & Misra, 2022). ZnO NPs can also be used as anti-inflammatory agents, in treating wounds, and in managing diabetes. Excellent potential in accelerating wound healing can be seen with the use of ZnO NPs by its role in wound infection prevention through its antibacterial effect, which promotes the proliferation and development of cells that aid in the healing process (Manuja et al., 2020). It also

greatly boosts the body's insulin absorption and protects insulin from degradation in diabetes patients (Bedi & Kaur, 2015).

1.4 Wound healing and ZnO NPs' potential for wound

The wound can be defined as normal anatomical skin structure and function disruption and damage (MC, 2001) . The normal healing process is a dynamic, complex series of coordinated events such as bleeding, coagulation, acute inflammatory initiation, regeneration, migration, and tissue proliferation with collagen deposition (Rivera & Spencer, 2007). In the case of chronic wounds, the healing process failing to progress across the normal stages, and the wound cannot be repaired (MC, 2001). An incomplete healing process will probably be due to factors that prolong the wound-healing phases of haemostasis, inflammation, proliferation, and remodelling (Vanwijck, 2001). One of the most important factors that delays and disturbs the healing process is infection, which increases inflammatory cytokine levels (Vanwijck, 2001).

Furthermore, the incomplete healing process and healing stage disturbance result from an uncoordinated healing process, which gives poor anatomical outcomes and frequent wound relapses (MC, 2001). An infected wound will progress to a complicated wound, tissue defects will occur if not treated, and bacterial growth, which is the cause of wound contamination. Therefore, it is necessary to prevent bacterial contamination by using different antibacterial agents to keep the wound-healing process coordinated without any disruption (Aldridge, 2015).

As mentioned in this section, infection is a major problem in wound healing. Therefore, developing a wound dressing incorporated with an antibacterial is important. In this study, ZnO NPs were chosen as a promising antibacterial agent since

they are known for their effect and their compatibility with the biological system (Kaushik et al., 2019).

1.5 Antibacterial activity of ZnO NPs

The antibacterial effect of ZnO NPs' has been studied and applied in various industries, including cosmetics, food packaging, textiles, and medicine (Islam et al., 2022; Mirzaei & Darroudi, 2017). ZnO NPs exhibit an excellent antibacterial effect against both Gram-positive and Gram-negative bacteria (Raghupathi et al., 2011). There are different mechanisms studied to explain the ZnO NPs' effect on bacterial growth (Lemire et al., 2013; Xie et al., 2011), as mentioned previously in Section 1.2.2. ZnO NPs' interaction with targeted bacterial cells leads to significant physical harm to the bacterial cell membrane immediately due to the relatively high surface-to-volume ratio of the NPs (Lemire et al., 2013). The efficacy of ZnO NPs depends on many parameters such as shape, size, concentration, and targeted bacteria species (Premanathan et al., 2011).

A study conducted by Kadiyala et al. (2018) showed the power of ZnO NPs to limit methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria growth. The mentioned study presented that ZnO NPs have strong efficacy in combating bacterial strains that are resistant to conventional drugs (Kadiyala et al., 2018). Consequently, more research and investigations are required to completely comprehend ZnO NPs' potential uses and implications in treating various infections.

1.6 Banana Fruit and Peel

Bananas (*Musa spp*.), members of the family *Musaceae*, are one of the world's most popular fruits. It is distinguished by its elongated shape, mild curvature, and

thick, leathery peel. The banana peel is usually yellow, green, red, or even purple, depending on the type, when ripe. The fruit flesh is smooth, creamy, and off-white to pale yellow, with a sweet flavor. Banana is considered to have originated in tropical parts of Southern Asia, particularly in the region encompassing Malaysia, Indonesia, and the Philippines; it is currently grown around the world, including India, China, the Philippines, Ecuador, and Brazil. Banana is one of the most widely consumed fruits, since it contains great nutritional value, such as vitamins and minerals, especially potassium, vitamin C and vitamin B_6 (Aurore et al., 2009).

Furthermore, polymers like lignin, cellulose, pectin, and hemicellulose, found in significant concentrations within banana fruit which are beneficial to human health. Many phytochemicals, such as tannins, alkaloids, glycosides, saponins, and flavonoids, are found in the aqueous extracts of this plant (Alzate Acevedo et al., 2021). Many phytochemicals can also be found in the peel, as illustrated in Figure 1.5.

Because banana peels contribute around 35% of the total weight of the fruit, approximately 36 million tonnes of banana peels are generated each year, which represents a potential resource for further usage (Vu et al., 2017). The peel has historically been used medicinally to cure various conditions, including burns, anemia, diarrhoea, ulcers, inflammation, diabetes, cough, snakebite, and excessive menstruation. It was discovered to be high in dietary fiber and phenolic compounds. The high pectin content of banana peel can be extracted and zed in various industrial applications, including in the food industry as gelling agents, thickeners, and stabilizers (Pereira & Maraschin, 2015).

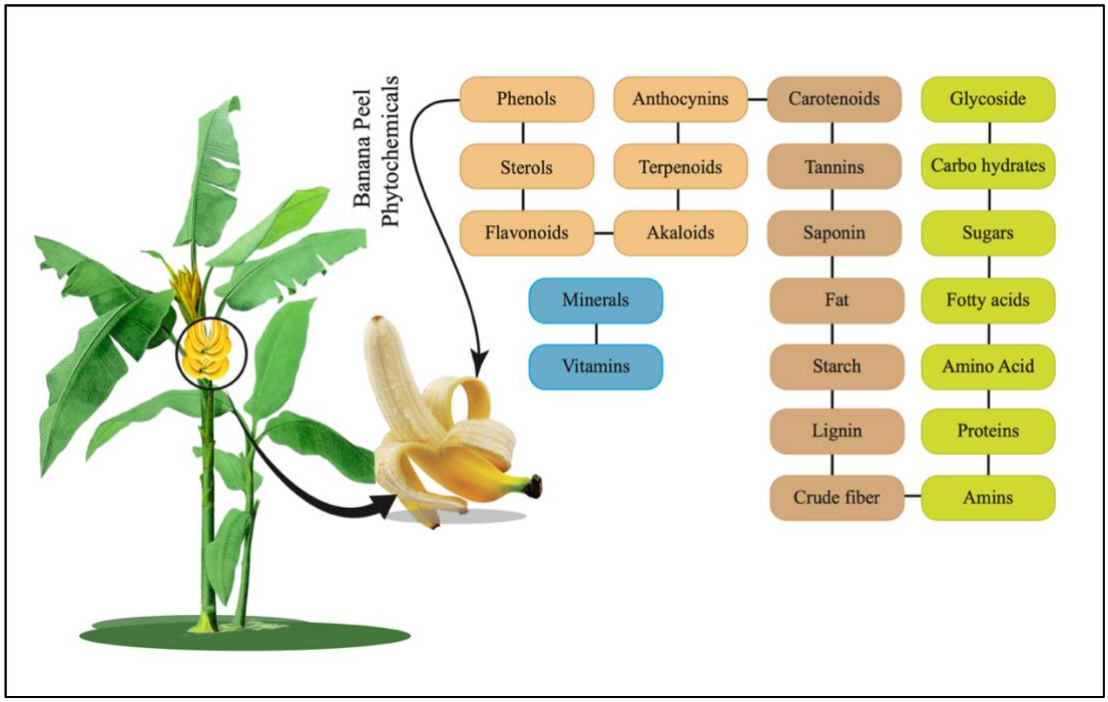

Figure 1.5 Schematic diagram of Banana peel phytochemical

Bananas, both fruit and peel, have been connected to a number of health benefits, making them potentially valuable in the pharmaceutical sector (Singh et al., 2016). Incorporating banana resources, particularly banana peels, into nanotechnology is a relatively new and emerging area of study. While application cases are restricted, bananas and, more typically, banana waste (such as peels) are utilized to make or assist in manufacturing nanoparticles (Chugh & Kaur, 2022). Banana peel extract has been used as a reducing agent for the biosynthesis of metallic nanoparticles (Kokila et al., 2015). These nanoparticles have various potential applications, including antimicrobial, anticancer, anti-inflammatory, and wound-healing properties (Bag et al., 2020; Ruangtong et al., 2020). Given the biocompatibility and natural abundance of bananas, the potential use of banana-based nanoparticles as drug delivery systems in pharmaceutical applications could be an effective field of research (Marfu'ah et al., 2020).

1.6.1 Banana peel Extract and its role in green synthesis

The peel of the banana fruit is a low-cost by-product of the food industry with appealing biological features. It is a valuable resource for many applications involving the pharmaceutical field. Banana peel has long been used as a medicinal ingredient to cure different diseases and cases, such as anemia, burns, diarrhoea, inflammation, ulcers, cough, diabetes, and excessive menstruation (Kumar, 2012). The peel's extract has the potential to reduce heart disease and cancer risks due to the presence of antioxidants such as polyphenols. The peel's extract also has an anti-inflammatory effect on wounds, with many benefits for the digestive system and blood sugar regulation due to the presence of pectin. Potassium is present in high amounts within the peel extract, which is vital in maintaining fluid balance, muscle control, and nerve function (Singh et al., 2016).

Banana peel extract by eco-friendly methods using non-organic solvents would be a valuable technique in pharmaceutical industries and research, especially in the NP field. Banana peel and its extract can be considered an excellent source of polyphenolic compounds such as quercetin, ferulic acid, and catechin; flavonoids are also found in good quantity in banana peel extract, in addition to many polymers such as hemicellulose, lignin, and pectin. These compounds act as bond coordinators and ligands when the extract is used in metallic nanoparticle production. The presence of secondary metabolites in banana peel extract, like polyphenols, flavonoids and alkaloids, will influence the formation and production of many types of nanoparticles (Chugh & Kaur, 2022).

The use of extract from the banana peel, one of the many biological resources that currently exists, has shown potential in producing metallic nanoparticles, as

mentioned in section 1.4. Banana peels are high in bioactive chemicals such as phenols, carotenoids, biogenic amines, and other antioxidants that can act as both reducing and capping agents in the creation of nanoparticles (Chugh & Kaur, 2022). The functional groups in banana peel extract, such as hydroxyl, carbonyl, amine, and carboxyl groups, can act as capping and reducing agents in the production of nanoparticles. The size and stability of the NPs can be controlled by using BPE in the NPs production process. In addition to reducing the metallic ions, as shown in Figure 1.4, the phytochemicals in the extract also cap the nanoparticles, preventing them from aggregating and regulating their size. Green-NPs synthesized via BPE exhibit antibacterial, antioxidant, and catalytic activities. As a result, they are fit to be applied in different fields, such as environmental remediation, medicine, and catalysis (Ruangtong et al., 2020).

1.7 Electrospinning technique

Electrospinning is an effective technique for fibre production within micro- to nanoscale in diameter using different polymeric materials (Yang et al., 2018). The Electrospun process involves the application of a voltage current to a polymer solution, resulting in the expelling of a charged stream from the tip of a nozzle (Asmatulu & Khan, 2018). As the jet progresses toward a grounded collector, the solvent undergoes evaporation, forming a network of fibers (Dos Santos et al., 2020). The liquid's electrostatic attraction by using a current first documented by the scientist William Gilbert in the year 1600. During the period from 1964 to 1969, Sir Geoffrey Ingram Taylor made significant contributions to the development of a theoretical framework for electrospinning. His research includes mathematical modeling of the form of the fluid droplet's cone, also known as the Taylor cone, which happens when an electric

field is applied. During the early 1990s, numerous study groups conducted experiments that showed the production of nanofibers through a process now commonly referred to as electrospinning. The quantity of scholarly articles related to the technique of electrospinning has exhibited an ongoing trend of exponential growth since the year 1995 (Tucker et al., 2012). The electrospinning instrument's principle of operation with the role of each part can be seen in Figure 1.6.

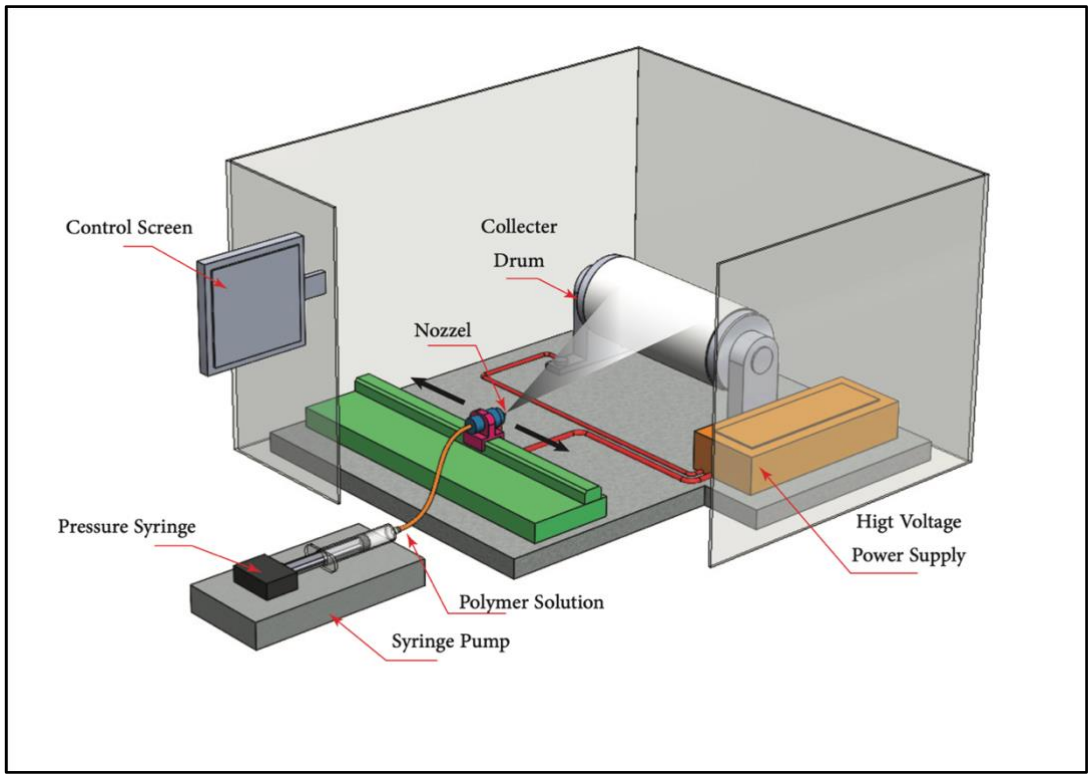

Figure 1.6 A 3D diagram of an electrospinning instrument and its parts. This is a self-constructed diagram with information obtained from Tan et al. (2024)

The formulation of various drug delivery systems through this technique has attracted researchers' attention recently due to its distinctive properties, simplicity, and advantages (Khoshnevisan et al., 2018). Since the Electrospinning technique can generate fibers with significant surface area-to-volume ratios and diverse morphologies, it can be used in different applications such as tissue engineering, drug delivery, filtration, and textiles (Huang et al., 2003).

Electrospinning parameters, such as applied voltage, feed rate, spinneret-collector distance, polymer concentration, polymer viscosity, and polymer conductivity, can be controlled easily and affect the physical and chemical characteristics of the resulting fibers (Deitzel et al., 2001). Since electrospinning is the technique of interest, it is important to study its challenges and limitations, which include scalability, control over fiber diameter, and the ability to create three-dimensional structures. Nevertheless, recent technological advancements such as multi-jet electrospinning, needleless electrospinning, and co-axial electrospinning have been developed to address these challenges (Dos Santos et al., 2020).

1.8 Problem statement

As mentioned, there has been a notable increase in the advancement and usage of nanoparticles, driven by their exceptional characteristics that hold great potential for various fields, including medicine, electronics, and environmental applications. More precisely, zinc oxide nanoparticles (ZnO NPs) are widely applied nanoparticles due to their noteworthy antibacterial, antifungal, photocatalytic, and UV-blocking characteristics. Presently, the predominant techniques employed for producing ZnO NPs encompass physical and chemical methodologies. However, these methods frequently give rise to adverse environmental consequences, such as pollution and significant energy expenses. These undesirable outcomes can be attributed to applying toxic chemicals and elevated temperatures, respectively.

Therefore, there is a pressing requirement for developing a clean, efficient, and environmentally sustainable approach to synthesizing ZnO NPs. Within this particular context, using natural resources in green synthesis techniques has emerged as a highly promising and viable alternative. Banana peels, which are often thought of as wasted

material, possess a significant abundance of phytochemicals that exhibit the ability to reduce metal ions into nanoparticles. Nevertheless, a full investigation of the potential of banana peel extract for synthesizing ZnO nanoparticles has not yet been achieved.

Hence, it is important to examine the ability to produce ZnO NPs through BP extract as a sustainable, environmentally friendly, and economically efficient method. It is also interesting to investigate the synthesis protocol and analyze the resulting nanoparticles to determine their characteristics and assess their physical and chemical properties.

Wound management is a crucial aspect of healthcare. In order to promote good wound healing and avoid wound-related problems, including infection, suitable wound dressing materials must be developed and applied. There are many different types of wound dressing materials available on the market right now. However, many lack the requirements for biocompatibility, antibacterial properties, moisture balance, and the ability to accelerate the healing process.

Incorporating greenly synthesized ZnO-NPs into the electrospun fibres is a potential technique for treating wound bacterial infections. The choice of polymer shall be focused on compatibility and biodegradability by creating non-woven, nano- to micro-scale fibre mats using the electrospinning technique.

1.9 Objectives of the thesis

General objectives: In general, this study explores the green synthesis of metallic NPs using local green sources for the synthesis process, characterizes the produced green NPs, and explores their application. Then, incorporate the produced eco-friendly NPs into a green polymer by fabricating them as a fibre mat. Moreover,

the research aims to produce functional electrospun mats by embedding NPs into microfibre polymer, emphasizing their potential use in biomedical applications, such as wound care. The produced wound dressing product activity has to be assessed through comprehensive characterization and *in vitro* testing.

Specific objectives: The scope of the research described in this thesis is to synthesize ZnO NPs using BP extract in an environmentally friendly manner and investigate the characteristics and antibacterial efficacy of the synthesized ZnO NPs against both gram-positive and gram-negative bacteria. Additionally, investigate the feasibility of incorporating greenly synthesized ZnO NPs into electrospun fibre mats with cellulose acetate as the electrospun polymer. To create a distinctive fiber mat that can be formulated and subjected to various characterization tests as well as in vitro testing that demonstrates its efficacy against some bacterial species.

The following is a summary of this thesis's main objectives:

- To extract banana peel and identify its species, phytochemicals, and functional groups that can be used for NPs synthesis.
- To synthesize ZnO NPs using a green method, utilizing Banana peel extract and characterizing it.
- Fabricate a microfibre mat by incorporating the synthesized ZnO NPs using cellulose acetate polymer and an electrospinning technique.
- To evaluate the in vitro antibacterial efficacy of the synthesized ZnO and the fabricated electrospun Cellulose acetate mat incorporated with ZnO NPs against gram-negative and gram-positive bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In developing an optimum NPs formula, it is important to study the characteristics and features of the greenly synthesized NPs, as well as the electrospun microfiber mat with the incorporated NPs. In the current study, there are different techniques are used to assess and characterize the physicochemical properties of the raw materials used and the final formulations. The characterization and analysis conducted in this work involve the following: liquid chromatography-mass spectrometry to study the plant extract phytochemicals, attenuated total reflectance-Fourier transfer infrared (ATR-FTIR), Powder X-ray diffraction (XRD), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), tensile strength, and contact angle measurement. Viscosity measurement, conductivity measurement, and surface tension tests were also used to characterize the electrospun polymer solution.

On top of the previously covered topic in Chapter 1, the current chapter reviews the used material. It delineates the basic understanding and steps of the used equipment and its usage in developing nanoparticle formulations for antibacterial purposes. This aims to ease the reader and provide a basic understanding of the equipment used in the subsequent chapters. To avoid redundant content presentation, the principles of nanoparticle synthesis methods introduced in Chapter 1 will not be covered here. The extraction process of banana peel, the preparation of ZnO NPs, and the preparation of electrospinning polymer solutions and their electrospinning processes will be covered briefly in principle; further details will be explained in their corresponding chapters 2

and 3, respectively. Other characterization techniques, principles of operation, and the philosophy of in vitro antibacterial studies will be introduced in more detail herein.

2.2 Materials

In general, nanoparticles could be synthesized via physical, chemical, and biological methods, as previously mentioned in Chapter 1, Section 1.2.3. In this study, we will use the green method, which belongs to the biological method, by utilizing Zinc acetate dihydrate (ZAD) as a precursor of ZnO NPs, as well as a plant extract and cellulose acetate, which served as the spinning carrier system in the process of fibre formation.

The process of synthesizing metallic oxide NPs necessitates the use of a precursor; the precursor used will be determined by the type of NPs. Salt precursors are commonly utilized in preparing most types of metallic NPs due to their ability to control particle size and shape by adjusting numerous variables such as salt concentration, pH, and solution temperature. Metal salts, such as acetate, nitrates, chlorides, or sulfates, can be readily dissolved and managed in a solution-based synthesis technique (Jamkhande et al., 2019). To produce metallic NPs, salts of the desired metal have to be used as a precursor in the synthesis process. The metal ion was converted to oxide NPs under controlled conditions to ensure the high purity of the synthesized NPs (Kaur & Kalia, 2016). The main NPs synthesis steps were dissociation of metal ions, oxidation-reduction, precipitation, and purification of the final product (Kaur & Kalia, 2016).

Moreover, the mentioned method can ensure NPs uniformity since the metal source from the salt was consistent. Each ion in the reaction released from the precursor salt will contribute to the formation of NPs, resulting in a high production

yield. On the other hand, salt precursors are also easily available and cost-effective compared to other precursors. In summary, the precursor can significantly affect the biological, physical, and other NPs properties (Hameed et al., 2021).

In this study, ZnO NPs' synthesis process will depend on zinc acetate dihydrate (ZAD) as salt precursor due to its advantages in terms of bioactivity and morphology over other Zn precursors like zinc chloride and zinc nitrate. The resulting NPs from this ZAD salt precursor will give a uniform spherical NPs shape with excellent antibacterial activity (Bahtoun et al., 2023; Mayekar et al., 2014). The synthesis process must be properly controlled, including precursor concentration and reaction conditions, to create high-quality metallic oxide NPs.

2.2.1 Banana plant

2.2.1(a) Taxonomy

The banana plant is a member of the *Musaceae* family in the *Zingiberales* order. The *Musaceae* family is categorized into three main genera: *Musa, Musella,* and *Ensete.* The genus *Musa* is divided into two distinct categories: *Musa* and *Callimusa.* The classification of various genera and divisions within the banana family can be determined by examining morphological characteristics (Inta et al., 2023). The taxonomy of wild banana species was revised, resulting in the reclassification of these species into two sections: *Musa* (which combines *Eumusa* and *Rhodochlamys*) and *Callimusa* (with a chromosome count of $x = 9, 10$). Additionally, Australimusa and Ingentimusa were included (Shetty et al., 2016).

In the current study, the genus *Musa* will be used. This Genus includes four species, each with its own genome letter. These species are *Musa acuminata*