DEVELOPMENT OF MODIFIED CUO AND MGO BASED ANTIBACTERIAL COATINGS FOR FABRICS TO PREVENT BODY ODOUR

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DOCTOR OF PHILOSOPHY

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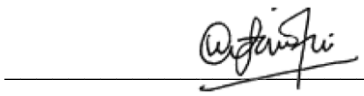

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Thesis submitted in fulfillment of the requirements

for the award of the degree of

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In the name of Allah, the Most Gracious and the Most Merciful.

Peace be upon our prophet Muhammad SAW.

This thesis is wholeheartedly dedicated to my cherished parents and family, whose unwavering love, boundless patience, steadfast support, and blessings have been my pillar of strength. It is also with deep gratitude that I dedicate this thesis to my dearest friend, Nur Ayuni Arifin, for her encouragement, and to my supervisor, Dr. Wan Norfazilah Wan Ismail, for her invaluable guidance and motivation throughout my doctoral odyssey.

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ABSTRAK

Setiap individu mempunyai bau badan semulajadi yang boleh menyebabkan rasa rendah diri, malu serta dapat menjejaskan hubungan antara individu. Penggunaan fabrik antibakteria berpotensi untuk menawarkan penyelesaian terhadap masalah ini. Justeru, pendekatan baru diperkenalkan dengan menggunakan ekstrak kulit delima (PRE) sebagai agen antibakteria untuk mengoptimumkan parameter sintesis sol-gel dan sintesis hijau bagi menghasilkan zarah antibakteria untuk salutan fabrik. Masalah yang ditangani adalah keperluan untuk kaedah sintesis yang cekap, mesra alam, dan ekonomik untuk menghasilkan salutan antibakteria untuk fabrik. Penyelidikan ini tertumpu kepada kesan prekursor; zarah kuprum oksida (CuO) dan magnesium oksida (MgO), dan kaedah sintesis terhadap sifat antibakteria zarah yang disalut ke atas fabrik kapas, poliester, dan fabrik bulu biri-biri campuran. Sebatian alkaloid, fenolik, dan polifenol yang bertindak sebagai ejen antibakteria telah dikenal pasti dalam PRE. Kehadiran unsur CuO dan MgO dalam larutan zarah masing-masing telah disahkan oleh Sinar-X Berpendarflour (XRF). Kaedah sol-gel menghasilkan zarah yang lebih kecil dengan saiz 325.9 nm (CuO) dan 317.7 nm (MgO) berbanding kaedah sintesis hijau (CuO, 374.5 nm; MgO, 325 nm). Imej daripada Mikroskopi Pengimbasan Elektron (SEM) menunjukkan permukaan lapisan yang seragam bagi fabrik kapas yang disaluti dengan zarah MgO, manakala zarah CuO menunjukkan sedikit pengaglomeratan. Sementara itu, pengendapan zarah yang tidak homogen dan tidak seragam diperhatikan pada semua fabrik poliester dan fabrik bulu biri-biri campuran. Spektrum Serakan Tenaga Sinar-X (EDX) telah mengesahkan kehadiran zarah MgO dan CuO dalam fabrik yang disaluti. Ujian antibakteria mendapati keberkesanan zarah MgO terhadap bakteria gram-positif (*B. linens*, *C. acnes*, dan *S. epidermidis*) untuk semua jenis fabrik. Sementara itu, aktiviti antibakteria tertinggi (7 mm) terhadap *B. linens* diperhatikan pada fabrik kapas yang dilapisi zarah CuO dari kaedah sol-gel . Kekuatan regangan dipengaruhi oleh jenis fabrik dan kaedah sintesis salutan, dengan peningkatan tertinggi kekuatan regangan lidah sebanyak 33.73% (arah lungsin) serta beban putus sebanyak 13.59 % (arah lungsin) dan 10.67 % (arah pakan) diperhatikan pada fabrik kapas yang disaluti zarah CuO yang dihasilkan melalui sintesis hijau. Kebolehtelapan udara berkurang pada fabrik kapas yang disaluti dengan zarah MgO dari kaedah sol-gel (42.82%), manakala fabrik polyester yang disaluti partikal CuO dari kaedah sol-gel menunjukkan peningkatan tertinggi sebanyak 12.01%. Ketahanan salutan adalah berlainan, dengan zarah MgO dari kaedah sol-gel menunjukkan ketahanan basuhan yang lebih baik. Analisa Kromatografi Gas-Spektrometri Jisim (GC-MS) menunjukkan kehadiran sebatian organik mudah meruap (VOCs) dalam fabrik kapas yang tidak disalut (asid fosforik, asid heksanoik, dan asid oktanoik), fabrik poliester yang tidak disalut (etil karbamat), dan kain bulu biri-biri campuran yang tidak disalut (asid asetik). Walau bagaimanapun, tiada VOCs yang dikesan dalam semua fabrik yang telah disalut, menunjukkan keberkesanan dalam mencegah bakteria penyebab bau badan. Berdasarkan keseragaman salutan, sifat antibakteria, ketahanan basuhan, peningkatan kebolehtelapan udara dan kekuatan regangan, zarah MgO dari kaedah sol-gel dan disaluti pada fabrik kapas dianggap sebagai fabrik antibakteria yang paling berkesan. Hasil kajian ini menunjukkan potensi PRE dalam sintesis partikel antibakteria. Penyelidikan ini menyumbang kepada penyediaan kaedah yang mampan dan berkesan untuk menghasilkan salutan fabrik antibakteria. Kajian lanjutan boleh ditumpukan kepada peningkatan sifat antibakteria dan ketahanan salutan pada fabrik untuk menghasilkan fabrik antibakteria berkualiti tinggi.

ABSTRACT

Every person has a natural body odour, which may lead to low self-esteem, embarrassment, and even affecting interpersonal relationships. The use of antibacterial fabric offers a potential solution to this problem. Thus, this study introduces a novel approach by utilizing pomegranate rind extract (PRE) as an antibacterial agent to optimize sol-gel and green synthesis parameters for producing antibacterial particles designed for fabric coatings. The problem addressed is the need for an efficient, eco-friendly, and economical synthesis method to fabricate antibacterial coatings for fabrics. The research focuses on the effects of precursors; copper oxide particles (CuO) and magnesium oxide particles (MgO) and synthesis methods on the antibacterial properties of the synthesized particles coated onto cotton, polyester, and blend wool fabrics. The alkaloid, phenolic, and polyphenols compounds which act as antibacterial agent were identified in pomegranate rind extract (PRE). The presence of CuO and MgO elements in respective particle's solution was confirmed by X-Ray Fluorescent (XRF). The sol-gel method produced smaller particle with the size of 325.9 nm (CuO) and 317.7 nm (MgO) compared to green synthesis method (CuO, 374.5 nm; MgO, 325 nm). Scanning Electron Microscopy (SEM) images showed uniform coating surfaces for MgO-coated cotton fabrics, while CuO particles exhibited small agglomerations. Meanwhile, non-homogenize and non-uniform depositions of particles were observed in all coated polyester and coated blend wool fabrics. The Energy Dispersive X-Ray (EDX) spectra has verified the presence of MgO particles and CuO particles in the coated fabrics. Antibacterial testing demonstrated the effectiveness of MgO particles against gram-positive bacteria (*B. linens*, *C. acnes*, and *S. epidermidis*) for all fabric types. In the meantime, the highest antibacterial activity was observed (7 mm) against *B. linens* on CuO-coated cotton fabric synthesized via sol-gel method. Tensile strength was influenced by fabric type and coating synthesis method, with the highest increment of tongue tear strength was 33.73 % in warp direction and breaking load was 13.59% (warp direction) and 10.67% (weft direction) observed for CuO-coated cotton fabrics synthesized via green synthesis. Air permeability decreased in MgO-coated cotton fabrics synthesized via sol-gel method (42.82%), while CuO-coated polyester fabrics showed the highest increment of 12.01%. The durability of the coatings varied, with the MgO particles from sol-gel method exhibiting better washing durability. Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed the presence of volatile organic compounds (VOCs) in uncoated cotton (phosphoric acid, hexanoic acid, and octanoic acid), uncoated polyester (ethyl carbamate), and uncoated blend wool fabric (acetic acid). However, none of the VOCs were detected in all coated fabrics, indicating effective prevention of bacteria-causing body odour. Owing to the coating uniformity, antibacterial properties, washing durability and the increment in air permeability and tensile strength, the MgO particles synthesized via sol-gel method and coated onto cotton fabric were identified as the most effective antibacterial coated fabric. The knowledge gained from this study demonstrates the potential of PRE in the synthesis of antibacterial particles. This research contributes to the field by providing a sustainable and effective method for producing antibacterial fabric coatings. Further research can focus on enhancing the antibacterial properties and durability in order to produce high quality antibacterial coated fabrics.

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LIST OF SYMBOLS

Ag	Silver
$AlCl_3$	Aluminum chloride
$BaCl_2$	Barium chloride
CFU/mL	Colony Forming Unit per milliliter
cm^{-1}	Wavenumber
cP	Centipoise
Cu	Copper
CuO	Copper oxide
Cu_2O	Cupric oxide
$Cu(NO_3)_2.3H_2O$	Copper nitrate
Da	Dalton
eV	Electronvolt
G	Gram
g/mol	Grams per mole
H	Hour
H_2SO_4	Sulfuric acid
K	Kilo or thousand
Kg	Kilogram
kV	Kilovolt
L	Liter
M	Meter
Mg	Magnesium
MgO	Magnesium oxide
MgX_2 or MgF_2	Magnesium halides
$Mg(NO_3)_2.6H_2O$	Magnesium nitrate
Mins	Minutes
mL	Milliliter
Mm	Millimeter
mm/min	Millimeter per minute
MMT	Million metric tons
N	Newton

N	Nitrogen
NH_4OH	Ammonium hydroxide
Ni	Nickel
Nm	Nanometer
O	Oxygen
Pa	Pascal
Ppm	Part per million
S	Sulfur
SiO_2	Silicon dioxide
TiO	Titanium oxide
TiO_2	Titanium dioxide
ZnO	Zinc oxide
°C	Degree Celsius
–OH	Hydroxyl group
µg	Microgram
µg/m³	Microgram per cubic meter
µm	Millimeter
%	Percentage
ß	Beta
Ɛ	Epsilon
±	Plus-minus

LIST OF ABBREVIATIONS

AMPs	Antimicrobial peptides
Approx.	Approximately
ATP	Adenosine triphosphate
ATR	Attenuated total reflection
BC	Before Christ
BHI	Brain Heart Infusion
CAGR	Compound annual growth rate
CASB	Columbia Agar with 5% Sheep Blood
DNA	Deoxyribonucleic acid
EGCG	Epigallocatechin gallate
GC-MS	Gas Chromatography-Mass Spectrometry
LAB	Lactic acid bacteria
LSD	Least Significant Difference
LSPR	Localized Surface Plasmon Resonance
MCFAs	Medium chain fatty acid
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
NAD+	Nicotinamide adenine dinucleotide
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
N/A	Not available
OVAT	One-variable-at-a-time
PEG	Polyethylene-glyco
PET	Poly(ethylene terephthalate)
PRE	Pomegranate rind extract
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RPM	Revolutions per minute
SEM-EDX	Scanning Electron Microscopy with Energy Dispersive X-Ray
SCFAs	Short chain fatty acids
SDA	Structure directing agent
SOD	Superoxide dismutase

SPSS	Statistical Package for Social Science
sp.	Species
TSYE	Trypticase Soy-Yeast Extract
UPLC-QTOF-MS	Ultra Performance Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry
USD	United States Dollar
UTM	Universal Tensile Machine
UV	Ultraviolet
VOCs	Volatile organic compounds
XRF	X-Ray Fluorescent

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CHAPTER 1

INTRODUCTION

1.1 Introduction

This chapter serves as an introduction to the thesis, providing essential background information to elucidate the context and scope of the study. It outlines the research problem, objectives, and the significance of this research. Section 1.2 delves into the motivation, rationale, and main concepts and theories that support the research topic. Additionally, Section 1.3 expresses the main problem, justifying existing gaps in knowledge, which lead to the aims and objectives of this research (Section 1.4). Section 1.5 depicts the scopes of this research, providing an overview of its implementation. Lastly, Section 1.6 highlights the contribution and value of this study to the existing literature, theory and practical applications.

1.2 Background of the Study

Every person has a body odour. It is caused by a combination of exocrine and the growth of bacteria which, largely linked to the apocrine glands (James, 2020; Pickett, 2017). The condition of poor hygiene with a beneficial environment promotes the growth of certain microbes, producing odorous acids. Common acids that produce odours are lactic acid, acetic acid, propanoic acid, and ammonia (Smallegange et al., 2011). Most of these acids come from healthy skin microbial flora such as *Propionibacterium*, *Staphylococcus*, and *Bacilli*, which perform fermentation on carbohydrates, proteins, and fatty acids that come from dead skin cells on people's feet (Abedi & Hashemi, 2020; Baker, 2019). The density and species of bacteria are linked to the intensity and volatile composition of odour (Verhulst et al., 2018). Odour is associated with *Brevibacterium* species, which are commensals on the human skin that thrive in humid environments such as the webs of the toes (van Vuuren et al., 2019). The presence of *Brevibacterium sp.* or *Staphylococcus sp.* attributed to "cheese-like"

odour due to the release of isovaleric acid (3-methyl-butanoic acid) (Öztürkoğlu et al., 2018). At the same time, the "vinegar" odour occurred due to the presence of propionic acid and acetic acid, resulted from the metabolite action of *Propionibacterium sp.*, which presents in the ducts of the sebaceous glands of the skin (Chen et al., 2020). The use of amino acid methionine sulphur by *Brevibacterium sp.* to generate methanethiol has contributed to "ammonia" odour (Abdelraof et al., 2020).

Another contributor to odour is the type of fabric used. It plays an essential role because it can be a potential site for microorganism's propagation. The optimal conditions of temperature, moisture affinity, and nutrient source of fabric could be favourable for microorganism's hostility, which can lead to deterioration of fabric strength, defacement, and odour (Tan, et al., 2019). There are three primary sources of fabric, which are animal-based, plant-based, and synthetic-based fabric. Animal-based fabric like wool mainly consists of keratin, which contains more sulphur than other types of protein. It has improved the strength and thermal stability of the fabric. However, keratin acts as a nutrition and energy source for microbes and bacteria growth which could lead to bad odour (Sanders et al., 2021). A plant-based fabric; cotton, is the most abundant natural polymer on earth. Cotton is susceptible to microbial degradation as it composes of celluloses and hemicelluloses, which is one of the carbon sources (Tan, et al., 2019). The biodegradation of cotton could lead to foul-smelling, discoloration, appearance disfiguration, and hygiene issues in the products (Yae, 2018). Synthetic-based fabrics like polyester, nylon, and acrylic give less ventilation to the foot than cotton or wool, which can lead to increased perspiration and odour, although they can also reduce the incidence of blisters by wicking away perspiration (Deopura & Padaki, 2015; Ma & Sun, 2005).

Coating of fabric with particles is an approach to the production of highly active surfaces with antibacterial properties. Compared to other materials, metals oxide is receiving huge attention as antibacterial agents because of their stability, non-toxicity, and efficient biological properties (Javed et al., 2022; Kumar et al., 2017). Copper oxide (CuO) particles are preferably coated with a plant extract to sustain the efficiency and durability of the antibacterial performance. The plant extract helps to cap the particles on the material by facilitating the slow release of ions (Sebastian & Arruebo, 2019).

Besides that, the high surface area also helps in creating a high probability of reaching and inhibiting the surrounding bacteria in the external environment (Pandey et al., 2022; Tamayo et al., 2016). Magnesium oxide (MgO) has an advantage of being non-toxic. It has been recognized as safe by regulatory agencies such as the United States Food and Drug (Abdallah et al., 2022; Altaee, 2022). MgO particles are able to damage and destroy the cell membrane of bacteria resulting in the leakage of intracellular content which can cause the death of the cell (Choudhary et al., 2022; Jin & He, 2011).

The utilization of plant extract in synthesis methods may enhance the antibacterial properties of the particles. Pomegranate rind extract (PRE), commonly deemed as an agricultural waste product, is known for its high content of bioactive compounds associated with antibacterial properties (Kumar et al., 2022). This is attributed to the presence of ellagitannins and other secondary polyphenolic compounds, which exhibit broad-spectrum activities against microbes (Celiksoy & Heard, 2021). The use of PRE together with metal and metal oxide particles could potentially improve its efficiency against antibiotic-resistant pathogens (McCarrell et al., 2008). The interaction of PRE with bacteria cell walls may inhibit enzymes and directly disrupt the co-aggregation of bacteria (Janani & Estherlydia, 2013). Additionally, the bioactive compounds presence in PRE could reduce the biofilm formation and eradicate pre-formed biofilms of certain bacterial species (Celiksoy et al., 2022).

The synthesis method of antibacterial agents may influence its mechanism of action and activity, which can indirectly affect the subsequent antibacterial effect. The sol-gel is one of the well-established synthetic approaches for the preparation of metal oxide. It creates new surface properties, controls stoichiometry, obtains coatings' homogeneity, large area of substrate coating, and the ability to scale up in the fabrication industries (Simon et al., 2021). The sol-gel method involves hydrolysis, polymerization, and condensation followed by drying to produce the final metal oxide (Rex & dos Santos, 2023). There are two routes of the sol-gel method, which are aqueous sol-gel and non-aqueous sol-gel, depending on the nature of the solvent (Bakar et al., 2023). Another method to synthesize metal oxide particles is the green synthesis method. It is a reliable, biocompatible, and green method which has gained more

attention from the scientific community (Jain et al., 2011). It involves the use of numerous natural resources such as plants, plants tissues, fruits, microorganisms, and algae to synthesize the metal oxide particles (Alhaji & Sujatha, 2022). Furthermore, the green synthesis method is environmentally friendly, cost-effective, and a fast alternative that avoids the use of harsh conditions, toxic reagents, and expensive chemicals (Mohd Yusop & Wan Ismail, 2021).

Therefore, this research was conducted to fabricate an antibacterial coating for fabrics in order to eliminate or reduce odour. Three types of fabrics which are 100% polyester, 100 % cotton and blend wool which consist of 20 % wool, 33 % of tencel, and 47 % of anti-pilling acrylic were used in this research. In addition, sol-gel and green synthesis methods were used to synthesize two different types of modified metal oxide particles, namely CuO and MgO to coat into the fabrics. Easily obtained and economical are the criteria for the selection of these metal oxides. Besides, there have been very few studies on the antibacterial activity of these two metals oxide compared to zinc oxide (ZnO) and silver (Ag). To date, there are very few studies on the use of modified CuO particles and modified MgO particles as an antibacterial coating for fabrics to prevent body odour. The modification of metal oxide particles involved the use of bio extracts from natural resources which is pomegranate rind extract. Pomegranate rind extract has an antimicrobial property that can exhibit certain species of gram-positive bacteria and fungus, which cause body odour (Celiksoy, 2022; Celiksoy & Heard, 2021). The modified metal oxide particles were then coated onto the fabrics to test its antibacterial and mechanicals performance. The optimization of the coating parameters and properties have been considered to produce particles for fabrics coating with enhanced antibacterial effects. Lastly, in order to understand the odorant composition, an analysis using GC-MS was conducted. Results obtained from this study may contribute to the understanding of the relationship between the antibacterial activity of the modified metal oxide particles with the types of fabric and synthesis methods.

1.3 Problem Statement

Every person has a natural body odour that is produced from a range of substances that carry a smell. The accumulation of these substance is important for regular bodily function, but excessive accumulation can result in noticeable smells. Body odour may change for several reasons such as diet, medications, stress, lifestyle, and others. It becomes more apparent to the teenagers as their sweat glands and hormones become more active during this time.

Body odour may contribute to low self-esteem, embarrassment, and even affect the personal relationships. The combination of sweat and the growth of certain microbes may produce offensive odour (McQueen et al., 2022; Lam et al., 2018). However, sweat itself is odourless and provides a cooling effect when the body's temperature rises too high (Baker, 2019). The warmth and dark conditions also provide an ideal environment for microorganisms to grow. Offensive odours are released when the microorganisms break down the substances contained in sweat (Pessemier et al., 2022). Besides, body odour can cause rashes, itchy skin, Athlete's foot, and other bacterial infections (Shastri et al., 2012). These infections can also increase the severity of punctured wound and cut injuries, especially in diabetic patients (Semkova et al., 2015).

Additionally, in Malaysia, there is an increasing concern for an active lifestyle. People tend to do physical exercises to keep fit and healthy. Sweat from physical activity not merely releases water and salt but also chemicals that, in combination with water and salt, can attract certain bacteria to grow (Liu et al., 2022). Clothes that absorb this type of sweat can cause foul-smelling due to the reaction of sweat, bacteria, and fabric. Therefore, choosing the right types of fabric for physical activity is vital in building comfort for the wearer, as the combination of sweat from the skin and the growth of bacteria may cause odour.

The use of deodorants and antiperspirants also can control odour. However, excessive use of these products can worsen the situation. It can lead to an increase in bacterial diversity, which reduces the density of existing bacteria and open up space for new species (Callewaert et al., 2014). This leads to the growth of malodour-causing

bacteria that are able to withstand the anaerobic environment in the sweat glands around the hair roots (Knight et al., 2017)

Therefore, the production of antibacterial coating fabrics has been widely studied as a potential solution to this problem. The direct interaction between particles and bacteria cells on fabric surfaces disrupts cell walls and biological processes, thereby reducing odours by affecting proteins and deoxyribonucleic acid (DNA), hence disrupting cell functions. Despite the advancements, several research gaps remain in this area.

One prevalent research gap involves the absence of a systematic comparison regarding the utilization of plant extract to optimize the sol-gel and green synthesis parameters for producing antibacterial particles designed for fabric coating. For instance, the study conducted by Perveen et al. (2020) compared the antimicrobial activity of the sol-gel and green synthesis methods against a range of pathogens but did not integrate plant extract in sol-gel synthesis. Similarly, Haque et al. (2020) did not employ plant extract in the preparation of particles using sol-gel method and solely discussed the differences in characterization of ZnO, antibacterial and photolytic activities between sol-gel and green synthesis methods. A study by Samat & Md Nor (2013), showed that ZnO particles produced from sol-gel synthesis method ranged in size from 50-200 nm with a spherical shape, while the ZnO particles produced via the green synthesis method had a nanorod shape and a size of 100 nm (Rafaie et al., 2014). Both synthesis methods utilized *Citrus aurantifolia* extracts in the process, but the synthesis parameters differed. Therefore, this study aims to improve the efficiency and effectiveness of both processes by systematically comparing the optimization of synthesis process using plant extract for the production of antibacterial particles, thereby expanding their potential applications.

Another notable research gap is the limited comprehension of how antibacterial coatings impact different types of fabric, their tensile strength, and the generated odorants. A study conducted by Gokarneshan et al. (2012) discussed the comparison of antibacterial activity on different type of fabrics, but used a different synthesis method and failed to report the tensile strength and odour composition of the coated fabrics.

Meanwhile, Tan et al. (2019) only reported the antibacterial activity and tensile strength of the cotton fabric without comparative analysis with other fabric types. The volatile profile of the coated fabrics was not reported in the paper, which limit the determination of the effectiveness of coated fabrics in preventing body odour. Furthermore, Richardson et al. (2022) discussed the antibacterial activity across various type of fabrics and concluded that the antibacterial coated fabric effectively prevents body odour. However, no comparison of odorant profile between the fabric types was indicated, nor the effect of coating on tensile strength. Consequently, there exists research gaps in exploring how antibacterial coatings on different fabric types influence tensile strength and evaluating their effectiveness in preventing body odour.

1.4 Objectives of the Study

The aim of this research is to fabricate antibacterial coatings for various types of fabrics in order to minimize or eliminate odour. The objectives of the research are as follows:

i. To synthesize modified PRE-metal oxide particles using sol-gel and green synthesis methods by optimizing the parameters such as pH, amount of plant extract and number of coatings.

ii. To evaluate the antibacterial behaviour of the coated and uncoated fabric against different species of gram-positive bacteria.

iii. To investigate the correlation between the performance of particles on different types of fabric and their mechanical properties.

iv. To analyse the odorant compositions of the coated and uncoated fabrics using GC-MS.

1.5 Scope of the Study

This study aims to synthesize modified CuO and MgO particles using sol-gel and green synthesis methods to produce antibacterial fabric. The study focuses on the use of pomegranate rind extract as an antibacterial agent in the modification of

particles. The study optimizes the extraction method of the plant extract, by varying factors such as temperature and amount of pomegranate rind powder used. The compounds presence in the extract is identified using UPLC-QTOF-MS. Several important synthesis parameters of modified metal oxide particles such as pH, volume of plant extract, and coating cycles were optimized, and the optimum conditions were applied to the fabrics in order to create new surface properties with enhanced antibacterial properties.

The modified metal oxide particles performance on different types of fabric were evaluated based on the antibacterial performance, mechanical characteristics and washing durability. The characterization of modified metal oxide particles is performed using XRF and SEM-EDX, while the size of particles was measured using particle size analyser. The antibacterial activity of the coated fabrics towards three species of gram-positive bacteria that cause body odour, namely *Staphylococcus epidermidis*, *Brevibacterium linens*, and *Cutibacterium acnes* were studied. The mechanical testing of coated and uncoated fabric samples is carried out using a universal tensile testing machine. The tongue tear strength test was run for both cotton and polyester fabrics, while the breaking load test was carried out for cotton, polyester, and blended wool fabric samples. The air permeability of the fabric samples was determined using an air permeability test device to ensure ventilation. The washing durability of the coated fabric were conducted to evaluate the effectiveness and durability of the modified particles on the fabric. Finally, the study analysed the odorant composition of the coated and uncoated fabrics using GC-MS for a better understanding of their odour compounds.

1.6 Significant of the Study

Fabric can serve as a breeding ground for microorganisms due to its ability to provide a favourable environment for microbial growth when in contact with skin, leading to unpleasant odours. This is due to the fact that fabrics can trap odorous compound, thereby increasing the surface area on which bacteria can thrive (Van Herreweghen et al., 2020; Revathi et al., 2015). Coating fabrics with metal oxide particles offers an excellent solution since it provides a large surface area that can

interact with microbial membranes and has unique physical and chemical properties compared to bulk materials (Sharma et al., 2022; Subhankari & Nayak, 2013). Therefore, developing antibacterial fabric is essential in order to ensure the safety and well-being of wearers.

Recent advancements in the field of technology have opened up a new era for the utilization of materials as antibacterial agents. The new modified metal oxide particles synthesis method is environmentally benign since it uses water as a solvent. Both synthesis methods are also straightforward, easy to carry out under mild conditions and inexpensive. The use of MgO and CuO as precursors is expected to reduce operating costs since they are less expensive compared to other metal oxide precursors. Moreover, both substances have antibacterial properties, and optimizing the synthesis parameters can enhance the antibacterial property of the coated fabric. Besides, the use of pomegranate rind extract as antibacterial agent is anticipated to preserve the fabric's softness and smooth texture, which indirectly enhances the wearer's comfort.

Examining the antibacterial activity on different types of coated fabric is essential since it may give a solution for eliminating or minimizing odour. For instance, very limited research has been conducted in determining the antibacterial activity on different types of coated fabric to prevent odour. Therefore, this study aims to determine the efficacy of antibacterial activities of coated fabric in preventing odour, leading to new knowledge in this field. Furthermore, the research's impact on society and the nation can be significant by enhancing the quality of fabric used by athletes, militaries, and others to prevent odours. This research is also expected to be internationally competitive, emphasizing innovation that advances new knowledge. Lastly, the results obtained from this research can be used to transform knowledge into products or solutions for the industry, directly upgrading the quality of fabric.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter attempts to review the relevant literatures and researches related to the production of antibacterial coatings for fabrics using various synthesis methods. It begins by discussing the concept of body odour in Section 2.2, followed by an exploration of its underlying causes in Section 2.3, and strategies to mitigate body odour in Section 2.4. Subsequently, the discussion delves into textiles, a key focal point of this study, outlined in Section 2.5. Section 2.6 focuses on the antibacterial agents, particularly metal oxides and natural-based agents specifically pomegranate, and examining their mechanisms in bacteria inhibition or eradication. Furthermore, Section 2.7 emphasize on the significance of sol-gel synthesis and green synthesis methods in generating antibacterial agents, outlining their advantages and limitations. Finally, Section 2.8 discusses the effect of coatings on the mechanical properties of the fabrics, such as air permeability and tensile strength.

2.2 Body Odour

Body odour can be defined as the unpleasant odour coming from the human body. It is a characteristic trait of humans and there are several factors influencing it, such as sex, genetics, age, diet, and disease. Body odour is a widespread problem suffered by many people. It is not a disease but rather a symptom. Generally, men produce more sweat and intensified odour due to larger sweat glands and higher quantities of volatile fatty acids than women. According to Pandey & Kim (2011), body odour can be categorized into three types: skin odour (perspiration), exhaled breath odour (released from the human oral cavity) and urine (released from human excreta). Body odour, especially from perspiration, is considered as a social taboo in most

cultures and may contribute to low self-esteem and even affect interpersonal social skills.

The surface of human skin has about 2-4 million sweat glands comprising eccrine, apoeccrine and apocrine sweat glands (Ezure et al., 2021). A human's foot alone has over 250,000 sweat glands, which can produce up to a half-pint of sweat per day (Mishra et al., 2021). The major types of sweat glands found on the surface of skin is eccrine which mainly distributed in the axilla (Chen et al., 2020). Concurrently, the apocrine sweat glands also can be found in the axilla, as well as breasts and perineum (Berth-Jones & Tebbs, 2022). The skin surface condition and regulation of body temperature is controlled by the eccrine sweat glands (Cramer et al., 2022) while the human skin niche is colonized by a diverse species of bacteria (Natsch & Emter, 2020). The complex interaction between the biochemical pathway of skin glands and bacterial enzymes initiates the production of odorant compounds, thus causing the body odour (Pickett, 2017).

2.3 Main Cause of Body Odour

Initially, sweat is odourless, but after being degraded by the bacteria present on human skin, it becomes odorous. The odorous compounds are caused by gram-positive microbial metabolisms such as *Propionibacteria*, *Staphylococcus*, *Bacilli*, and *Brevibacterium* (James, 2020; Mayer et al., 2021). The microflora presents on the skin surface eventually find the secreted amino acids to break down via multiple metabolic processes (Natsch & Emter, 2020). The branched free amino acids responsible for odour are dehydrogenated by microbes into volatile fatty acids to create particular odoriferous compounds (Kim et al., 2021). Odour is produced with an association between high population densities of *Staphylococci*, along with aerobic *Coryneform* bacteria (James, 2020). It is also occurred due to the high population densities of microbial exo-enzymes, like lipases, proteases, and callous degrading enzymes (Pickett, 2017). The intensity of odour depends on the quantity of the enzymes for the degradation of sweat components and the number of bacteria present during that process (de Oliveira et al., 2021; Mark & Harding, 2013). Body odour is composed of volatile organic compounds (VOCs) that contain various free fatty acids such as

propionic, isobutyric, butyric acids, hexanoic acid, octanoic acid and isovaleric acid, as well as thioalcohols (Hand, 2019). The presence of VOCs in the human body is shown in Table 2.1

According to Ara et al. (2006), human sensory tests detected *S. epidermidis*, *S. hominis*, and *Corynebacterium minutissimum* to produce mild odour. On the other hand, *S. aureus*, *P. granulosum*, *P. avidum*, and *Bacillus sp*. were detected to produce intense odour, particularly the genus *Bacillus.* The characteristic of odour is attributed to the presence of *S. epidermis* and *P. acnes*, along with the quantity of isovaleric and propionic acids, and the intensity can be increased with the increasing population of *Bacillus sp*. (Stevens et al., 2014). Isovaleric acid and isobutyric acid are the main contributors to plantar malodour due to their pungencies (Pickett, 2017). These acids can be detected at a low concentration of about 0.17 µg/m³ and 0.72 µg/m³, respectively. Ara et al. (2006) reported that less than 3% of foot odour composition is from isovaleric acid, which is nearly 1/2000 the concentration of acetic acid detected in individuals. Meanwhile, acetic acid and hexanoic acid have a pungent odour (Wang et al., 2021; Kanlayavattanakul & Lourith, 2011), while octanoic acid and carbamic acid have a mild odour (Stevens et al., 2014).

Although microbial metabolism causes odour, the problem also aggravated by poor ventilation of the skin covered by the attire. This is because it retains the skin's moisture, creating an environment for microbial growth. The flourishing microbial is responsible for body odour, which is expected to increase in amount when there is poor ventilation of the attire and high level of sweat. Poor hygiene and engaging in sports, mainly running, may create a more significant problem because sweat provides an ideal environment for microbial growth (Van Vuuren et al., 2019). Proper hygiene may eliminate some of the microbes that cause body odour. However, excessive hygiene may disrupt the skin's environment, leading to other problems such as fungal infections. Besides, the diet also may affect body odour by excreting the foul-smelling or VOCs compounds through sweat glands (Bontempi et al., 2023). To combat this problem, it is essential to understand the cause that contributes to this problem, as there is no quick fix cure for body odour problems.

Table 2.1 The volatile organic compounds presence in human body.

Chemical class	Compound	Possible origin	References
Acid	Octanoic acid	Breath, skin (sebaceous gland secretions)	(Girod et al., 2012; Vishinkin et al., 2021)
	Acetic acid	Breath, skin (human metabolism, microbial metabolism)	(Kruza & Carslaw, 2019)
	Propanoic acid	Breath, skin (microbial metabolism)	(Showering et al., 2022)
	Hexanoic acid	Breath, skin (microbial metabolism)	(Drabińska et al., 2021)
	Decanoic acid	Breath, skin (sebaceous gland secretions)	(Vishinkin & Hossam Haick, 2022)
	Dodecanoic acid	Skin (diet)	(Mohd Kamal et al., 2020)
	Nonanoic acid	Breath, skin (sebaceous gland secretions)	(Drabińska et al., 2021)
	Benzoic acid	Breath, skin (sebaceous gland secretions)	(Vishinkin & Hossam Haick, 2022)
	Isovaleric acid	Skin (microbial metabolite)	(Showering et al., 2022)
	Isobutyric acid	Breath, skin (microbial metabolite)	(Zhang et al., 2022; Zou & Yang, 2022a)
	Valeric acid	Skin (microbial metabolite)	(Mohd Kamal et al., 2020)

Table 2.1 Continued

Chemical class	Compound	Possible origin	References
Alcohol	Isocaproic acid	Breath, skin (microbial metabolite)	(Gio-Batta et al., 2020)
	Ethanol, 2-butoxy	Skin (microbial metabolite)	(Rankin-Turner & McMeniman, 2022)
	Benzyl alcohol	Breath, skin (toluene metabolism, microbial metabolite)	(Zou & Yang, 2022a)
	1-Dodecanol	Breath, skin (microbial metabolite)	(Mitra et al., 2022)
	Phenylethyl alcohol	Skin (microbial metabolite)	(Rankin-Turner & McMeniman, 2022)
	2-Ethyl-1-hexanol	Breath, skin (exogenous)	(Mitra et al., 2022)
Aldehyde	Decanal	Breath, skin (fatty acid degradation, microbial metabolite)	(Kruza & Carslaw, 2019)
	Hexanal	Breath, skin (fatty acid degradation)	(Zou & Yang, 2022a)
	Benzaldehyde	Breath, skin (benzyl alcohol oxidation, microbial metabolite)	(Willems et al., 2022)
	Nonanal	Breath, skin (fatty acid degradation)	(Zou & Yang, 2022a)
	Octanal	Breath, skin (fatty acid degradation)	(Zou & Yang, 2022b)
	Furfural	Breath, skin (unknown)	(Wilkinson et al., 2020)
	Heptanal	Breath, skin (fatty acid degradation)	(Haze et al., 2001)

Table 2.1 Continued

Chemical class	Compound	Possible origin	References
Aromatic	Xylenes	Breath, skin (unknown)	(Vishinkin & Hossam 2022)
	Cymene	Breath (diet)	(Papaefstathiou et al., 2020)
	Naphthalene	Skin (microbial metabolite)	(Zou & Yang, 2022b)
	Styrene	Breath, skin (microbial metabolite)	(Rankin-Turner & McMeniman, 2022)
	Ethylbenzene	Breath, skin (exogenous)	(Rankin-Turner & McMeniman, 2022)
	Phenol	Breath, skin (microbial metabolite)	(Fitzgerald et al., 2020)
Hydrocarbon	Undecane	Breath, skin (lipid peroxidation, microbial metabolite)	(Eshima et al., 2020)
	Dodecane	Breath, skin (microbial metabolite)	(Eshima et al., 2020)
	Pentadecane	Breath, skin (lipid peroxidation, microbial metabolite)	(Fitzgerald et al., 2020)
	Hexadecane	Breath, skin (lipid peroxidation)	(Eshima et al., 2020)
	Decane	Breath, skin (lipid peroxidation, microbial metabolite)	(Eshima et al., 2020)
	Heptadecane	Breath, skin (lipid peroxidation)	(Rankin-Turner & McMeniman, 2022)

Table 2.1 Continued

Chemical class	Compound	Possible origin	References
Hydrocarbon	Tridecane	Breath, skin (microbial metabolite)	(Fitzgerald et al., 2020)
	Octadecane	Breath, skin (lipid peroxidation)	
	Tetradecane	Breath, skin (microbial metabolite)	(Eshima et al., 2020)
Ketone	6-Methyl-5-hepten-2-one (sulcatone)	Skin (oxidation of squalene, breath)	(Dalvi & Rossky, 2010)
	3-Hydroxy-2-butanone (acetoin)	Breath, skin (microbial metabolite)	(Fitzgerald et al., 2020)
	6,10-Dimethyl-5,9-undecadien-2-one (geranylacetone)	Skin (oxidation of squalene)	(Dalvi & Rossky, 2010)
	Acetophenone	Breath, skin (unknown)	(Rankin-Turner & McMeniman, 2022)
Terpene	Limonene	Breath, skin,(diet, cleaning product)	(Amann & Smith, 2013)
	Pinene	Breath, skin (exogenous)	(King et al., 2010)
Terpenoid	Linalool	Breath, skin (unknown)	(Rankin-Turner & McMeniman, 2022)
	Terpineol	Breath, skin (exogenous)	(Amann & Smith, 2013)
Thiozole	Benzothiazole	Breath, skin (exogenous)	(Rankin-Turner & McMeniman, 2022)

2.4 Minimizing Body Odour

Deodorants and antiperspirants are commonly used to minimize or prevent body odour. Deodorant acts by killing skin flora and blocking the production of malodour compounds produced by bacteria metabolites (Yuan et al., 2021). The use of antiperspirants may help in reducing sweat as they can keep the skin dry, which eventually inhibits the growth of microbes on the skin (Srikrishnan, 2022). The aluminium chloride ($AlCl_3$) contained in antiperspirants helps in clogging the sweat ducts in the skin, thus reducing the production of sweat (Shukla et al., 2019). However, the propylene glycol, and benzalkonium chloride contained in most of these products could decrease the bacteria growth and, in long term, alter the skin microbiome (Wood & Cock, 2022). According to McLoughlin et al. (2022), the use of antiperspirants for a long time was found to increase of the presence of odour-producing *Actinobacteria* in individuals. Moreover, the reaction of sweat and $AlCl_3$ causes the de-coloration and degradation of the fabric fibres (Srikrishnan, 2022).

Maintaining proper hygiene is crucial to minimize body odour resulting from microbial digestion of sweat. Regular showers and the use of antibacterial soaps can aid in reducing body odour by eliminating unpleasant smells. The antibacterial soaps are effective in killing microbes on the skin, thereby directly reducing the microbes responsible for body odour. However, according to Food and Drug Administration (2019), the presence of triclosan and triclocarban in antibacterial soaps can contribute to bacterial resistance and environmental pollution. Additionally, changing attire regularly is important, as the microbes could adhere to clothing and contribute to body odour. Wearing clean clothes may help to decrease the number of microbes on the skin. Nevertheless, due to busy schedules related to work or school activities, it is often challenging to shower and change the attire frequently.

The use of appropriate fabric in the manufacturing of attire is also important in order to prevent body odour. Fabric can be a host for odour causing microbes and the odoriferous compounds. According to McQueen & Vaezafshar (2020), the reaction of skin microbiome with textile could produce more intense odour than in axillae. The chemical composition of fabric materials plays a crucial role in the trap and release

actions of body odour (Yao et al., 2015). Cotton is susceptible to microbial degradation and may cause discoloration, hygiene issues, and bad odour (Karim et al., 2020). The use of polyester and nylon fabric may increase perspiration and directly increase the body odour (Thilagavathi & Rathinamoorthy, 2022). Bamboo fibre possesses antimicrobial properties and acts as a bacteriostatic bio-agent due to the presence of bamboo cane substance in the fibre (Jais et al., 2023). However, its limited availability in the market and higher cost makes it less accessible for widespread use. Blending both natural and synthetic fibres presents a potential solution to address body odour issues and enhance the absorption of sweat (Wang et al., 2020). Additionally, this blending approach can mitigate the negative characteristics of individual fibres by producing a diverse range of yarns with desirable attributes (Kahoush & Kadi, 2022). Nevertheless, the utilization of blended fibres remains restricted due to challenges for commercially separating them back into their original components (Peterson et al., 2022).

The removal of sweat glands can aid in reducing body odour by eliminating the medium for bacteria propagation, namely sweat (Wang et al., 2022). The results of sweat gland removal are typically immediate and permanent in the treated area. However, sweat glands removal surgery can lead to both temporary and, in rare cases, lasting side effects. The temporary side effects may include infection, bruising and pain at the surgical site (Song et al., 2023). Rare negative side effects may involve nerve damage leading to loss of sensation under the arm, underarm scarring, and low blood pressure (Schlereth, 2009). The heat intolerance may arise post-surgery due to inability of the body to produce sweat for cooling purposes and maintaining body temperature (Baker, 2019). Besides, the excessive sweating may occur in other parts of the body, and causing discomfort for some individuals. Moreover, the sweat glands removal surgery is costly, making it unaffordable for many.

Recently, there has been a growing interest in the use of antibacterial fabric prepared using antibacterial agents such as metal ions and natural resources. These fabrics act as a medium to kill or inhibit skin bacteria (Bakar et al., 2023). In addition, fabric coated with metal ions can help in avoiding skin injuries and promote regeneration of the damaged tissue (Thampi et al., 2015). Research conducted by Dykes (2015), has found that CuO particles coated on fabric can improve skin elasticity by

reducing the fine lines and wrinkles, resulting in a younger-looking appearance. Moreover, a low concentration of metal ions is required in the production of antibacterial fabric in order to destroy the key intracellular protein of bacteria (Li et al., 2021). The destructive mechanisms involved in metal ions antibacterial activity include disruption of the cell wall membrane, damage of bacteria membrane, oxidation of key proteins, and interruption of electron transport chains (Godoy-Gallardo et al., 2021). A natural defensive amino acid found in natural resources is considered as a promising antibacterial candidate (Li et al., 2021; Kaskow et al., 2020). While numerous studies have explored antibacterial coatings for fabrics, there remains a scarcity of research investigating the effect of antibacterial coatings on natural, synthetic and blend fabrics concerning their effectiveness in preventing body odour.

2.5 Textiles

Textiles have been widely used in many industries for several decades, including clothings, furnishings, automotive textiles, sports equipment, medical devices, and hygiene applications (Antinate et al., 2023). They are composed of a natural or synthetic yarn fibres that are interlaced through processes such as weaving, knitting, crocheting, knotting, or pressing (Jahandideh et al., 2021). Various types of fibres used in the production of textile is shown in Figure 2.1.

The production of textiles can be traced back to ancient times, with women primarily manufacturing them manually at home until the seventeenth century (Sinclair, 2015). The industrial revolution in the late 18th century marked the beginning of mass textile production, with the invention and patenting of the world's first sewing machine by Thomas Saint in 1790 (Nayak & Padhye, 2015). In the 1850s, Isaac Merritt Singer developed the high-speed sewing machine, surpassing human capabilities in the production of textile (Strauven, 2020). Further advancements occurred in the 20th century, with the introduction of steam engines replacing manual power for sewing machines (Aloviddinovich, 2020). Nowadays, modern electronic machines have led the rapid growth of the textile industry, resulting in competitive pricing and the production of technical textiles.

The global production of textile fibres has witnessed substantial growth, increasing from 34 million metric tons (MMT) in 1975 to 109 MMT in 2020, and the demand for textiles is expected to reach 149 MMT by 2030 (Statista Research Department, 2022). In 2021, the sales values for global technical textile were USD 993.6 billion and is estimated to increase by 4.0% at compound annual growth rate (CAGR) from 2022 to 2030 by Asia Pacific region became the largest worldwide market with over 48% revenue share due to the increase and high demand for clothing and apparel (Grand View Research, 2020). The presence of multiple e-commerce platforms such as Shopee, Lazada and others has added the positive growth in the textile market. It is estimated that the consumption of textile will increase every year due to the growing population, rapid urbanization in economic, favourable government policies and significant technological advancement in conducive textile (Shaw, 2022).

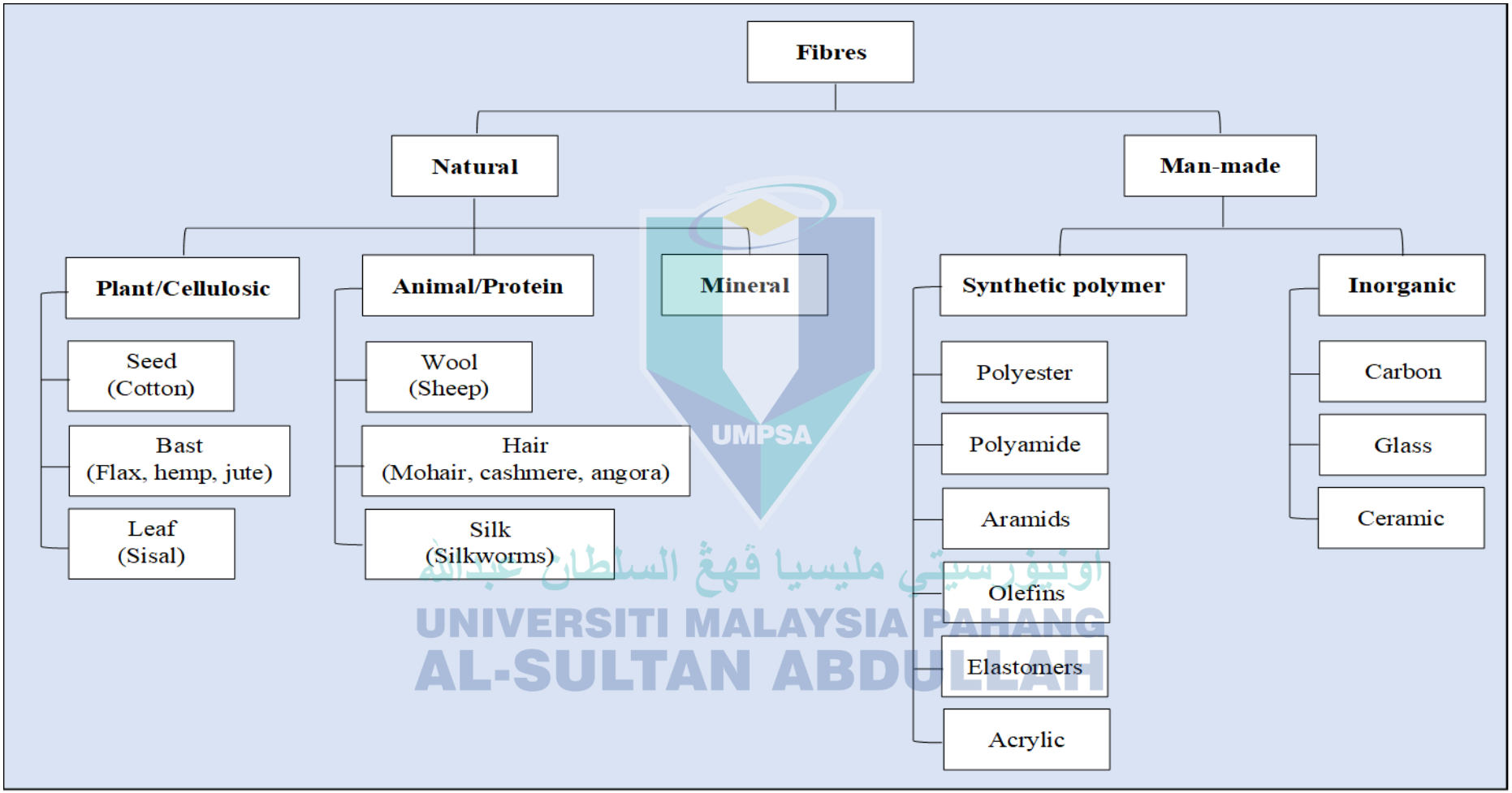


Figure 2.1 The types and sources of fibres for the production of textiles.

2.5.1 Natural-based Textiles

Natural-based textiles can be classified into three types: plant-based textiles, animal-based textiles, and mineral-based textiles. They are produced from natural sources of fibres, either from plants, animals, or minerals (Lopes et al., 2021). However, mineral-based textiles will not be further discussed as they are not commonly used in textile manufacturing. Natural-based textiles are differed from each other in terms of colour, surface contour, chemical structure, and cross-sectional shape depending on the origin of the fibres (Nayak et al., 2020).

Recently, the demand for natural-based textile has been rising, and thus becoming a key factor to the growth of textile industry. The fact that natural-based textiles are stronger than synthetic-based textiles have risen their popularity (Shaw, 2022). Many manufacturing sectors have increased their usage of natural fibres due to the demand for long-lasting products (Wankhede et al., 2023). The natural-based textiles have a wide range of applications and versatility (Patil et al., 2022). They can be used in the production of clothing, furnishings, car upholsteries, sports equipment, medical devices, and many more. In addition, the natural-based textiles are considered sustainable, renewable, and eco-friendly as they able to break down in the environment over time (Shaw, 2022). Due to the growing concern about environmental impact among the consumer, the production of eco-friendly materials has had a positive impact on manufacturers, as these materials have become more popular amid the end users.

2.5.1.1 Plant-based Textiles

Plant-based textiles are derived from different parts of plants, such as seeds, bast, and leaves. These components mainly composed of cellulose, lignin, and hemicellulose (Promhuad et al., 2022). The physical and chemical properties of the plant-based textiles depend on the chemical composition of the fibres, growing conditions of the plant, and extraction process (Muthu & Gardetti, 2020). Due to their abundance, comfortability, softness, and breathability, plant-based textiles have gained popularity in clothing applications (Syduzzaman et al., 2020).

Cotton is the most commonly used plant fibre in the production of textiles, accounting for about half of the textile production. It belongs to the Malvaceae family and the *Gossypium* genus (Wendel & Grover, 2015). Cotton is known for its softness, biocompatibility, breathability, and comfort, making it suitable for human use (Imran et al., 2020). It is the most abundant natural polymer on earth and mainly composed of cellulose, accounting for about 80-90%, along with other macromolecules such as hemicelluloses, waxes, protein, and pectin (Pullas Navarrete & de la Torre, 2022; Tan et al., 2019). It is also composed of a lengthy series of glucose units connected through oxygen bridges between the C−1 and C−4 positions (Peter, 2021). This connection, known as a glycosidic bond, is formed with the removal of a water molecule (Etale et al., 2023). Hydrogen bonding, which occurs between the hydroxyl groups of adjacent molecules, typically stabilizes the cellulose chains present within cotton fibers (Poletto et al., 2014). This interaction contributes to the strength and durability of the cotton fabric.

Also, owing to the richness of cellulose content in the fibres, cotton has high absorption efficiency. This is due to the high number of free hydroxyl groups exist in the fibres (Rosli et al., 2019). Begum et al. (2021), have reported that cotton can absorb moisture approximately 24 to 27 times its own weight. Besides, cellulose is also known as one of the renewable carbon sources which can be degraded by microorganisms (Abe et al., 2021). The deterioration of cotton in terms of weight and strength occurs due to the depolymerization of the cotton fibres (Pullas Navarrete & de la Torre, 2022). The degradation of cotton by fungi begins from the inner to the outer layer of fibres, while the degradation by bacteria starts from the fibre surface to the inner layer (Zayed et al., 2021). In order to enhance the quality of the cotton fabric, numerous studies on antimicrobial treatments using metal and metal oxide particles have been conducted. Such research includes the use of Ag/chitosan composite (Gao et al., 2022), nano silica dioxide (SiO_2) loaded Ag particles (Amibo et al., 2022), chitosan Schiff base-titanium dioxide-zinc oxide (base-TiO_2-ZnO) nanocomposites (Refaee et al., 2022), ZnO particles (Shehabeldine et al., 2022), Ag-CuO-zeolite particles (Sk et al., 2023), titanium oxide (TiO) particles (El-Naggar et al., 2022), MgO particles (Ramezani Farani et al., 2023), and many more.

2.5.1.2 Animal-based Textiles

Animal fibres, also known as natural protein fibres, such as wool, hair, and silk, are largely composed of certain proteins (Roy Choudhury, 2023). They are formed through polymerization of peptide bonds by a series of amino acids into macromolecular chains (Xueliang, 2020). The physical characteristics of these fibres is differed in terms of length, fineness, internal structure, and shape (Nayak et al., 2020). However, the chemical structure of these fibres is associated with wool (Nayak & Padhye, 2015). Among all animal fibres, wool plays an important role in textile industry. It has excellent properties which endow it with different unique styles. Such properties are high water absorption, elasticity, thermal stability, and stain resistance (Li et al., 2023). Wool fabrics are originated from sheep, camel, rabbit, and goat (Xueliang, 2020). Comprising roughly 97% protein and 3% fat, wool possesses unique characteristics that make it particularly suitable for specific uses (Das & Das, 2022). The wool fibres are commonly used to produce technical products such as apparel, blankets, cushions, carpets and many more (Allafi et al., 2022). Wool fibre is widely used in textile applications due to its thermal characteristics. Additionally, wool fabric is eco-friendly, soft, durable, and tear-resistant (Bharath et al., 2019). Wool fibre possesses a unique and versatile physical and chemical property due to its composition and macromolecule spatial structure (Zhu et al., 2021). The shape of wool fabric remains the same at temperature below 100 °C (Xueliang, 2020). Wool fabric has high moisture absorption due to the large amount of hydroxyl groups, which make it comfortable to the wearer (Khosravi & Montazer, 2023).

The main component of wool fibres is a fibrous protein substance called keratin (Navone et al., 2020). Keratin can be found in cuticles and cortical cells (Andra et al., 2021). Keratin contains sulphur, which is bound to a lipid membrane cell complex (Giteru et al., 2023). The strength and thermal stability of the fabric are improved through the crosslinking of keratin fibres and polypeptide chains (Zhu et al., 2023). However, the presence of cysteine bonds in keratin, which are susceptible to sunlight, may weaken the fabric (Wilkie et al., 2016). Keratin also acts as a nutrient and energy source for microbial growth, which can indirectly cause damage to the fabric. Infected fabric may induce health problems such as irritation to the consumers (Rohani Shirvan

et al., 2022). In terms of chemical characteristics, wool exhibits resistance to all concentrations of mineral acids, even at elevated temperatures, although it can be damaged by oxidation due to nitric acids (Lakshmanan, 2022). On the other hand, wool is notably susceptible to substances with alkaline properties. While strong alkaline substances can impact wool fibers, those with a weak alkaline nature do not have an effect (Mostafizur Rahman et al., 2023). Given the unique chemical characteristics of wool, numerous research studies have been conducted to functionalize the wool fabric with different types of particles. The bonding formation of wool with metal oxide particles such as ZnO, MgO, and TiO_2 could provide hydrophilic, UV protection, and good colourfastness properties to the fabric (Abdelrahman et al., 2020). These new characteristics could facilitate the dyeing and finishing process of the fabric. Coating wool fabric with particles such as Ag (Hasan et al., 2021; Sadeghi-Kiakhani et al., 2021), ZnO (Abdelghaffar et al., 2021; Hassabo et al., 2023), and MgO (Kafafy et al., 2021) offers excellent antimicrobial characteristics.

2.5.2 Synthetic-based Textiles

Synthetic-based textiles are produced through various processes by adding chemicals to natural processing materials. This process combines monomers into polymer chains to form fabrics with different characteristics (Egan & Salmon, 2022). Examples of synthetic fabrics are polyester, nylon, polyamide, and acrylic. Synthetic fabrics are easy to maintain, lightweight and poor conductors of heat (Karimah et al., 2021). They are often considered inexpensive and have specific characteristics not found in their natural counterparts like waterproofing, flame resistance, elasticity, and wrinkle resistance (Pironti et al., 2021). However, unlike the natural-based textiles, the manufacturing of synthetic textiles involved the use of toxic chemicals in the polymerization process, which is harmful to the environment (Patti et al., 2020). The synthetic textiles also serve as a medium for microbial growth (Andra et al., 2021). According to Jeevanandam et al. (2022), the chemical-based synthetic textiles exhibit toxicity towards microorganisms, which can help control the growth of microbes. The synthetic textiles have a rod-like structure with long and circular filament fibres, giving them a smooth and shiny appearance (Afzal et al., 2020).

Polyester is the most well-known and widely used synthetic fabric, synthesized through a chemical process that involves various elements such as coal, water, air, and petroleum (Lee, 2017). It is primarily composed of compounds within the ester functional group. The typical method of producing polyesters involves a condensation reaction between an organic alcohol, which contains hydroxyl (OH) groups, and a carboxylic acid, which contains carboxyl (COOH) groups (Kobayashi & Uyama, 2019). The reaction between these two functional groups results in the formation of the distinctive ester linkage. Poly(ethylene terephthalate), commonly referred to as PET, is a frequently used type of polyester (Singh et al., 2022). The creation of this type of polyester involves a reaction between an acid, benzene-1,4-dicarboxylic acid (also known as terephthalic acid), which has two −COOH groups, and an alcohol, ethane-1,2-diol (also known as ethylene glycol), which has two −OH groups (Wang et al., 2024). In the process of forming ester linkages, these substances undergo a reaction, and each formation of an ester bond results in the expulsion of a water molecule (Wang et al., 2022).

Polyester fabric is commonly used as an alternative to cotton and linen fabrics. According to Business Research Insight, (2022), the global market size of polyester was USD 805.9 million in 2022, and it is expected to reach 973.6 million in 2028. Polyester is widely used in apparel applications such as children's wear, lingerie, pants, dresses, and raincoats. This is due to its elastic characteristics, wrinkle and shrinkage resistance, pleat retention, easy-care properties, and resistance to damage from sunlight (Tan et al., 2019). According to Ketema & Worku (2020), polyester fabric is hydrophobic and not capable to form hydrogen bonds with other molecules, making it resistant to microbial growth. However, the presence of fibre lubricants and spinning oil used during the finishing process can provide sufficient nutrients for the growth of microorganisms (Andra et al., 2021).

Blending polyester with natural-based fibres could enhance the quality of the polyester fabric. For example, blending polyester with wool could reduce fading and eliminate crushing, making it suitable for furniture applications and pillow filling (Deopura & Padaki, 2015). However, the lack of hydrophobic properties in polyester can lead to poor adhesion of coatings and shorten the durability of finishing treatments,

which resulted in hygiene and health issues of the consumers (Prorokova et al., 2022). Thus, research on modification of polyester to have more hydrophilic characteristics has been conducted to overcome this problem using materials such as Ag (Zhao et al., 2023) and titanium dioxide (TiO_2) (Abdelghaffar et al., 2020). Meanwhile, blending polyester with cotton resulted in high moisture absorption and easy-care characteristics, although it reduces moisture absorption from polyester and weakens wrinkle recovery from cotton (Tan et al., 2019). The blending fabrics can cover up the negative characteristics of both fibres.

Coating fabrics with metal oxide particles can also enhance their quality. Hanh et al. (2016) determined that the antimicrobial efficacy of silver (Ag) is enhanced when bound to polyester/cotton fabric, although this efficacy varies with the fabric type. Wool has the lowest antimicrobial performance, followed by polyester/wool, and polyester fabric, due to the different concentrations of Ag absorbed by the fabric (Klemenčič et al., 2013).

2.6 Particles

Particles can be defined as small pieces or amount of a substance characterized by their physical or chemical properties such as volume, density, or mass (Gross-Rother et al., 2020). It can be divided into metal-based, metal oxide-based, natural-based, carbon-based, and ceramic-based depending on their composition (Joudeh & Linke, 2022). However, only metal oxide-based and natural-based particles will be further discussed in this study. As for size and shape of particles, it can be varied and are generally classified based on their sizes (Amidon et al., 2009). The particles within the range of 1-1000 µm are called microparticles, while those between 2500 to 10,000 nm are considered coarse particles (Kumbhar et al., 2023; Lengyel et al., 2019). The fine particles typically range from 100 to 2500 nm, while nanoparticles possess sizes smaller than 100 nm (Kumbhar et al., 2023). The size of particles is important as it can influence various properties such as surface area, porosity, optical properties, electrical conductivity, magnetic behaviour, and biological attributes, that directly affecting the properties and functionalities of the materials or products (Nguyen et al., 2021; Zwijnenburg, 2021).

2.6.1 Metal oxide-based Particles as Antibacterial Agent

Metal oxides have been used as antibacterial agents since ancient times, when Egyptians, Romans, Greeks, and Persian kings used them for preservation of food and water disinfection, with records dating back to 450 BC (Gold et al., 2018). The discovery of antibiotics in 1920 reduced the use of metal oxides as antibacterial agents in many applications (Sharma et al., 2022). However, long-term use of antibiotics caused mutation of bacteria cells resulting in the resistance of bacteria from multiple mechanisms towards antibiotics (Figure 2.2) (Pulingam et al., 2022; Sánchez-López et al., 2020; Bakkeren et al., 2020). This is due to the agglomeration of bacteria, irreversibly adhere to substrates, and forms biofilms (Gold et al., 2018).

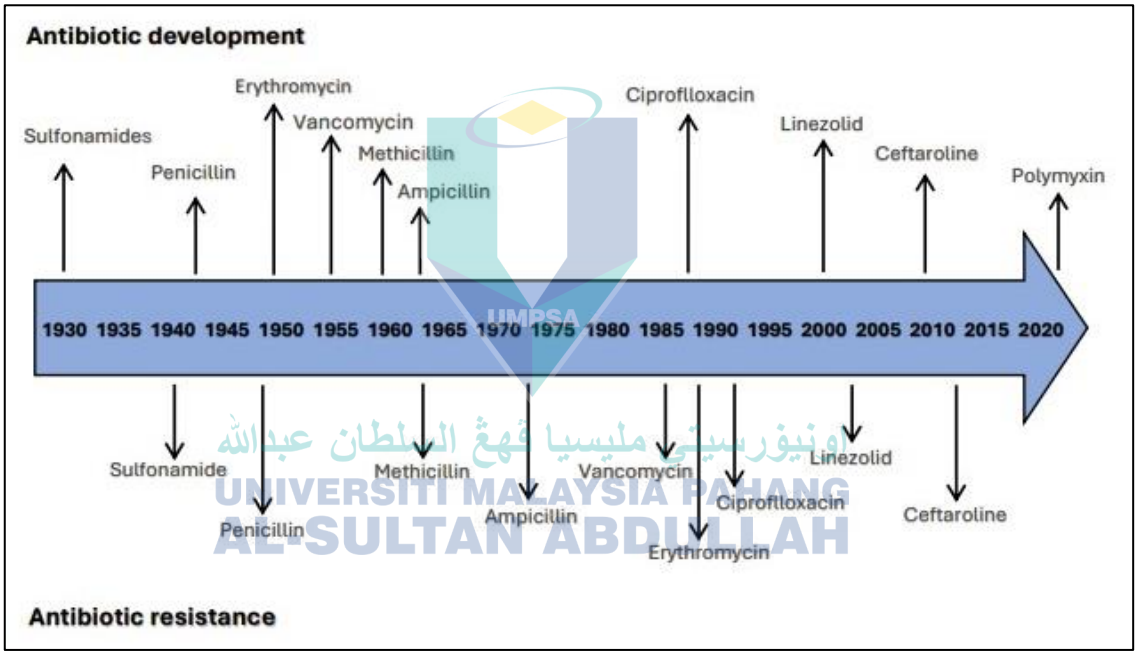


Figure 2.2 The timeline of the discovery of antibiotic and antibiotic resistance.
Source: Dam (2018)

With growing concern of bacterial resistance, metal oxides have been explored as antibacterial agents due to their application history. Metal oxide-based particles are often used in the form of salt-based additives due to the high cost of metal oxide in its pure form (Jamshideasli, 2022). Metal oxide-based particles are not a new technology in the textile industry. They have been widely used to develop efficient antibacterial textiles in recent years (Bhandari et al., 2022). Metal oxide-based particles are

considered a promising solution against antibiotics as they can address the gaps where antibiotics often fail (Khalil et al., 2021). Both antibiotics and metal oxide-based particles able to recognize bacterial cells through the presence of bacteria's metal transport system and metalloproteins (Gold et al., 2018; Yin et al., 2023). However, unlike antibiotics, the antibacterial activity of metal oxide-based particles occurs via multiple mechanisms (Subhan, 2020).

Metal oxide-based particles typically have a size ranging from 1 to 100 nm, yet it depends on their source or application (Battistini et al., 2020). The small dosage of metal oxide-based particles is required in order to inhibit the bacteria growth as it provides a strong and targeted antibacterial activity (Rosli et al., 2021). Their small particle size and large surface area to volume ratio have permit the interaction with biofilms and bacteria cells (Gold et al., 2018). Metal oxide-based particles also can be dissolved faster in a given solution and be easily embedded into fibre's polymeric matrices (Zakhireh et al., 2022). Therefore, they could give a higher release of metal ions, and a more potent antimicrobial effect to the fabric (Stanić & Tanasković, 2020). The antibacterial activities of metal oxide particles also are greatly affected by its physicochemical properties including size, shape, chemical modification, coating, and mixture ratios with other particles and solvent (Dediu et al., 2022). Besides, the physiological state of the bacteria such as growth rate, biofilm, planktonic, stationary, or starvation phase also affect the sensitivity of the bacteria towards metal oxide particles (Chakraborty et al., 2022). The ratio of the bacteria to particles and environmental factors such as aeration, pH, and temperature further contribute to the toxicity of particles to bacteria (Khorsandi, Keyvani-Ghamsari, et al., 2021).

The toxicological effects of metal oxide-based particles on bacteria depend on the types of bacteria being targeted (Ameen et al., 2021). This is due to the direct contact of metal oxide-based particles with the cell wall of gram-positive and gram-negative (Awassa et al., 2022). Gram-positive bacteria have a negatively charged surface and a thick layer of peptidoglycan (Rohde, 2019). Meanwhile, gram-negative bacteria have a more complex structure with a negatively charged surface (Alfei & Schito, 2020). Positively charged particles and the negatively charged bacterial cell wall attracted to each other due to electrostatic interactions (Figure 2.3) (Fang et al., 2019; Li

et al., 2019), leading to the disruption of cell walls and increased membrane permeability (Karnwal et al., 2023). Metal oxide-based particles can also release metal ions into extracellular space, inducing the production of reactive oxygen species (ROS) and causing oxidative stress that inhibits the antioxidant defence mechanisms of bacteria (Yusop et al., 2023; Soheili et al., 2022; Stensberg et al., 2011). The interaction of metal ions with cellular structures such as membranes, proteins, and deoxyribonucleic acid (DNA) can further disrupt cell functions (Rajagopalachar et al., 2022). Metal oxide-based particles exhibit broad-spectrum antibacterial activity due to their ability to form strong bonds with nitrogen (N), oxygen (O), or sulfur (S) atoms in biomolecules and organic compounds (Chidre et al., 2023; Yuan et al., 2018).

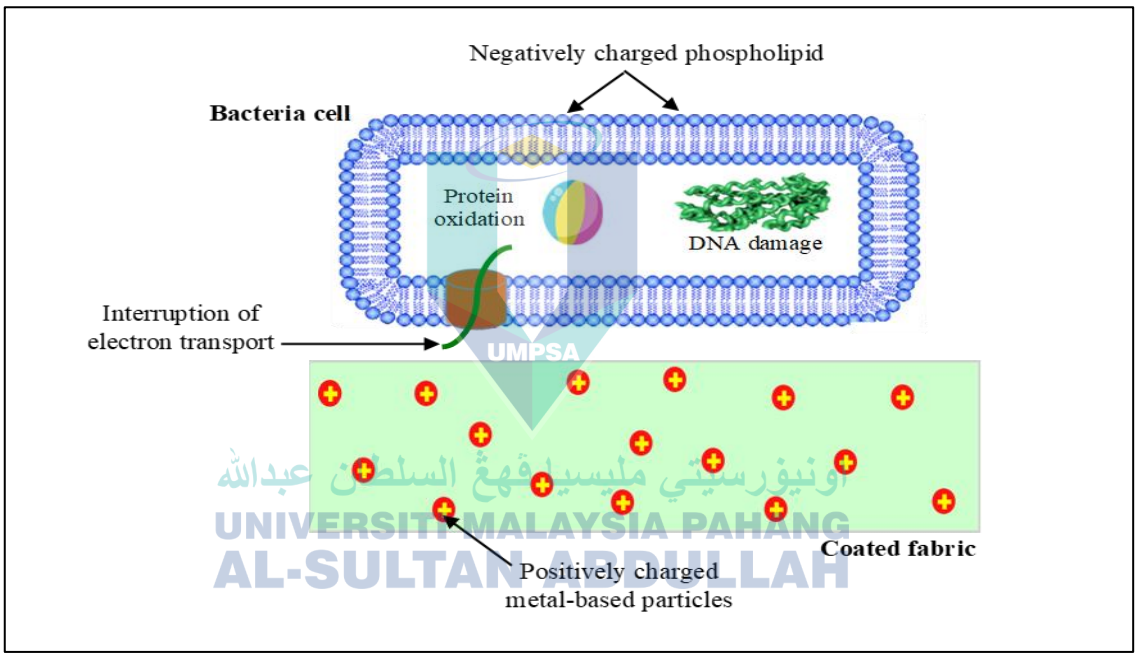


Figure 2.3 Damage to Gram-positive bacteria cell through electrostatic interactions. Source: Li et al. (2019)

2.6.1.1 Copper Oxide

Copper oxide is one of the metal oxide-based particles which possesses antimicrobial properties (Manjunatha et al., 2021). It consists of copper and oxygen elements (Singh et al., 2016). Copper oxide is considered as an excellent candidate for the synthesis of metal oxide-based particles due to its ease of synthesis using plant

extracts, algae, bacteria, and fungi (Chand Mali et al., 2023). Copper is a semiconductor with excellent electrical and thermal conductivity (El Nahrawy et al., 2019). It has been widely used in various applications such as in electronics, sensors, catalysis, and biomedicine. Copper is resistant to heat, reliable, cheap, and stable (Zhang et al., 2020). Both copper oxide (CuO) and cupric oxides (Cu_2O), commonly known as copper (II) oxide, have a monoclinic structure (Jun et al., 2021) and can take on numerous shapes such as rods, wires, spheres, flowers and many more (Majumdar & Ghosh, 2021). The shape and size of CuO particles can affect their absorption and fluorescence spectra (El-Trass et al., 2012). CuO is more attractive than Cu_2O due to its stability, simplicity, and photovoltaic properties (Wang, Liu, et al., 2021). CuO is capable of withstanding high temperatures because it has a more stable valence state compared to Cu_2O (Lupan et al., 2021)

There are three mechanisms associated with the antimicrobial activities of CuO particles including the ROS generation, release of copper ions, and contact killing. CuO particles exert their antimicrobial activity through disruption of membranes, proteins, and DNA of microorganisms due to the production of ROS (Jagadeeshan & Parsanathan, 2019). The interaction of copper ions with thiol groups of proteins and enzymes also may disrupt the cell functions, leading to cell death (Godoy-Gallardo et al., 2021). Besides, the adherence of CuO particles to the cell surface can cause physical damage such as membrane rupture, cytoplasm leakage and cell lysis (Bezza et al., 2020). The CuO particles can also prevent the formation and growth of biofilms by disrupting them and reducing their viability by generating ROS, releasing copper ions, and causing contact killing (Padmavathi et al., 2019). Generally, the CuO particles activity depends on the bacteria species. According to Rehan et al. (2015), *Bacillus subtilis* and *Bacillus anthracis* are more sensitive to CuO particles because they have rich amine and carboxyl groups in their cell walls, which bind more strongly to CuO particles. Coating CuO particles with supporting polymer matrices such as chitosan (Ancona et al., 2014), epoxy resin (Das et al., 2014), cellulose (Llorens et al., 2012), and bovine serum albumin (Rastogi & Arunachalam, 2013) can enhance their antimicrobial performance efficiency and durability. The surface area and morphology

of CuO particles also play important roles in their antimicrobial performance (Janani et al., 2022).

The selection of synthesis method could influence the antimicrobial performance of metal oxide particles, resulting in differences in size and shape. In a wet chemical synthesis method conducted by Ananth et al. (2015), CuO particles synthesized with polyethylene-glyco (PEG) as a surfactant exhibited grain-like particles at 75 °C and needle-like particles at 100 °C. Both samples appeared in fine dispersion due to the reduction of Gibb's free energy facilitated by PEG. However, the hydrothermal synthesis method showed a plate-like CuO particles with aggregated particles. They appeared to have a larger inhibitory zone compared to CuO particles with PEG. This is due to morphology-dependent interaction with the bacterial cell wall, which damages the bacteria and inhibits their growth. Muthuvel et al. (2020) founded that CuO particles synthesized by the green synthesis method exhibited a spherical morphology with a size of 25 nm. They also showed significant antibacterial activity against gram-negative bacteria (*Pseudomonas aeruginosa*, and *Escherichia coli*) compared to gram-positive bacteria (*Staphylococcus. saprophyticus*, and *Bacillus subtilis*).

2.6.1.2 Magnesium Oxide

Magnesium oxide (MgO) is one of the antibacterial metal oxides that is economical, easy to obtain, biocompatible and non-toxic (Ali et al., 2023; Hassan et al., 2021). MgO particles have been listed as safe materials by the United States Food and Drug Administration (Fahmy et al., 2020). MgO particles have a cubic crystal structure with a rock salt-type array (Prado et al., 2020). They can be used in various forms, such as MgO or magnesium halides (MgX_2 or MgF_2) (Khorsandi et al., 2021). MgO particles are efficient against both gram-positive and gram-negative bacteria, viruses, and spores (Abhishek Singh et al., 2022). MgO particles are usually used in medicines to initiate post-activation of bone repair scaffolds, heartburn reliever, and act as hyperthermia agents in cancer therapy (Al-Karam & Yousef, 2021). Additionally, MgO particles can exert antioxidant properties (Faizan et al., 2022). MgO particles can be used as adsorbents, catalysts, and supports for various chemical processes due to their high

specific surface area and adsorption capacity (Ali et al., 2023; Balakrishnan et al., 2020). MgO particles are able to decompose chlorinated and fluorinated compounds, and adsorb heavy metal ions, phosphorus compounds and dyes from wastewater (Owusu Adjei et al., 2021). MgO particles are ideal for refractory applications due to their high melting point of 2800 °C and boiling point of 3600 °C, and their wide band gap also makes them suitable for optical and electronic applications (Balakrishnan et al., 2020).

The action mechanisms of MgO particles depend on pH and Mg^{2+} ions. Higher concentrations of MgO particles resulted in their dissociation in microbial cells due to an increase in OH− ions and broth pH (Saied et al., 2021). However, this is not the primary mechanism of microbial killing. The production of ROS is another mechanism responsible for the antimicrobial action of MgO particles (Hassan et al., 2021). Bacteria undergoing for aerobic respiration generates superoxide anions and other ROS, which are toxic to them (Kim et al., 2019). In order to neutralize the ROS, bacteria produce superoxide dismutase (SOD) (Palmieri et al., 2019). Higher concentrations of MgO particles could damage bacterial cells when not all the ROS could be timely neutralized by SOD (Prado et al., 2020). In some microbes, such as *E. Coli*, the inhibition was observed even without the presence of ROS (Leung et al., 2014). The MgO particles could inhibit gram-negative bacteria more efficiently than gram-positive bacteria (Zhang et al., 2021). This is due to the presence of a thinner layer of peptidoglycan in gram-negative bacteria, allowing MgO particles to penetrate the cell wall and bind to the cell membrane (Khorsandi, Keyvani-Ghamsari, et al., 2021), resulting in shape alteration and cell death. In gram-positive bacteria, the MgO particles may disrupt the interactions among the bacteria and inhibit their function and activity (Li et al., 2023). MgO particles could also disrupt the extracellular matrix by chelating with enzymes and acting as a catalyst to degrade the matrix in biofilms (Ramezani Farani et al., 2023).

Several studies have been conducted on the synthesis of MgO particles using plant extracts. The MgO particles synthesized using plant extract acted as excellent sorbents, and a higher concentration of MgO (0.1 g/mL) was able to completely inhibit bacteria (Anantharaman et al., 2016). According to Khan et al. (2021), MgO particles exhibited antibacterial activity against both gram-positive and gram-negative bacteria,

such as *Escherichia. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Klebsiella pneumoniae*, and *Streptococcus. pyogenes*, when synthesized with green tea. MgO synthesized using *Amaranthus tricolor*, *Amaranthus blitum* and *Andrographis paniculate* showed varying antibacterial activity against *E. coli*, even when using the same concentration of MgO. This variation has attributed to the number of bioactive compounds present in the leaf extract, including phenols, terpenoids, and flavonoids, which possess antibacterial activity (Govindarajan et al., 2023). Thus, these bioactive compounds contribute to the action mechanism of MgO particles. Furthermore, bioactive compounds act as reducing and stabilizing agents for particles synthesis, thereby influencing the particles concentration in the colloidal solution (Gebre, 2023).

2.6.2 Natural-based Antimicrobial Agent

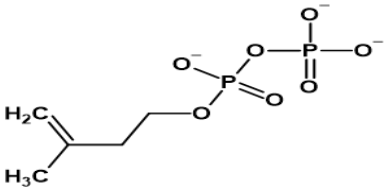
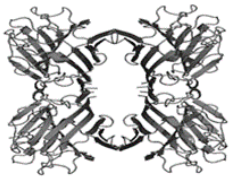
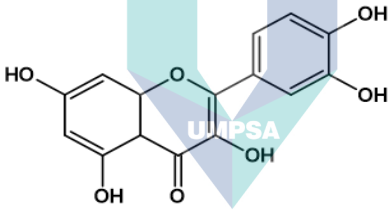
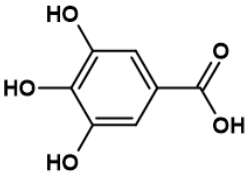
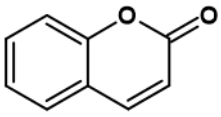
Mother nature provides numerous natural antibacterial agents that can be obtained from various resources such as plants, animals, bacteria, algae, and fungi. Among these, plants provide a wide range of antibacterial compounds that can be extracted from various parts of plants, such as seed, peels, leaves, flowers, pulps, and husks (Singh, 2022). Most of the plant-derived compounds are phenolic compounds, with the main groups of compounds include flavonoid, phenolic acids, tannins, stilbenes, quinones, alkaloids, and lignans (Saranraj et al., 2019). According to Fan et al. (2018), most of the plant-derived antibacterial compounds come from secondary metabolites through the shikimate pathway, mevalonate and methylerythritol phosphate pathway. These compounds play an important role in biochemistry and physiology of plants as they act as natural defences of plants against microorganisms (Zehra et al., 2021). The plant-derived antibacterial compounds are natural; thus, they are safe, non-toxic to the skin, environmentally-friendly, and easy to obtain (Kamarudin et al., 2022). Besides, they do not exhibit the side effects associated with synthetic chemicals.

The natural compounds from plants have a broad activity spectrum against invading pathogens such as bacteria, fungi, and viruses (Keita et al., 2022). The natural defensive amino acids and peptides have the ability to inhibit various microorganisms, including both gram-negative, and gram-positive bacteria (Matos et al., 2023). Phenolic

compounds were reported to have antimicrobial activity towards human pathogens (bacteria, fungi, and viruses) and food spoilage pathogens (bacteria and fungi) (Deabes et al., 2021). The antibacterial compounds from plants are an alternative therapeutic to combat bacterial growth on textiles (Hemthanon & Ungcharoenwiwat, 2022). Benzoic acid and sorbic acid have been used for ages in food industry in order to minimize food spoilage and extend the shelf-life of food (Marrez et al., 2022).

There are about 12 to 50 amino acids, and they are classified based on size, predominant acid structure, or conformational structure (D'Aloisio et al., 2021). The structure of natural compounds and the composition of plant extracts have a significant effect on the antibacterial activities of the compounds (Table 2.2). Amphiphilicity is the key feature of an antibacterial compound as it consists of hydrophilic and lipophilic properties (Zhang & Ma, 2019). These properties permit the movement of antibacterial compounds through the cell membrane (Zhang et al., 2020). Also, an amphiphilic characteristic resulted in the solubility of antibacterial compounds in the aqueous phase (Echeverría et al., 2017). According to Fan et al. (2018), molecular size, polar groups, functional groups, and solubility in non-polar solvent were found to affect the antibacterial activities of plant compounds. Both polar groups and molecular solubility are related to each other, thus, the higher the polarity of the molecule, the better the solubility in polar solvents, which indirectly affects the antibacterial activity. The functional group such as hydroxyl group (–OH) acts as a proton exchanger in order to interact with the cell membrane of the bacteria (Park et al., 2021). The interaction causes destabilization of the cytoplasmic membrane due to the decrease in pH and leads to the leakage of cellular components and ultimately cell death (Lobiuc et al., 2023). The location of the −OH group also influences the antibacterial activity. The –OH group located at the meta position have higher antibacterial properties than those located at the ortho position (Synowiec et al., 2021). Moreover, the number and position of double bonds in the chain also can impact the antibacterial activities (Lee et al., 2019). Compound with one double bond, such as citronellol (phenolic), are less effective than those with two double bonds, such as geraniol (phenolic) (Gyawali et al., 2015), indicating that the degree of unsaturation is an important factor affecting the activity of natural antibacterial compounds.

Table 2.2 Plant-based antimicrobial agents and its antimicrobial spectrums.

Plant-based Antimicrobial Agents	Chemical Structure	Antimicrobial Spectrum
Alkaloids		
Terpenoids		- *Staphylococcus aures* - *Pseudomonas aeruginosa* - *Vibrio cholera*
Lectin and polypeptides		- *Staphylococcus aures* - *Bacillus subtilis* - *Escherichia coli* - *Pseudomonas aeruginosa*
Phenolics and Polyphenols		
Flavonoids		- *Klebsiella pneumonia* - *Salmonella enterica* - *Pseudomonas aeruginosa* - *Staphylococcus aureus* - *Escherichia coli*
Quinones		- *Staphylococcus aureus* - *Bacillus subtilis* - *Pseudomonas aeruginosa*
Tannins		- *Bacillus cereus* - *Listeria monocytogenes* - *Staphylococcus aureus* - *Salmonella enterica*
Coumarins		- *Staphylococcus aureus* - *Escherichia coli* - *Vibrio parahaemolyticus*

Source: Morais et al. (2016)

Various mechanisms are involved in the antibacterial activities of plant-derived compounds. However, the exact mechanism for most of the compounds remains unclear due to the large variation in compositions and chemical structures. Some antimicrobial peptides (AMPs) are bacteriocins such as nisin, pediocin and reuterin, which are produced through bacterial fermentation (Mora-Villalobos et al., 2020). These compounds inhibit the growth of their related species of bacteria. The mechanism of action of AMPs mainly involves the disruption of cell membranes by binding to them and leading to destabilization of membrane (Seyfi et al., 2020).

There are three models that describe the binding of AMPs to membranes which are barrel-stave, toroidal pore wormhole and carpet model (Figure 2.4) (Corrêa et al., 2019). The formation of ion channel occurred in the barrel-stave model is due to the accumulation and placement of peptides in same direction within the membrane (Guidelli & Becucci, 2022). In the toroidal pore wormhole model, the peptides initially accumulated parallel to the membrane (Kamal et al., 2023), leading to bending stress and the formation of pores that cause the eruption and lysis of cytoplasmic (Seyfi et al., 2020; Corrêa et al., 2019). The carpet mechanism model resembles the action of detergents, where the peptides accumulated on the surface of the cell membrane, then destabilizing it due to the increase of fluid concentration and thus forming micelles (Lāce et al., 2022). Another mechanism of AMPs is the inhibition of DNA, ribonucleic acid (RNA), and protein synthesis. They affect several internal processes in actively respiring cells, resulting to the loss of adenosine triphosphate (ATP) (Yu et al., 2022). The loss of ATP resulted in the production of ROS, which are toxic and harmful to bacteria cells (Oliveira et al., 2019).

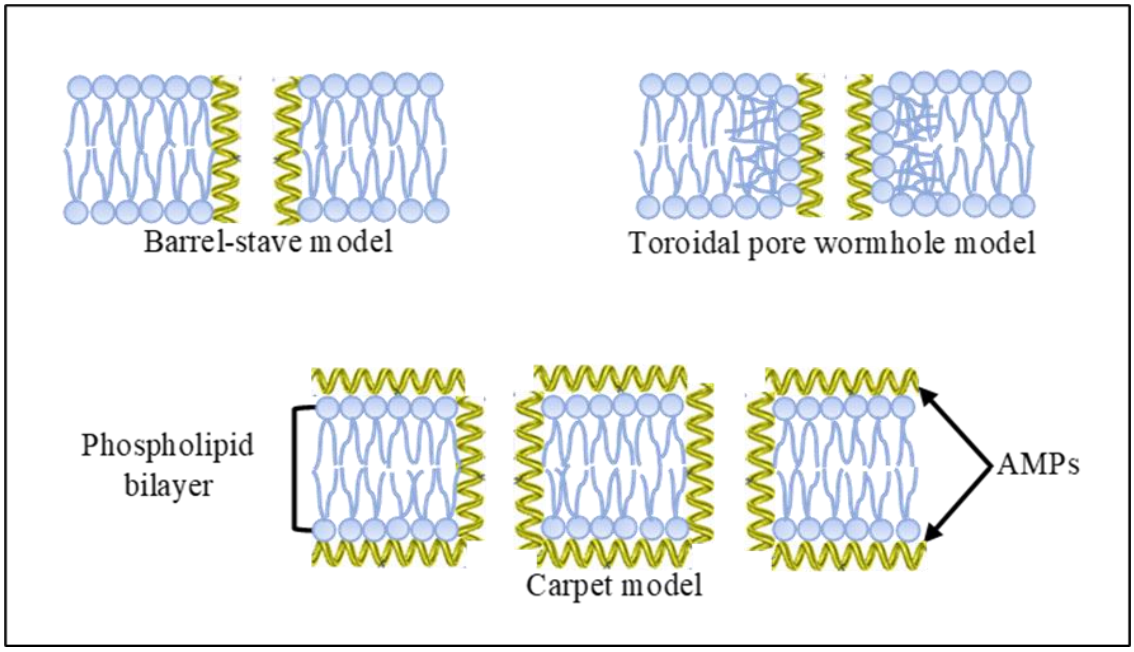


Figure 2.4 Models of antibacterial mechanisms of AMPs towards membranes. Source: Zhang et al. (2021).

2.6.2.1 Pomegranate

Pomegranate (*Punica granatum L*) belongs to the family of Punicaceae, which is the native fruit to the Mediterranean region (Laaraj et al., 2022). Due to its multi-functionality and vast nutritional benefits, it is currently grown globally in many geographical regions (Kandylis & Kokkinomagoulos, 2020). Pomegranate has been used by Egyptians for thousands of years as a traditional remedy for treating several different infections (Ge et al., 2021). The extract from the rind of fruit and bark of the tree has been used to treat diarrhoea and dysentery (Howell & D'Souza, 2013). Pomegranate is also known for its potent antioxidant properties, which have shown anti-cancer activity against human cancer cells and anti-inflammatory effects (Akbari et al., 2022). In addition, pomegranate peel extract contains tannins and flavonoids, which exhibit high antioxidant activity (Saroj et al., 2020). The bioactive compounds found in various parts of the pomegranate tree are listed in Table 2.3.

Table 2.3 Bioactive compounds of pomegranate.

Plant components	Constituents
- Pomegranate juice	- Anthocyanins, glucose, ascorbic acid, ellagic acid, gallic acid; caffeic acid; catechin, Epigallocatechin gallate (EGCG), quercetin, rutin; numerous minerals, particularly iron; amino acids.
- Pomegranate seed oil	- 95% of punicic acid; other constituents, including ellagic acid; other fatty acids; sterols
- Peel, rind	- Phenolic punicalagins; gallic acid and other fatty acids; catechin, EGCG; quercetin, rutin and other flavonols; flavones, flavonones; anthocyanidins.
- Pomegranate leaves	- Tannins (punicalin and punicafolin); and flavones glycosides, including luteolin and apgenin.
- Pomegranate flower	- Tannins (punicalin and punicafolin); and flavones glycosides, including luteolin and apgenin.
- Pomegranate roots and barks	- Ellagitannins, including punicalin and punicalagin; numerous piperidine alkaloids.

Source: Julie (2008).

Pomegranate has demonstrated antimicrobial activity against several highly pathogenic and drug-resistant bacteria strains (Hanafy et al., 2021). Research conducted by Sabbar Dahham et al. (2010) showed that pomegranate rind had the highest antimicrobial activity against *S. aureus*, with an inhibition zone of 20 mm, compared to *B. coagulans, B. cereus B. subtilis, E. coli*, and *K. pneumoniae*. The white seed exhibited a lesser antimicrobial effect, with an inhibition zone of 8 mm against *E. coli*, while pomegranate juice showed antimicrobial effects against *S. epidermidis* and *K. pneumoniae*. The results varied depending on the content of phenolic compounds, citric acid, and pigments in the juice. Oligomeric ellagitannins are the most potent antimicrobial compounds found in pomegranate (Andishmand et al., 2023). Other compounds that synergistically contribute to the antimicrobial effects include flavanols (quercetin and myricetin) and anthocyanins (pelargonidin-3-galactose and cyanidin-3-

glucose) (Fahmy et al., 2020). According to Rongai et al. (2019), pomegranate also has an inhibitory effect on mycelial fungal growth. The peel extract of pomegranate contains punicalagin compound, which acts as antifungal agent to reduce or inhibit citrus mold (Salem et al., 2022).

The extraction methods used for pomegranate have a significant effect on its bioactive composition, antioxidant potential, and antimicrobial activity (Alexandre et al., 2019; Chen et al., 2020). Factors that can influence the extraction method are types of solvent used, temperature, pressure, duration and frequency of extraction, and the presence of enzymes (Campos et al., 2022). Different extraction methods resulted in differences of yields and bioactive compounds, such as phenolics, flavonoids, and tannins (Olvera-Aguirre et al., 2022). The extraction efficiency and quality can be improved by using high pressure and enzymatic-assisted extraction methods (Kumar et al., 2021). In the meantime, comparing to conventional extraction methods, the sonication-assisted extraction method was found to enhance the antioxidant and antimicrobial properties of pomegranate rind extract (Campos et al., 2022). However, the optimal extraction method depends on the cultivar of pomegranate as it may affect the bioactive compounds (Rosas-Burgos et al., 2017).

2.7 Synthesizing Method of Antibacterial Particles

The metal oxide-based particles have been a topic of concern in the scientific community for a few decades. Their versatility has initiated ongoing research into new compositions, synthesis methods, and applications. Among antibacterial particles, Ag particles are the most commonly used due to their wide spectrum of antibacterial activity against various bacteria (Akintelu et al., 2020). According to Sánchez-López et al. (2020), transition of metals such as zinc, copper, iron and gold are ideal for synthesizing metal oxide-based particles because they have partially-filled orbitals, which enable particles aggregation.

The synthesis methods of antibacterial particles can be categorized into three, namely, the physical method, chemical method, and biological method. In the physical method, the bulk metals are fragmented into smaller fragments, which are then transformed into particles using physical forces such as light, electricity, heat, and

sound (Shah et al., 2022). However, this method is not suitable for the synthesis of metal oxide-based particles due to the size of particles, which is a crucial factor for their activity (Wang & Xia, 2004). Additionally, this method is expensive, energy-intensive and requires sophisticated equipment (Vishwanath & Negi, 2021). Chemical method involves the use of organic solvents to produce metal and metal oxide particles (Esmaeilzadeh et al., 2021). This method is relatively cheaper and faster compared to the physical method (Parashar et al., 2020). However, it may involve the use of toxic chemicals, which can pose environmental and health risks (Vishwanath & Negi, 2021). At the same time, biological method focuses on green-synthesis processes using different types of microorganisms such as plants, bacteria, or fungi (Kumar et al., 2021). This method is typically considered eco-friendly, bio-compatible, and cost-effective (Mohd Yusop & Wan Ismail, 2021). However, it may result in lower yields and less stable particles compared to other methods (Vishwanath & Negi, 2021).

2.7.1 Sol-gel Synthesis

Sol-gel method is an established wet-chemical technique used to synthesize metal oxides and produce particles. The history of the sol-gel method dates back to 1921 when Geffcken and Berger prepared single oxide coatings (Sakka, 2022; Dislich & Hinz, 1982). However, it was not regarded as a significant sol-gel product. The first significant sol-gel product, known as aerogels, was invented in 1931 by Kistler (Sakka, 2016). He used water glass as a silica source and dried using supercritical drying technique. In the 1960s, the development of sol-gel method continued to meet the new demands in the nuclear industry (Valverde, 2019). The revolution of the sol-gel method occurred in 1971 with the fabrication of transparent sodium aluminoborosilicate glass plates by Dislich (Sakka, 2022). Since then, it has gained popularity and has been extensively applied in a wide range of functional and high-tech materials, including electronic, photonic, chemical, micromechanical, and bionic materials (Sakka, 2022; Qian & Lu, 2020).

The sol-gel method involves the conversion of a liquid “sol” into a solid “gel” phase (Bokov et al., 2021). Inorganic metal salts or metal-organic compounds such as metal alkoxides are typically used in the preparation of the sol (Gautam et al., 2020).

The sol-gel process consists of three steps, namely hydrolysis, condensation, and drying. Initially, the metal hydroxide solution is produced through the hydrolysis of the metal precursor, followed by the condensation process to create three-dimensional gels. Subsequently, the drying process takes place, converting the product into xerogel or aerogel based on the drying method employed (Esposito, 2019).

The sol-gel method can be classified into two routes based on the nature of the solvent, which is aqueous sol-gel and non-aqueous sol-gel. The term “aqueous” refers to the use of water as the reaction medium, while “non-aqueous” indicates the use of organic solvents (Parashar et al., 2020). In the aqueous sol-gel route, water serves as the solvent, supplying oxygen for the formation of metal oxides (Mohammad, 2020). Metal alkoxides are normally used as precursors due to their high reactivity with water (Yorov et al., 2022). Other types of metal precursors are metal acetates, sulphates, nitrates, and chlorides (Gager et al., 2022). However, there are disadvantages of the aqueous sol-gel route when applied to nanoscale materials. In most cases, it is challenging to control particle morphology and achieve reproducibility in the final protocol due to the simultaneous process of hydrolysis, condensation, and drying (Vioux & Hubert Mutin, 2018). Thus, the aqueous sol-gel route is only suggested for the synthesis of bulk metal oxides, as it has less impact on the synthesis process.

In the non-aqueous sol-gel route, solvents such as alcohols, aldehydes, ketones, or solvents provided by the metal precursors supply the required oxygen for the formation of metal oxide (Mohammad, 2020). Besides, these organic solvents play a role in modifying various components such as particle size, morphology, composition, and surface properties of the metal oxide particles (Rao et al., 2017). The non-aqueous sol-gel route is more suitable for the production of nano oxides compared to the aqueous sol-gel route. There are two significant approaches for producing metal oxides particles in this route: surfactant controlled and solvent controlled methods. Surfactant control involves the hot injection method at high-temperatures for the conversion of metal precursor into the respective metal oxide (Soni et al., 2021).The control of the shape and growth of particles and to avoid their agglomeration are permits through this method (Rao et al., 2017). Meanwhile, the reaction between metal halide and alcohols

is involved in the solvent-controlled sol-gel route to produce metal oxide nanostructures (Niederberger, 2007).

The sol-gel method is a versatile technique that offers many advantages over conventional method. It enables precise control of composition and structure, and particle size (Sakka, 2016). There is a possibility of incorporating organic materials and particles into the sol-gel matrix (Mura et al., 2020). Besides, materials can be moulded into complex geometries, and this can improve adhesion between substrate and topcoat (Zhu et al., 2012). The sol-gel process requires less energy consumption since it can be achieved at low temperatures (Bokov et al., 2021). The high homogeneity of the sol-gel method resulted in the production of high purity products by dissolving oxides precursors in an appropriate solvent during the transformation of sol-gel (Esposito, 2019). Moreover, it allows for the fabrication of various oxide compositions as well as non-oxide and hybrid organic-inorganic materials (Bakar et al., 2023). Sol-gel is a cost-effective method that does not require specialized or expensive equipment (Za'im et al., 2021). It is simple and effective, capable of producing high-quality coatings in various forms such as thin films, fibres, monoliths, porous membranes, composites, and powders (Ismail, 2016).

However, as stated by Rao et al. (2017), due to some limitations, the sol-gel method cannot be applied in certain industries. Some of the problems are having weak bonding, difficulty in controlling porosity, low wear-resistance, and high permeability. During the thermal process, thick coatings might be problematic because the maximum coating thickness limit for crack-free coatings is 0.5 μm. In the meantime, the shrinkage of wet gel during the drying process might lead to cracking due to capillary stress, making it challenging to prepare for massive monoliths (Buisson, 2003). The sol-gel method requires expensive raw materials like precursors, and the drying and sintering processes can be time-consuming (Modan & Schiopu, 2020). In multicomponent materials, the preferential precipitation of a particular oxide during sol formation may occur due to different reactivities of each precursor (Voon et al., 2020). Currently, sol-gel technology faces challenges due to a lack of scientific understanding of its complex reactions. Despites its limitations, the sol-gel method remains a popular choice in many

industries due to its versatility and cost-effectiveness in terms of machinery. Further research could help overcome the problems faced by the industry.

2.7.2 Green Synthesis

Currently, green synthesis has received enormous attention from the scientific community as an alternative to physical and chemical synthesis for the production of metal oxide particles. Chemical synthesis often generates hazardous by-products, making the use of "green chemicals" that are clean, non-toxic, and environmentally friendly a desirable option for particles synthesis (Akintelu et al., 2020). Green synthesis offers a simple, cheap, and easy method for quick large-scale synthesis, making it suitable for various applications (Mohd Yusop & Wan Ismail, 2021). The history of green synthesis of metal oxide particles can be traced back to ancient times when gold and silver particles were used for colouring glass and ceramics (Schröfel & Kratošová, 2011). However, modern scientific interest in the green synthesis of metal oxide particles began in the late 20th century with the discovery of microorganism's ability to produce particles (Saravanan et al., 2021). Since then, numerous research studies have been conducted to explore the use of biological sources for the synthesis of metal oxide particles (El-Seedi et al., 2019).

Microorganisms, algae, plants materials, and bio-waste have been utilized as synthesizing agents to produce particles (Jadoun et al., 2021). Plant extracts have the edge over microorganisms as synthesizing agents because green synthesis of particles occurs extracellularly (Küünal et al., 2018). Extracellular synthesis is faster, easier, and more scalable than intracellular synthesis, which is more complex (Ahmad et al., 2021; Lahiri et al., 2021). Besides, plant extracts may act as both stabilizing and reducing agents in the synthesis of particles due to the presence of hydroxyl and carbonyl groups found in bioactive compounds (Sharma et al., 2019). The source of plant extracts can greatly influence the morphology of the synthesized particles due to variations in the concentrations of biochemical reducing agents present in different plant extracts (Qamar & Ahmad, 2021). The composition of biochemical reducing agents in most plant extracts differs seasonally and regionally, due to the nutrient uptake of the plants

(Chatterjee et al., 2020). As a result, there can be variations in the properties of particles produced in each batch.

According to Mittal et al. 2013, there are three steps involved in the bio-reduction of metal particles using plant extracts, namely activation, growth, and termination. In the activation step, also known as nucleation, metal ions are reduced and nucleated to form small-sized particles. This process is facilitated by bioactive compounds present in plant extract that donate electrons to the metal ions. In the growth phase, the small particles aggregate and join together to form larger particle sizes, while the thermodynamic stability of the particles increases. This step is also referred to as the aggregation process. The bio-reduction of metal particles is completed in the termination phase, where the shape of the particles is formed, and the active metabolites play a role. In this step, the stabilization of the metal oxide particles occurs through the formation of a protective layer of bioactive compounds that can prevent aggregation of the metal oxide particles.

The morphology, size, and stability of the particles depend on various factors. These factors include the type of plant extract, concentration of metal ions, pH, temperature, and reaction time (Kaur et al., 2022). As stated by Adeyemi et al. (2022), different plant extracts may affect the reduction and stabilization of metal ions due to variations in their bioactive compositions and concentration. The rate of nucleation and growth of particles are controlled by the concentration of metal ions, while the solubility and charge of metal ions, and bioactive compositions in the solution are affected by pH (Mittal et al., 2013). The synthesis temperature affects the kinetics and thermodynamics of the reaction (Kaur et al., 2022). The duration of the nucleation, growth, and stabilization processes depends on the reaction time (Mittal et al., 2013).

Similar to other synthesis methods, the green synthesis of metal oxide particles also has advantages and limitations. The green synthesis method is cost-effective and environmentally friendly as it can be performed in aqueous media at standard temperature and pressure (Schröfel et al., 2014). The use of biological sources may enhance the novel properties of the metal oxide particles (Sharma & Tagad, 2022). Furthermore, different shapes and sizes of particles can be produced by varying the

biological sources and reaction conditions (Maťátková et al., 2022). However, the use of biological sources may influence the quality, quantity, and stability of the particles produced due to source variability, while additional procedures or chemicals may be needed to separate or purify the particles from the biological matrix (Mohd Yusof et al., 2019). Thus, further research is needed to better understand the green synthesis process, optimize the production and properties of metal oxide particles, and overcome the challenges and limitation of this method.

2.8 Fabric Coatings

Coating is a process of applying a polymeric layer onto the surface of a fabric (Billah, 2019). This procedure could enhance the functional properties of the fabric, such as antibacterial, water repellent, flame retardancy, UV resistance, and many more (Tania & Ali, 2021; Paul, 2015). Coating of fabrics offers various advantages, such as protection from the environment and hazardous chemicals, improved performance, functionality, and aesthetic appeal (Singha, 2012). However, coating also poses some problems, such as elevated cost and complexity of fabrication, impact on the environmental compatibility and recyclability of fabrics, and the need for more rigorous quality and process control standards (Elzaabalawy & Meguid, 2020; Rosenberg et al., 2019).

There are several methods available for coating of fabrics, such as direct coating, immersion coating, direct roll coating, transfer coating, heat lamination, and adhesive lamination (Billah, 2019; Meirowitz, 2016). The choice of coating method depends on the types and characteristics of the fabric as well as coating materials (Shim, 2019). Coating of fabrics can affect their tensile strength and air permeability (Xu et al., 2020). The effectiveness of the coating can be influenced by many factors, including coating thickness, types of fabric, coating method and environmental conditions (Billah, 2019). Nowadays, coating is widely used in various fields and applications in order to meet the demands and challenges of modern technology and society.

2.8.1 Effect of Coating on Air Permeability

Air permeability is a measure of air flow passing through a specific area at a given time (Mishra et al., 2019). It is an important property for the production of fabric as it may influence the thermal comfort of the fabric. Generally, the air permeability of a fabric depends on its porous openings (Kumar & T., 2022). The fabric material, structure, density, and thickness also play a role in determining air permeability (Islam et al., 2019). Fabrics with higher porosity and lower thickness usually have higher air permeability (Kumar & T., 2022). Coating the fabric can also affect air permeability as it may alter the pore size and shape of the fabric. The main factors that affect the air permeability of the coated fabric are the coating materials and the types of fabric (Hu et al., 2006).

According to Hu et al. (2006), the air permeability of coated fabric can be influenced by the coating materials. Different coating materials have distinct properties. The viscosity, density, and surface tension of the coating material may affect the thickness and uniformity of the coating layer, thereby altering the size and shape of pores in the fabric (Mavukkandy et al., 2020). Higher viscosity, density, and surface tension of the coating material may reduce the air permeability of the fabric, and thus reducing breathability and increasing thermal properties (M'chaar et al., 2021). The pH level of the coating material may also affect the air permeability by modifying the structure of the fabric (Benltoufa et al., 2020). An acidic coating material may increase the porosity of the fabric, hence enhancing the air permeability (Ahirrao et al., 2021).

Types of fabric also has a significant effect on the air permeability of the coated fabric. Fabrics with fibres that have radial expansion exhibit better air permeability than fabrics with circular section fibres (Zhou et al., 2022). This unalike is due to variations in moisture regain. Higher moisture regain in fibres may decrease air permeability by reducing the air space between fibres and yarns (Ivanovska et al., 2022). The type of yarn used can also affect the air permeability. The yarns with finer, lower crimp, and higher twist have lower air permeability than yarns with coarser, higher crimp, and lower twist (Corbin et al., 2021). According to Jamshaid et al. (2020), knitted fabrics generally have higher air permeability compared to woven fabrics. This is due to the

loop structure of knitted fabrics that provides more porosity and directly influences the air permeability of the fabric (Wen et al., 2021).

2.8.2 Effect of Coating on Tensile Strength

Tensile strength and tongue tear strength are two commonly used indicators for measuring the strength and durability of the fabric. The tensile strength of the fabric can be defined as the ability of the fabric to withstand force and stretching before breaking, while, tongue tear strength is defined as the force required to initiate or continue tearing of the fabric in either weft or warp direction under specified conditions (Zegan & Ayele, 2022). The high tensile strength and tear resistance of fabric are important for the production of architectural fabrics that are mainly used under tension (Shi et al., 2020). Furthermore, the fabrics with these properties are also suitable for producing protective textiles, parachutes, tents, and furniture (Maity et al., 2023). Basically, tensile and tear strength may affect the durability, comfortability, and performance of the fabric (Motlogelwa, 2018).

The strength of the yarns has a significant impact on both tensile and tear strength of the coated fabric. Finer yarns with higher twist tend to have greater strength than the coarse yarn (Shahzad et al., 2022). Normally, the fabrics with smooth surface have higher strength compared to textured surface fabrics due to the increased amount of twist (Irfan et al., 2023). However, beyond the optimum twist level, the strength of the yarn reduces and becomes more prone to breakage (Afroz & Islam, 2021). Additionally, the weave and fabric structure may also affect the fabric strength. Interlacing patterns and the tightness of the weave and fabric structure may influence stress distribution and deformation of the fabric (Begum & Milašius, 2022). The fabric strength increases with an increase in interlacement points in the weave structure, while a loose fabric structure allows for increased thread density, leading to high tearing strength (Mobarak Hossain, 2016). Although the warp and weft directions of the fabric may have the same weave structure, their strength for both directions can differ due to the differences in yarn count, density, and material (Begum & Milašius, 2022).

Both tensile and tear strength can be affected by coating materials, either in positive or negative ways. The adhesion effect of coating materials can enhance the

fabric strength (Croll, 2020). It provides a strong bond between fibres and the coating material, restricting yarn movement (Shinde & Sampath, 2022; Eltahan, 2018). However, according to Yuksekkaya et al. (2016), the acidity of coating materials may result in the degradation of the fabric structure. This is due to the diffusion of acid molecules into the fabric structure, causing the breakdown of non-crystalline domains and inter-crystalline contacts, ultimately resulting in the damage of yarn fibres (Zheng et al., 2023; Ji et al., 2016). However, the affinity of acid to the fabric depends on the concentration, duration of the coating treatment, and the types of fabric (Ji et al., 2016). Reduction in tensile strength can occur due to defects, stress, or cracks during the coating process (Volkhonsky et al., 2020).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the methodology employed to fabricate antibacterial coatings for various fabric types aimed at minimizing or preventing body odour. Employing a mixed methods approach combining qualitative and quantitative data analysis, this study explored the impact of synthesis methods and formulations on the appearance, antibacterial activities, tensile strength, and odorant composition of cotton, polyester and blend wool fabrics. The experimental design (Section 3.2) facilitated comparison among synthesis methods and formulations to assess these fabric properties. Chemicals used in the study are presented in Section 3.3. The study commenced with parameter optimization for extraction and synthesis (Section 3.4) followed by pre-antibacterial activity testing of coated fabrics (Section 3.5). Characterization of particle solutions (Section 3.6) preceded scaling up solutions exhibiting antibacterial activity against tested bacteria species (Section 3.7) for fabric coating (Section 3.8). Effectiveness of the coatings in inhibiting the bacteria was confirmed via disc diffusion assay (Section 3.9). Tensile and tear strength (Section 3.10), air permeability (Section 3.11), and durability of the coatings after washing (Section 3.12) were evaluated following ISO standards. Finally, the volatile odorant composition of the coated and uncoated fabrics was assessed using artificial sweat (Section 3.13). Statistical analysis of data on antibacterial activity, tensile and tear strength, and air permeability was conducted using Statistical Package for Social Science (SPSS) software (Section 3.14).

3.2 Experimental Design

Two synthesis methods, namely sol-gel and green synthesis method, were employed to produce modified metal oxide (CuO and MgO) particles. The

modifications were achieved using PRE as antibacterial agent. Two sets of modified metal oxide particle formulations were prepared using each method. However, to assess the antibacterial property of PRE, one set of Cu and Mg was synthesized without the addition of PRE using sol-gel method, resulting in a total of six sets of formulations. For each set, triplicate samples were prepared to estimate experimental error. The formulations of the modified metal oxide particles are presented in Table 3.1.

Table 3.1 The formulations of modified metal oxide particles with synthesizing methods.

Methods	Formulations
Sol-gel	Cu
	Mg
	Cu + PRE
	Mg + PRE
Green synthesis	Cu + PRE
	Mg + PRE

In each formulation using sol-gel method, the pH, volume of PRE and coating cycle were optimized, while for each formulation using green synthesis method, only the volume of PRE and coating cycle were optimized to maximize antibacterial activity before further analysis. The flowchart of the experimental design is presented in Appendix A.

3.3 Experimental

3.3.1 Reagent

Copper nitrate [$Cu(NO_3)_2.3H_2O$] with molecular weight of 241.6 g/mol and a purity of 99.6%, and magnesium nitrate [$Mg(NO_3)_2.6H_2O$] with molecular weight of 256.41 g/mol and a purity of 99.1% were purchased from Bendosen (Malaysia). Citric acid with a molecular weight of 210.14 g/mol and a purity of 100% and ethylene glycol with molecular weight of 62.07 g/mol and a purity of 99.9% were also obtained from Bendosen (Malaysia). Diethyl ether (purity: 99.6 %; molecular weight: 74.12 g/mol)

was obtained from R & M Chemicals (India). Ammonium hydroxide (NH_4OH) with purity of 28-30% and molecular weight of 35.046 g/mol was purchased from Merck (United State). The pomegranate (*Punica granatum*) rind was purchased from Ayurvedic Pharmacopoeia (India) in powder form. Deionized water, obtained from a Milli-Q water purification system (Millipore), was used as a solvent, while distilled water was used to extract the pomegranate rind. Denatured ethanol with a purity of 95% and a molecular weight of 46.069 %, purchased from DChemie, was used to wash the fabrics (cotton, polyester, and blend wool). Both cotton and polyester fabrics were purchased from Kamdar Group of Company, while the blend wool fabric was purchased from Guangzhou Ntg Textile Co., Ltd (China). Anhydrous barium chloride ($BaCl_2$) with molecular weight of 208.227 g/mol and a purity of 97%, and sulfuric acid (H_2SO_4) with a molecular weight of 98.08 g/mol and a purity of 99.99% were purchased from Sigma-Aldrich (United States) and were used for the preparation of McFarland solution.

3.4 Sample Preparation

Sample preparation consisted of two steps: extraction of pomegranate rind and preparation of modified metal oxide particles using sol-gel and green synthesis methods.

3.4.1 Extraction of Pomegranate Rind

Pomegranate rind was extracted using solvent extraction method (Garcia-Vaquero et al., 2020). It was conducted by adding 4 g of pomegranate rind powder into 100 mL of distilled water and stirring vigorously at 450 rpm for 1 hour at 100 °C. The solution was then filtered through Whatman No. 1 filter paper to separate the solid residue from the extract. The extract was then stored in a sterile Duran bottle covered with aluminium foil (dark packaging) at 4 °C in order to maintain high storage stability.

3.4.1.1 Optimization of Pomegranate Rind Extraction

The optimization of pomegranate rind extraction involved two parameters which are temperature (30 °C, 80 °C and 100 °C) and amount of pomegranate rind powder (2

g, 4 g and 8 g) while the stirring rate was kept constant. 30 °C was selected as one of the temperature parameters as it can help to preserve the stability and activity of bioactive compounds from oxidation or heat degradation (Theocharis et al., 2012). Meanwhile, the high temperature (80 °C and 100 °C) were selected for optimization of PRE as it can increase the solubility of some bioactive compounds and finally affecting the antibacterial property (Antony & Farid, 2022). The parameters with the best antibacterial efficiency against *B. linens*, *C. acnes*, and *S. epidermidis* were selected for the synthesis of modified metal oxide particles and subjected to UPLC-QTOF-MS analysis.

3.4.1.2 Analysis of Bioactive Compounds of Optimized Pomegranate Rind Extract

The bioactive compounds of PRE, which consist of flavonoids, alkaloids, phenols, and polyphenols, were analysed using an ACQUITY UPLC® I-Class system coupled to an ion mobility mass spectrometer Vion IMS QTOF with electrospray ionization mode (ESI) from Waters in Wilmslow, UK. The UPLC conditions were modified according to Shao et al. (2020). A silica-based column, ACQUITY UPLC HSS T3, with dimensions of 2.1 mm × 100 mm and a particle size of 1.8 µm, was used with a flow rate of 0.6 mL/min at 40 °C. The ACQUITY UPLC® I-Class system is equipped with a Binary Solvent Manager that ensures precise and accurate solvent delivery in the UPLC system. As for mobile phase, 0.1 % formic acid with water and acetonitrile was used as solvent A and solvent B, respectively. The optimized gradient for mobile phase A was as follows: 99 % (0 min), 99 % (0.5 min), 65 % (16 min), 0 % (18 min), and 99 % (20 min). Meanwhile, for mobile phase B, the optimized gradient was as follows: 1 % (0 min), 1 % (0.5 min), 35 % (16 min), 100 % (18 min), 1 % (20 min). The Vion IMS QTOF was operated in ESI- ionisation sensitive mode. Nitrogen was used as the desolvation gas with a flow rate of 800 L/h and a capillary voltage of 1.5 kV. The source temperature was set to 120 °C, and the desolvation gas temperature was 500 °C. The data were acquired in High Definition MS^E ($HDMS^E$) acquisition mode. The scan range was from 50 m/z to 1500 m/z with a scan time of 0.1 s. The low collision energy was set at 4 eV, with a ramp of high collision energy from 10 eV to 40

eV. The acquisition and processing were performed using the UNIFI v2.0 Scientific Information System (Waters, Wilmslow, UK).

3.4.2 Preparation of Modified Metal Oxide Particles

3.4.2.1 Synthesis Methods

The modified CuO particles solution was prepared using sol-gel synthesis according to Saridewi et al., (2021). Initially, 0.59 g of $Cu(NO_3)_2$, 0.42 g of citric acid and 20 mL of deionized water were stirred vigorously at 30 °C and 450 rpm. Then, 1 mL of diethyl ether and 1 mL of ethylene glycol were added into the solution. After 30 mins, 10 mL of PRE was dropwise added into the solution and the pH of the solution was adjusted to pH 4 with NH_4OH. The solution was allowed to react for 24 h under constant stirring and temperature. Finally, the modified CuO particles were placed in sterile glass vial and stored at 4 °C until use. The same procedure was applied for the synthesis of modified MgO particles using 0.51 g of $Mg(NO_3)_2$.

The green synthesis method was used as control. In this method, the preparation of modified CuO particles only involved the use of precursor, PRE and water. The synthesis was conducted by diluting 0.59 g of $Cu(NO_3)_2$ into 20 mL of deionized water. Then, 10 mL of PRE was added to the solution. The mixture was allowed to react at 30 °C with constant stirring at 450 rpm until the colour of the solution changed completely from light green to dark green and lastly brownish black. For the synthesis of modified MgO particles, 0.51 g of $Mg(NO_3)_2$ was used and the same procedure was repeated. The colour change for MgO particles was yellowish to light brownish-orange. The change in colour after the addition of the PRE indicated that the synthesis had occurred and was completed when no further change in colour was observed (Aboyewa et al., 2021).

3.4.2.2 Optimization of Synthesis Process

Three effects of the sol-gel coating process on fabrics were studied, namely the volume of the PRE, pH, and the number of coatings. For green synthesis, only two parameters were studied: the volume of the PRE, and the number of coatings. The pH

was not considered as one of green synthesis parameters as it may not give a significant effect on the reduction and capping ability of biomolecules (Mohammadi & Ghasemi, 2018). Other synthesis conditions for both synthesis process was kept constant, including the amount of precursor, volume of solvent, volume of reagents (if any), and stirring conditions (temperature, and rpm). All experiments were conducted in triplicate to rule out the experimental bias. A total of 24 particles formulations for sol-gel and 6 formulations for green synthesis were prepared. The pre-antibacterial test of the coated and uncoated fabrics using disk diffusion assay against three species of gram-positive bacteria was conducted before proceeding with further characterization and the scaling-up process. Table 3.2 shows the summary of the sol-gel process and green synthesis process for each optimization used before scaling-up the process of the selected formulations.

Table 3.2 Summary of sol-gel and green synthesis process for each optimization.

Synthesis process	Precursor	Volume of PRE (mL)	pH	Number of coatings
Sol-gel	$Cu(NO_3)_2$	-	4, 5.5, 7	1×, 2×, 3×
	$Cu(NO_3)_2$	5, 10, 15	4, 5.5, 7	1×, 2×, 3×
	$Mg(NO_3)_2$	-	4, 5.5, 7	1×, 2×, 3×
	$Mg(NO_3)_2$	5, 10, 15	4, 5.5, 7	1×, 2×, 3×
Green synthesis	$Cu(NO_3)_2$	5, 10, 15	-	1×, 2×, 3×
	$Mg(NO_3)_2$	5, 10, 15	-	1×, 2×, 3×

3.5 Pre-antibacterial Activity Characterization

3.5.1 Cultivation of Bacteria

Three gram-positive bacteria, namely *Brevibacterium linens*, *Cutibacterium acnes*, and *Staphylococcus epidermidis*, were used to test the antibacterial property of the uncoated and coated fabrics. *B. linens* (isolated from harzer cheese) and *C. acnes* (isolated from an acne lesion on human facial skin) were both obtained from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany, in freeze dried form, while the live culture of *S. epidermidis* was obtained from the Food

and Feed Laboratory, Universiti Malaysia Pahang Al-Sultan Abdullah. Trypticase Soy-Yeast Extract (TSYE) broth powder and Brain Heart Infusion (BHI) broth powder were purchased from Shanghai Baiwei Chemicals (China). Both Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) were obtained from Shanghai Bio-way Technology (China). The prepared Columbia Agar with 5% Sheep Blood (CASB) was purchased from Isolab (Malaysia).

The cultivation of the bacteria started with the preparation of the medium culture. For each species of bacteria, the specific medium, which was in solid form (agar) and liquid form, was prepared. To prepare TSYE agar medium, 36 g of TSYE broth powder was dissolved in 1 L of distilled water. Then, 15 g of agar powder was added into the solution, mixed, and autoclaved at 121 °C for 15 mins. In the laminar air flow, the autoclaved agar solution was then poured into sterilized petri dish and allowed to solidify and cold down before starting the inoculation process. The same procedure was applied for the preparation of MHA by replacing the 38 g of TSYE broth powder with MHA powder. However, the agar powder was not added to the solution as it already contained the agars. As for CASB, the purchased agar is ready to use. Preparation of liquid culture medium involved a very simple step. The TSYE broth powder (36 g) was dissolved in 1 L of distilled water, mixed, and autoclaved at 121 °C for 15 mins. Meanwhile, the preparation of BHI medium and MH medium required the use of 37 g BHI powder and 24 g MHB powder. The medium was ready to use once it reached room temperature.

Initially, the freeze-dried bacteria *B. linens* and *C. acnes* were rehydrated. A specific liquid medium (0.5 mL) was added to the inner vial of the ampoules containing the dried pellet of bacteria and allowed to rehydrate for 30 mins. Later, the mixture was gently mixed using a Pasteur pipette by pressing and releasing the plunger a few times. Half of the hydrated bacteria were transferred to a sterilized test tube containing 5 mL of specific medium, while the other half was streaked and spread onto respective agar plates by using inoculation loop and a cell spreader, respectively. Both liquid and agar cultures were incubated. The culture was then used as inoculum to start the cultivation process.

In this study, the streaking technique was used for the cultivation of bacteria. It is a popular and widely used method to produce pure culture and discrete colonies. The bacteria were transferred to agar culture plates using an inoculation loop. The inoculation loop was flamed until red hot prior to each sterilization purpose. The plates were then sealed with parafilm and incubated at specific cultivation conditions in New BrunswickTM Innova® 42 incubator manufactured by Eppendorf Company (Germany). All apparatus used were sterilized and the experiments were conducted in an aseptic environment in an ESCO laminar airflow (Germany). The specific medium and cultivation conditions for each bacterial species are shown in Table 3.3.

Table 3.3 The specific medium in agar and liquid form and, culture conditions of each species of bacteria used in this study.

Bacteria species	**Agar medium**	**Liquid medium**	**Culture Conditions**		
			Temperature (°C)	**Incubation time (h)**	**Biological process**
B. linens	TSYE	TSYE	30	72	Aerobic
C. acnes	CASB	BHI	37	48	Anaerobic
S. epidermidis	MH	MH	37	48	Aerobic

3.5.2 Preparation of McFarland Standard

McFarland turbidity standard was used as a reference in antibacterial activities characterization in order to determine the density of bacterial suspension used in this study. The McFarland turbidity standard solution consists of $BaCl_2$ solution and H_2SO_4 solution.

To prepare McFarland turbidity standard, 1 g of $BaCl_2$ was diluted in 100 mL of distilled water. Then, 1 mL of concentrated H_2SO_4 was diluted in 99 mL of distilled water. The $BaCl_2$ and H_2SO_4 solutions were then mixed according to the required concentration to obtain the desired cell density (Table 3.4). The mixture was kept in screw cap vial covered with aluminium foil to prevent the evaporation of the solution. The McFarland turbidity standard solution was kept in a dark place at 4 °C to 25 °C and

could be used up to 6 months. Prior to each use, it was vigorously vortexed to prevent precipitation and clumps.

Table 3.4 Variation of McFarland turbidity standard solution and the approximate concentration of bacteria in suspension.

McFarland standard number	**0.5**	**1**	**2**	**3**	**4**
1% $BaCl_2$ (mL)	0.05	0.1	0.2	0.3	0.4
1% H_2SO_4 (mL)	9.95	9.9	9.8	9.7	9.6
Approx. cell density (1×10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0

3.5.3 Agar Disc Diffusion Assay (Disc Test)

The agar disk diffusion method is the most frequently used laboratory method to determine the susceptibility of the tested bacteria. In this method, a filter paper disc containing the test solution is placed on the inoculated agar medium and incubated. The diffusion of the test solution through the agar media during incubation resulted in inhibition of microbial growth, which is known as the inhibition zone surrounding the tested disc.

In this study, the susceptibility of *B. linens*, *C. acnes* and *S. epidermidis* on different types of uncoated and coated fabric (cotton, polyester, and blended wool) was determined. The susceptibility test was also conducted to optimize pomegranate rind extraction. The antimicrobial susceptibility test disc (10 µg of ampicillin, 10 µg of gentamicin, and 30 µg of vancomycin) obtained from OxoidTM (United Kingdom) was used as a positive control. The ampicillin, gentamicin, and vancomycin susceptibility disks were used to test the antibacterial activity towards *S. epidermidis*, *C. acnes* and *B. linens*, respectively. The uncoated fabric was used as the negative control, while the coated fabrics were used as tested discs. However, for the optimization of pomegranate rind extraction, filter paper was used as a blank disk (negative control) and tested disk (positive control).

To test the antibacterial activity of the fabric samples, a single colony of bacteria was picked using sterilized inoculation loop, transferred, and mixed into a sterilized

Eppendorf tube containing 3 mL of respective liquid medium. The density of the cultured bacterial suspension was compared with McFarland turbidity standard. Additional bacterial colonies were added to the suspension in case of insufficient bacterial density. However, the suspension was diluted with liquid media if it was too dense. After 15 to 20 mins, 300 µL of bacterial species with a concentration of 9.0×10^8 CFU/mL was spread on the respective agar media using sterilized cell spreader. A positive control, negative control and tested disc were placed on the inoculated petri dish. The agar plate was sealed with parafilm and incubated based on the specific culture conditions as shown in Table 3.3. All the antibacterial tests were performed in triplicate to confirm reproducibility.

3.6 Samples Characterization

3.6.1 XRF Analysis

The chemicals compositions of the sample solutions were analysed using X-Ray Fluorescence (XRF) (RIGAKU ZXS Primus II, Tokyo, Japan) with a Rh anode (4.0 kW). This method provided rapid and accurate determinations of elemental compositions, with detection limits for the elements ranging between 0.01 % to 100 %. All equipment settings were controlled by the software provided by RIGAKU. Prior to analysis, the sample was placed into a plastic sample holder with a Polyethylene plastic support film to ensure a flat surface for the X-ray analyser. This step is crucial as it can affect the transmission capabilities and helps in supporting the sample over the X-ray beam.

3.6.2 Particle Size Analysis

Measurement of particle size is essential to ensure the bioavailability, efficiency, and durability of the product. The particle size of the sample solutions was carried out using Zetasizer Nano S90 particle size analyser (Malvern Instrument, UK). The disposable cuvette cell (DTS0012) with measurement position of 4.65 mm was used for the measurement of dynamic light scattering (DLS). The parameters employed for the sample analysis included a temperature of 25 °C, a measurement time of 60 s, a refractive index of 1.59, and an adsorption value of 0.001. Water served as the

dispersant, with refractive index and viscosity values of 1.330 and 0.8872 cP, respectively.

3.6.3 Structure and Morphology Characterizations of the Coated and Uncoated Fabrics

The surface of the fabrics before and after the deposition of modified MgO and CuO particles and their chemical compositions were studied using a combination of two effective systems called Scanning Electron Microscopy-Energy Dispersive X-Ray (SEM-EDX). The microscopic structure of the sample was observed using a SEM model TM3030 Plus from Hitachi High-Tech (Japan) in high resolution magnifications between 500 to 5K. The coated and uncoated fabrics were coated with gold for 1 min prior to SEM processing. This was to ensure the stability of the sample towards electron bombardment without charging effect (Su et al., 2023). The EDX model SwiftED3000, also from Hitachi High-Tech (Japan), was used for compositional analysis of the sample. The interaction of electrons and the surface of the sample during SEM imaging produced the X-ray for EDX analysis. It analysed and identified all the elements contained in the sample based on the X-ray spectrum with an acceleration voltage of 15 kV and time of 30 seconds.

3.7 Scaling-up of Synthesis Process

The optimized formulations were used for the scaling-up process for both the sol-gel and green synthesis methods (Table 3.5). A total of 5 L of particles solutions of each formulation are needed to coat into 2 m of fabric.

Table 3.5 The optimized formulation for sol-gel synthesis and green synthesis process.

Synthesis process	Precursor	Amount of precursor (g)	Volume of solvent (mL)	Volume of PRE (mL)	pH
Sol-gel	$Cu(NO_3)_2$	0.59	20	10	4
	$Mg(NO_3)_2$	0.51	20	10	4
Green synthesis	$Cu(NO_3)_2$	0.59	20	10	-
	$Mg(NO_3)_2$	0.51	20	10	-

3.8 Fabric Coating Process

3.8.1 Fabrics Pre-treatment

Fabric pre-treatment is important in order to eliminate foreign residue or impurities from the fabric samples. The pre-treatment of the fabric was carried out using ultrasonication with the DSA ultrasonic cleaner (DSA200-GL_2-12L) (Fuzhou Desen Precision Ltd, China). To carry out the fabric pre-treatment process, 2 m of cotton fabric was fully immersed in 8 L of denatured ethanol and ultrasonicated at 35 °C for 15 mins. The fabric was then air dried at ambient temperature for 8 h before undergoing the coating process. Similar procedures were carried out for the pre-treatment of polyester and blended wool fabric. In each pre-treatment, new denatured ethanol was used to avoid contamination.

3.8.2 Fabrics Coating

The coating process was performed on fabric samples by the dip-dry method. The cotton, polyester, and blended wool fabrics were fully immersed in the prepared particles solution for 15 mins. Next, the fabric was air dried at 20 °C to 26 °C for 24 h. The fabric was dried in horizontal direction by folding the weft direction of the fabric to the drying rack (Figure 3.1). The plastic clothespins were used to hang up the fabric.

During each coating process, newly prepared particles solution was used. The volume of particles solution required for each coating process depended on the types and sizes of fabric to be coated. The procedures from coating to drying for each fabric were repeated depending on the number of coatings required.

Figure 3.1 Drying method of fabric after the dipping process.

3.9 Antibacterial Activity Characterization

The antibacterial activity test was conducted to verify the effectiveness of the coated fabrics in preventing bacterial growth. To test the antibacterial activity of the coated fabrics, the procedures were similar to those used in the pre-antibacterial characterization. However, following the incubation period, the inhibition zones of the bacterial colonies around the antibiotic, coated and uncoated fabrics were measured and recorded for comparison.

3.10 Tensile and Tear Strength Test

Tensile strength is defined as the strength and elongation properties of fabric, which are typically measured in terms of force given to the fabric per cross-sectional area. On the other hand, tear strength refers to the resistance of fabric against tearing and is associated with the individual yarns in the fabric. In this study, the tests were conducted using the Universal Tensile Machine (UTM) from Tinius Olsen H25KS (United States). The tensile strength was measured for all types of coated and uncoated fabric, while the tear strength was only measured for coated and uncoated cotton and

polyester fabrics. The tear strength was not measured for blend wool fabric because it is knitted fabric which has a loop structure. This structure can easily unravel, making it cannot be measured accurately (Ma et al., 2014). The tensile and tear tests were carried out for both warp and weft direction of the fabric samples (Figure 3.2).

The breaking load procedure used to measure the tensile strength was carried out for all coated and uncoated fabric samples according to ISO 13934-1:2013 - Textiles - Tensile properties of fabrics - Part 1: Determination of maximum force and elongation at maximum force using the strip method (International Organization for Standardization, 2013). According to this standard, the maximum force and elongation at the maximum force were determined using the strip method. The fabric sample was cut into a rectangular shape with the size of 400 mm × 50 mm. Then, the fabric sample was properly placed between the clamps by vertically aligning the sample from the upper clamp to the lower clamp in order to avoid side loading during the test. This is the crucial part of the test, as mishandling the fabric may lead to a negative result. The gauge length of the fabric used was 200 mm, with a speed of 10 mm/min. The fabric sample was slowly elongated and deformed in the middle of its length during this process. The testing was ended after the fabric sample was fractured. The breaking load of the fabric was determined by comparing the measurement of the fabric sample before and after coated with particles.

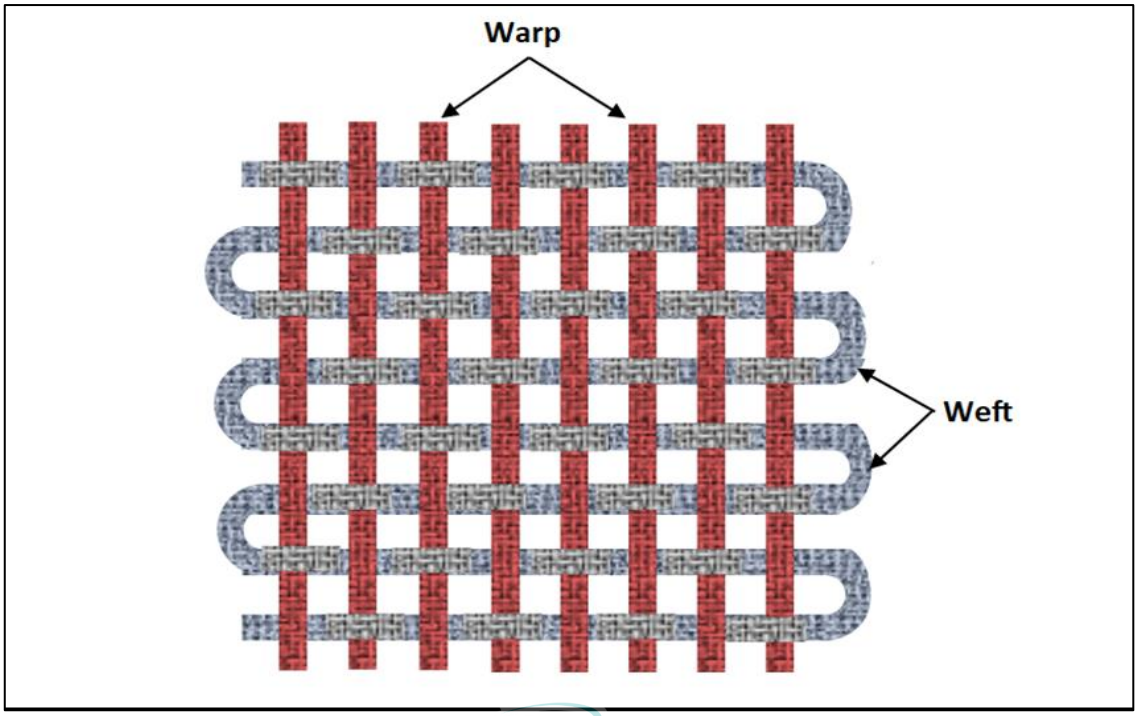


Figure 3.2 The warp and weft direction of the fabric.

The tear property of fabrics was determined according to ISO 13937-4:2000 - Textiles - Tear properties of fabrics - Part 4: Determination of tear force of tongue-shaped test specimens (Double tear test) (International Organization for Standardization, 2000). Based on this standard, the tongue-shape fabric sample (double tear test) was used for the determination of tear force. For each type of fabric, two sets of fabric samples with triplicates from warp and weft directions were prepared. The sample was cut into a rectangular shape with a size of 200 mm × 150 mm. Then, the sample was cut 100 mm parallel to the length direction from the middle of the width direction with a size of 100 mm × 50 mm. The size of the tear terminal was 25 mm. Figure 3.3 shows the standard measurement of fabric used in this study. To measure the tear strength, the fabric tongue was clamped symmetrically. The two legs of the fabric were then clamped symmetrically in parallel direction of tearing. The tongue of the fabric was pulled to the end marks of the sample with a stretch speed of 100 mm/min and an extension range of 200 mm. The result was recorded in newton (N) unit.

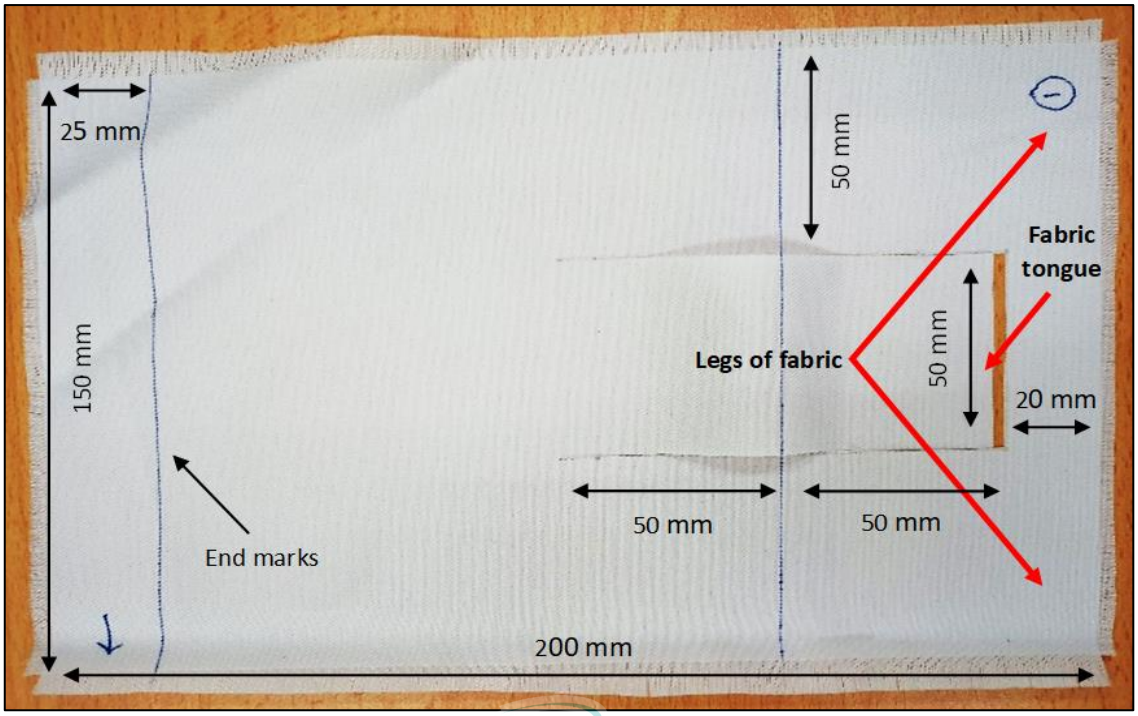


Figure 3.3 The standard measurement of fabric sample for tongue tear test.

3.11 Air Permeability Test

Air permeability is a fundamental property of fabric and can be defined as the volume of air passing through a specific area at a given time period (Mishra et al., 2019). In this study, the SDL Atlas M021A Air Permeability Tester (United States) was used. The air permeability of the cotton, polyester, and blended wool fabrics before and after coating was measured according to ISO 9237:1995 - Textiles - Determination of the permeability of fabrics to air (International Organization for Standardization, 1995). The fabric samples were kept at 20 ± 2 °C with a relative humidity of 65 ± 2 % for 24 h before the test procedure were carried out (ISO 139:1937 - Textiles - Standard atmospheres for conditioning and testing) (International Organization for Standardization, 1937).

Five tests were performed for each sample, and the test area for each sample was 20 cm^2. A pressure of 100 Pa was used in this study. To measure the air permeability, the fabric sample was clamped in the air permeability device with the right side facing the air inlet, using a vacuum. The air was then sucked through the

fabric sample and the air flow was adjusted to achieve the agreed pressure drop. The velocity of the air flow, which is the air permeability, was read in cfm unit.

3.12 Washing Durability

The washing durability test was conducted to evaluate the effectiveness and durability of the modified particles on the fabric. It tested the antibacterial efficiency of the coated fabric after several washing processes. The washing durability test was conducted following ISO 6330:2012(E) - Domestic Washing and Drying Procedure for Textile Testing (International Organization for Standardization, 2012).

The fabric samples were washed in a washing machine type C (vertical axis, top loading pulsator) with a water temperature inlet range of 20 °C to 25 °C. The relative humidity during the test was recorded at 65 ± 4 %. A total of 2 kg of fabric from the same types consisted of uncoated fabric and 20 cm × 20 cm of coated fabric was placed into the washing machine. A non-phosphate powder detergent with optical brightener and enzymes was used at a ratio of 1.33 g to 1 L of water. All the washing actions, such as water supply, washing, rinsing, and spinning cycles, were as programmed on the washing machine.

After the washing process completed, the fabric sample was immediately air-dried using the line dry method. Two corners of the fabric were hung unfolded with the fabric length in a vertical direction to avoid fabric distortion. The samples were dried at ambient temperature until completely dry. The washing and drying process was repeated 2, 3, 4, and 5 times and the antibacterial activities of the coated fabrics were recorded.

3.13 Identification of Volatile Active Compounds

3.13.1 Preparation of Samples

A simple method was used to identify odours from fabric samples (Verhulst et al., 2016). In this method, the non-stabilized artificial sweat with a pH of 4.5 was purchased from Nanochemazone (Canada) and used as the substrate. A 2 cm × 2 cm fabric was inserted into a 2 mL autosampler vial containing 1.0 mL of artificial sweat.

Then, 100 µL of each bacteria species (*B. linens*, *C. acnes* and *S. epidermidis*) with a concentration of 1.5×10^8 CFU/mL was added to the artificial sweat solution and gently mixed. The solution was then incubated for 24 h at 37 °C. The fabric sample was removed from the vial prior to GC-MS analysis. All of these procedures were conducted in a laminar air flow.

3.13.2 Gas Chromatography Mass Spectrometry analysis

The volatile active compounds were determined using gas chromatography-mass spectrometry (GC-MS) 6890 Series GC System (Agilent Technologies, USA). The GC was fitted with a 30 mm × 0.25 mm GC Capillary column (BPX5), with a film thickness of 0.25 µm and a maximum temperature of 360 °C to 370 °C. The GC-MS conditions were modified according to Xin et al. (2013). The injector was maintained at 250 °C, with a transfer line temperature of 280 °C. The scanning range was about 40 – 450 Da, and the ion energy of electron impact ionization of 70 eV was used. The ion source temperature was set to 230 °C. Helium gas was used as a carrier gas with a flow rate of 1.2 mL/min. The incubated artificial sweat solution which absorbed the analytes was introduced to the GC injector at 250 °C in the splitless mode for 3 min. The isothermal temperature was set at 40 °C for 3 min. The temperature was gradually increased by 3 °C/min until it reached 73 °C. The temperature was held for 3 min once it reached 73 °C. The temperature was then increased to 220 °C at a rate of 5 °C/min and was held for 1 min.

3.14 Statistical Analysis

The data on the tensile and tear strength, and air permeability of the uncoated and coated fabric samples were statistically analysed using Statistical Package for Social Science (SPSS) software. In this study, multiple range test was conducted using Least Significant Difference (LSD) as a post-hoc test to compare specific group means and identify any significant differences. The results are reported as mean ± standard deviation.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and interpretations derived from the experiments conducted on the antibacterial coating for fabrics in the prevention of body odour. The key finding of this study reveals that all coated fabrics exhibited antibacterial properties against all the tested bacterial species and did not generate volatile odorant compounds responsible for body odour. These findings directly aligned with the research objectives, aiming to fabricate antibacterial coatings for various types of fabric to minimize or eliminate body odour. The chapter employs various methods and techniques for data analysis and interpretation, including UPLC analysis, XRF analysis, particles size analysis, SEM-EDX, antibacterial activity test (disc diffusion assay), tensile strength testing, washing durability assessment, and GC-MS analysis. The selection of these methodologies was based on their capacity to offer a comprehensive and reliable assessment of the antibacterial properties and efficacy of the fabric coatings.

4.2 Optimization of Pomegranate Rind Extraction Parameters

Pomegranate rind extract was extracted using the solvent extraction method. The optimization of extraction parameters is crucial as it directly influence the composition of bioactive compounds within the extract. Consequently, this factor can ultimately influence the antibacterial efficiency of the particles. In this study, the temperature and amount of pomegranate rind powder used were optimized using one-variable-at-a-time (OVAT) methodology (Venkatachalam et al., 2021). Initially, the amount of pomegranate rind powder was optimized, followed by the extraction temperature. The antibacterial performance of all extracts was evaluated using the agar disk diffusion method.

Based on the observations, extracts obtained from 4 g and 8 g of pomegranate rind powder extracted at 100 °C showed an antibacterial activity against all tested species of bacteria (Table 4.1). However, the parameters of 4 g of pomegranate rind powder and a temperature of 100 °C were selected as the optimal parameters. This decision was based on the similar pattern of antibacterial efficiency observed between the use of 4 g and 8 g of pomegranate rind powder for extraction.

According to Cavalaro et al., (2019), the solid-to-solvent ratio may influence the bioactive compounds of the extract due to concentration differences between the solid and liquid phases which affect the mass transfer rate. The use of a lower amount of pomegranate rind powder for extraction enhances its surface contact with the solvent, leading to increased extraction yields (Erragued et al., 2022). Additionally, it can reduce the extraction time and overall production cost for particles preparation. On the other hand, using a higher amount of pomegranate rind powder could significantly affect the equilibrium constant, resulting in the increased extraction time, and overall particles production time (López et al., 2023). This is due to the fact that exponential phase requires more time to produce the maximum yield of bioactive compounds (Suparmaniam et al., 2023).

The extraction temperature also plays a role in influencing the antibacterial activity and cytotoxicity of the extract as it can impact the bioactive compounds present. Generally, increasing the extraction temperature can reduce the viscosity and surface tension of the liquid solvent, thereby improving the mass transfer and solubility of bioactive compounds (Rodríguez et al., 2020). Additionally, the polarity of water is reduced at high temperatures, allowing non-polar compounds to dissolve, and be extracted by water. The high extraction temperature may also lead to the release of reducing agents and sugars due to the breakdown of lignocellulose (Usmani et al., 2022). As stated by (Cacace & Mazza, 2003) the high extraction temperature resulted in an increase in bioactive compounds such as anthocyanin in the extract, thereby the use of 100 °C as an extraction temperature enhanced the antibacterial activity of PRE.

Table 4.1 The antibacterial activity of pomegranate rind extract towards *B. linens*, *C. acnes*, and *S. epidermidis*.

Bacteria	Temperature (° C)	Amount of pomegranate rind powder (g)		
		2	4	8
B. linens	30	×	×	×
	80	×	×	×
	100	a	a	a
C. acnes	30	×	×	×
	80	×	×	×
	100	×	a	a
S. epidermidis	30	×	×	×
	80	×	×	×
	100	×	a	a

Note: The symbol indicates; no antibacterial activity observed (×), presences of antibacterial activity (a).

4.2.1 Analysis of Bioactive Compounds in the Optimized Pomegranate Rind Extract

The identification of bioactive compounds within the optimized PRE was carried out using UPLC-QTOF-MS. This analysis holds its significance as it directly affects the antibacterial properties of the particle solutions and subsequently influences the effectiveness of the coated fabrics. Herein, a total of 82 bioactive compounds were identified in the extract which consists of alkaloids, phenolics and polyphenols compounds (Table 4.2), and the UPLC spectra are presented in Appendix B. These compounds, renowned for their antibacterial properties, potentially enhance the effectiveness of the particle solutions in eliminating or inhibiting the growth of skin's bacteria.

Among the identified compounds, tannins, a subset of phenolics and polyphenols, dominate the composition of the PRE, constituting 62.62%. Tannins are known for their ability to form strong complexes with proteins, leading to precipitation, which contributes to their antibacterial activity (Baron et al., 2019). This mechanism

makes tannins an effective antibacterial agent, as they can target and incapacitate proteins that are vital for bacterial survival. By precipitating proteins, tannins-incorporated coatings may not only inhibit bacterial growth but also prevent the formation of biofilms, enhancing the antibacterial efficacy of the treated fabrics. Examples of the tannins in the PRE include punicalagin (8.9%), ellagic acid (7.56%), penduculagin (4.06%), and terchebulin (22.37%). Terchebulin is particularly effective against anaerobic bacteria like *C. acnes* (Abozeid et al., 2022).

Flavonoids, another class of phenolics and polyphenols, represent the largest group of compounds identified in the extract, numbering 47. Despite this, they account for only 33.18% of the extract's total composition. Rutin, catechin, and quercetagetin are examples of flavonoids present in the extract, known for their efficacy against a wide range of bacteria, including both gram-positive and gram-negative strains, as well as antibiotic resistant strains such as Methicillin-resistant *Staphylococcus aureus* (MRSA) (Patra, 2012; Rasouli et al., 2019). Flavonoids exert their antibacterial activity through various mechanisms, including the inhibition of nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, and disruption of ion and nutrient transportation across the membrane (Górniak et al., 2019; Veiko et al., 2023). Meanwhile, Quinones, yet another subset of phenolic and polyphenols identified in the extract, also contribute to its antibacterial state (Rahman et al., 2021).

The presence of alkaloids compounds (2.27%) such as carbolin, harman, laevigating, and hypoxanthine in the extract has shown effectiveness in inhibiting both gram-positive and gram-negative bacteria (Tang et al., 2023). However, certain alkaloids like acutumidine and anhydroberberillic acid are effective solely against gram-positive bacteria (Mittal & Jaitak, 2019). Alkaloids act against bacteria through various mechanisms, including disrupting cell membranes, altering metabolic pathways, and interfering with the synthesis and function of nucleic acids, proteins, and enzymes within bacterial cells (Yan et al., 2021). Other compounds identified in PRE including xanthone (0.18%), quinone (0.52%), benzophenone (0.46%), phenol (0.07%), chalcone (0.05%), nucleoside (0.2%), and gingerol (0.32%).

Table 4.2 The identified alkaloids, phenolics and polyphenols compounds in the optimized pomegranate rind extract.

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds (%)
1	(-)- Gallocatechin	Flavonoids	$C_{15}H_{14}O_7$	4.16	306.0737	305.0664	0.35
2	2,5,7-Trihydroxy-6,8-dimethyl-3-(3',4'-methylenedioxybenzyl) chroman-4-one		$C_{19}H_{18}O_7$	0.94	358.1125	357.1053	0.20
				0.88	358.1111	357.1038	
				0.9	344.1322	343.1249	
3	2,5,7-Trihydroxy-6,8-dimethyl-3-(4'-methoxybenzyl) chroman-4-one		$C_{19}H_{20}O_6$	0.9	344.1322	343.1249	1.52
4	2′,6′-Dihydroxy-4,4′-dimethoxydihydrochalcone		$C_{17}H_{18}O_5$	9.77	302.1072	301.0999	0.12
5	3',4',7-Trihydroxyisoflavanone		$C_{15}H_{12}O_5$	2.6	272.0693	271.062	0.04
6	3,4-O-Dicaffeoylquinic acid		$C_{25}H_{24}O_{12}$	1.21	516.1361	515.1288	0.12
7	5,7,3',4'-Tetramethoxyflavone		$C_{19}H_{18}O_6$	0.74	342.1174	341.1101	0.85
				0.87	342.1159	341.1086	
8	5-Hydroxyauranetin		$C_{20}H_{20}O_8$	0.87	388.1215	387.1143	0.29

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
9	6-Aldehydo-isoophio-pogonone A	Flavonoids	$C_{19}H_{14}O_7$	0.84	354.0798	353.0725	0.45
				1.21	354.0817	353.0744	
10	Asebotin		$C_{22}H_{26}O_{10}$	0.9	450.159	449.1518	0.06
11	6-Methoxy-2-[2-(4'-methoxyphenyl) ethyl] chromone		$C_{20}H_{22}O_4$	16.89	326.1594	325.1521	0.08
12	6-Formyl-isoophiopogonanone A		$C_{19}H_{16}O_7$	1.21	356.097	355.0897	0.64
13	7,4′,7″,4‴-Tetra-O-amentoflavone		$C_{34}H_{26}O_{10}$	10.78	594.1621	593.1548	0.38
14	Cyanidin		$C_{15}H_{11}ClO_6$	18.69	322.0254	321.0181	0.03
15	Cytidine		$C_9H_{13}N_3O_5$	0.84	243.0864	242.0791	0.20
				0.84	243.0864	242.0792	
16	d-Catechin		$C_{15}H_{14}O_6$	6.17	290.0792	289.0719	0.07
17	Divaricatol		$C_{17}H_{18}O_7$	2.7	334.1094	333.1021	0.07

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
18	Feroxidin	Flavonoids	$C_{11}H_{14}O_3$	16.81	194.0955	193.0882	0.11
19	Furosin	Flavonoids	$C_{27}H_{22}O_{19}$	3.7	650.0855	649.0782	0.98
				1.21	650.0812	649.0739	
				3.72	650.0716	649.0643	
				3.69	650.0718	649.0645	
				3.68	650.0747	649.0675	
20	Gallocatechin	Flavonoids	$C_{15}H_{14}O_7$	5.75	306.0739	305.0666	0.39
				7.1	306.0766	305.0693	
21	Gallocatechin(4α→8)-epicatechin	Flavonoids	$C_{30}H_{26}O_{13}$	5.82	594.1389	593.1316	0.29
				4.94	594.1385	593.1313	
				4.49	594.1384	593.1312	
22	Gemin D	Flavonoids	$C_{27}H_{22}O_{18}$	5.47	634.0878	633.0805	9.34

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				5.43	634.0797	633.0724	
				5.25	634.08	633.0728	
				5.46	634.0877	633.0804	
				7.32	634.0845	633.0773	
23	Geraniin	Flavonoids	$C_{41}H_{28}O_{27}$	4.03	952.0755	951.0682	3.96
				4.78	952.0792	951.0719	
				5.5	952.0862	951.079	
				8.93	952.0905	951.0832	
				8.82	952.0886	951.0814	
				5.41	952.0848	951.0775	
				4.75	952.0844	951.0771	
				5.42	952.0885	951.0812	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				8.87	952.0899	951.0826	
				5.38	952.0829	951.0756	
24	Kuwanon L	Flavonoids	$C_{35}H_{30}O_{11}$	0.81	626.1747	625.1674	0.03
25	Laevigatin A	Flavonoids	$C_{34}H_{26}O_{23}$	6.48	802.0904	801.0832	1.95
				6.43	802.089	801.0817	
				6.49	802.0818	801.0745	
				6.64	802.0866	801.0793	
				6.45	802.0913	801.084	
26	Laevigatin G	Flavonoids	$C_{54}H_{42}O_{36}$	5.5	1266.154	1265.147	0.62
				5.98	1266.149	1265.141	
				6.31	1266.136	1265.129	
				5.68	1266.151	1265.144	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
27	Maclurin	Flavonoids	$C_{13}H_{10}O_6$	0.77	262.0419	261.0347	0.11
28	Mahuannin E	Flavonoids	$C_{30}H_{24}O_9$	5.22	528.1484	527.1412	0.15
				5.18	528.1488	527.1415	
29	Mahuannin G	Flavonoids	$C_{32}H_{22}O_{10}$	6.14	542.1249	541.1176	0.06
30	Mallotinic acid	Flavonoids	$C_{34}H_{26}O_{22}$	9.28	786.0931	785.0859	1.59
				5.99	786.0907	785.0834	
				5.97	786.092	785.0847	
				8.1	786.0956	785.0883	
				5.94	786.0942	785.0869	
				7.09	786.0906	785.0834	
31	Maltol	Flavonoids	$C_6H_6O_3$	2.55	126.0303	125.023	0.30
				2.53	126.0296	125.0224	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				2.57	126.0308	125.0236	
32	Mulberrofuran O	Flavonoids	$C_{39}H_{34}O_9$	2.75	646.2195	645.2123	0.06
33	Mururin A	Flavonoids	$C_{24}H_{16}O_9$	9.74	448.0696	447.0624	0.17
34	Myricetin	Flavonoids	$C_{15}H_{10}O_8$	3.82	318.037	317.0297	0.11
				16.84	318.0319	317.0246	
35	Nilocitin	Flavonoids	$C_{20}H_{20}O_{14}$	2.97	484.0819	483.0746	3.44
				4.01	484.0815	483.0742	
				4.78	484.0867	483.0795	
				4.05	484.0812	483.0739	
				4.03	484.0812	483.0739	
				6.29	484.0872	483.0799	
				5.94	484.0861	483.0789	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				5.23	484.0856	483.0783	
				5.7	484.0862	483.0789	
				6.08	484.0863	483.079	
36	Nobilin C	Flavonoids	$C_{18}H_{22}O_6$	18.69	334.1417	333.1344	0.14
				2.73	334.1358	333.1285	
37	Ophiopogonanone B	Flavonoids	$C_{18}H_{18}O_5$	0.92	314.1219	313.1146	0.07
38	Polygoacetophenoside	Flavonoids	$C_{14}H_{18}O_{10}$	7.37	346.091	345.0837	0.40
				8.65	346.0948	345.0875	
				2.35	346.0909	345.0836	
				8.61	346.0922	345.085	
39	Procyanidin B2_1	Flavonoids	$C_{30}H_{26}O_{12}$	5.67	578.1437	577.1364	0.06
40	Protosappanin A	Flavonoids	$C_{15}H_{12}O_5$	3.09	272.0609	271.0537	0.42

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				3.07	272.0608	271.0535	
41	Quercetagetin	Flavonoids	$C_{15}H_{10}O_8$	16.85	318.0319	317.0246	0.07
42	Rutin	Flavonoids	$C_{27}H_{30}O_{16}$	9.78	610.1577	609.1504	0.08
43	Tachioside	Flavonoids	$C_{13}H_{18}O_8$	9.77	302.1072	301.0999	0.16
44	Terchebin	Flavonoids	$C_{41}H_{30}O_{27}$	5.44	954.0914	953.0841	0.18
				9.95	954.1066	953.0993	
45	Terflavin A	Flavonoids	$C_{48}H_{30}O_{30}$	6.28	1086.087	1085.08	0.81
				6.01	1086.079	1085.072	
				5.05	1086.076	1085.069	
				6.39	1086.091	1085.083	
46	Viscidulin I	Flavonoids	$C_{15}H_{10}O_7$	10.03	302.0352	301.028	0.22
				9.8	302.0408	301.0335	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
47	Yakuchinone A	Flavonoids	$C_{20}H_{24}O_3$	16.88	312.1749	311.1676	1.57
				16.78	312.1742	311.167	
				16.74	312.1764	311.1691	
				16.85	312.177	311.1697	
				18.67	312.1749	311.1676	
48	1,2,6-Tri-O-galloyl-β-D-glucopyranoside	Tannins	$C_{27}H_{24}O_{18}$	6.94	636.0983	635.091	0.17
				7.6	636.1005	635.0932	
				7.72	636.0975	635.0902	
				7.72	636.0948	635.0875	
49	1-Galloyl-glucose	Tannins	$C_{13}H_{16}O_{10}$	3.05	332.0799	331.0727	2.60
				2.27	332.0747	331.0674	
				3.07	332.0819	331.0746	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				3.03	332.0786	331.0713	
				2.57	332.0741	331.0668	
				2.17	332.077	331.0697	
				2.12	332.0754	331.0681	
				1.2	332.076	331.0687	
				1.25	332.0744	331.0671	
50	1-O-Galloylpedun-culagin	Tannins	C_{41} H_{28} O_{26}	6.64	936.0884	935.0811	0.41
				8.1	936.0929	935.0857	
				6.7	936.0832	935.0759	
51	2,3-(S)-Hexahydroxydiphenoyl-D-glucose	Tannins	C_{20} H_{18} O_{14}	1.45	482.067	481.0597	13.75
				6.04	482.0687	481.0614	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				1.44	482.0778	481.0706	
				1.32	482.0706	481.0633	
				1.39	482.07	481.0628	
				1.42	482.071	481.0638	
				1.56	482.07	481.0627	
				1.5	482.0716	481.0643	
				1.21	482.0729	481.0656	
52	5-Desgalloylstachyurin	Tannins	$C_{34}H_{24}O_{22}$	6.94	784.0828	783.0755	1.41
				6.83	784.0803	783.073	
				6.73	784.0817	783.0744	
53	Castalagin	Tannins	$C_{41}H_{26}O_{26}$	4.06	934.0699	933.0626	0.74
				4.25	934.0726	933.0654	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
54	Corilagin	Tannins	$C_{27}H_{22}O_{18}$	4.68	634.0784	633.0711	0.48
				4.66	634.0796	633.0723	
55	Decaffeoylacteoside	Tannins	$C_{20}H_{30}O_{12}$	7.64	462.1766	461.1693	0.17
56	Ellagic acid	Tannins	$C_{14}H_6O_8$	5.07	302.0063	300.999	7.56
				16.84	301.9999	300.9926	
				6.04	302.0048	300.9975	
				5.81	302.0057	300.9984	
				9.9	302.0094	301.0021	
				9.85	302.0093	301.002	
				9.79	302.0092	301.002	
57	Pedunculagin	Tannins	$C_{34}H_{24}O_{22}$	3.54	784.0748	783.0675	4.06
				5.32	784.0848	783.0776	

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
				3.52	784.0736	783.0663	
				4.1	784.0768	783.0696	
58	Punicalagin	Tannins	$C_{48}H_{28}O_{30}$	5.04	1084.071	1083.064	8.90
				5.97	1084.069	1083.061	
				3.82	1084.069	1083.062	
				6.09	1084.068	1083.06	
				5.04	1084.071	1083.064	
				5.07	1084.069	1083.062	
59	Terchebulin	Tannins	$C_{48}H_{28}O_{30}$	4.01	1084.065	1083.058	22.37
				6.01	1084.072	1083.065	
				6.01	1084.07	1083.063	
60	1-Acetyl-β-carboline	Alkaloids	$C_{13}H_{10}N_2O$	11.23	210.0873	209.08	0.18

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
61	1-Formyl-β-carboline	Alkaloids	$C_{12}H_8N_2O$	0.8	196.0581	195.0508	1.40
				1.21	196.0592	195.0519	
				0.82	196.0566	195.0493	
				0.8	196.0581	195.0508	
				0.82	196.058	195.0507	
62	Acutumidine	Alkaloids	$C_{18}H_{22}ClNO_6$	0.79	383.1131	382.1059	0.08
63	Anhydroberberillic acid	Alkaloids	$C_{20}H_{17}NO_8$	0.8	399.1054	398.0981	0.18
64	Harman	Alkaloids	$C_{12}H_{10}N_2$	0.94	182.0792	181.0719	0.16
				1.12	182.0792	181.072	
65	Hordenine-O-α-L-rhamnopyranoside	Alkaloids	$C_{16}H_{25}NO_5$	18.7	311.1816	310.1743	0.10
66	Hypoxanthine	Alkaloids	$C_5H_4N_4O$	0.86	136.0367	135.0294	0.03

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
67	Kukoamine A	Alkaloids	$C_{28}H_{42}N_4O_6$	18.67	530.3086	529.3013	0.04
68	Lysicamine	Alkaloids	$C_{18}H_{13}NO_3$	1.21	291.0965	290.0893	0.07
69	Tribulusamide A	Alkaloids	$C_{36}H_{36}N_2O_8$	11.87	624.245	623.2377	0.03
70	1-Hydroxy-2,3,7-trimethoxyxanthone	Xanthone	$C_{16}H_{14}O_6$	9.77	302.0779	301.0706	0.10
71	3-Hydroxy-2,8-dimethoxyxanthone	Xanthone	$C_{15}H_{12}O_5$	3.06	272.061	271.0537	0.08
72	Belladonnine	Quinone	$C_{34}H_{42}N_2O_4$	6.03	542.3063	541.299	0.35
				6.01	542.3103	541.3031	
				5.06	542.3094	541.3022	
73	Arbutin	Quinone	$C_{12}H_{16}O_7$	2.6	272.0835	271.0763	0.06
74	Protohypericin	Quinone	$C_{30}H_{18}O_8$	1.21	506.0955	505.0882	0.11
75	3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone	Benzophenone	$C_{10}H_{12}O_4$	0.81	196.0734	195.0661	0.05
76	2,4,4′,6′-Tetrahydroxy-benzophenone	Benzophenone	$C_{13}H_{10}O_5$	0.8	246.0505	245.0432	0.04

Table 4.2 Continued

No	Component name	Classification	Molecular formula	Retention time (min)	Observed neutral mass	Observed m/z	Percentage of compounds
77	2,4-Dihydroxyaceto-phenone	Benzophenone	$C_8H_8O_3$	10.87	152.0481	151.0408	0.37
				10.82	152.0485	151.0413	
				10.9	152.0484	151.0411	
78	6-Gingerol	Gingerol	$C_{17}H_{26}O_4$	16.79	294.1856	293.1783	0.32
				18.53	294.1857	293.1784	
				16.74	294.1865	293.1793	
				18.71	294.1825	293.1753	
79	7-Hydroxy-2,3,5-trimethoxy-9,10-dihydrophenanthrene	Phenols	$C_{17}H_{18}O_4$	1.02	286.1161	285.1088	0.07
80	Bavachalcone	Chalcone	$C_{20}H_{20}O_4$	3.67	324.1396	323.1323	0.05
81	6-Isoinosine	Nucleoside	$C_{10}H_{12}N_4O_5$	0.91	268.0801	267.0728	0.16
82	Cordycepin	Nucleoside	$C_{10}H_{13}N_5O_3$	0.74	251.1014	250.0941	0.04

4.3 Optimization of Synthesis and Coating Cycles Parameters

The antibacterial properties of cotton, polyester and blend wool fabrics were enhanced through the optimization of process parameters in both sol-gel and green synthesis methods. For the sol-gel process, the volume of PRE, pH, and number of coating cycles were optimized. As for the green synthesis process, the optimization involved the volume of PRE and number of coating cycles.

Table 4.3 shows the parameters for both sol-gel synthesis and green synthesis, focusing on their effects on the antibacterial activity against gram-positive bacteria. Based on the observations, the use of PRE in the sol-gel solution has greatly affected the antibacterial activity of the particles, as none of the formulations containing Cu and Mg particles (which acted as controls) exhibited antibacterial activities. This is probably due to the lesser amount of precursor used in the synthesis process (Parashar et al., 2020). According to Kessler & Seisenbaeva (2023), the efficacy of metal oxide particles in inhibiting bacterial growth is frequently associated with their surface area and reactivity, which is related to the amount of precursor used during the synthesis process. Thus, the optimized parameters selected for both methods were 10 mL of PRE, 1× coating cycle, and pH 4 for sol-gel synthesis.

According to Gopal et al. (2018), the hydrolysis and condensation of precursors, such as metal oxide can be influenced by the amount of plant extract used in the synthesis process, resulting in variations in the size, shape, and distribution of particles. Using a low amount of plant extract in the synthesis process may resulted in incomplete reduction and stabilization of metal oxide precursors, leading to the formation of large and non-uniform particles, which can impact their performance (Chaillot et al., 2019) and explain the absent of antibacterial activities when 5 mL of PRE was used. The small and uniform particles can be produced using high amount of plant extract due to the enhancement of reduction and stabilization of metal oxide precursors (Innocenzi, 2019). However, the use of 15 mL of PRE resulted in fungal contamination in the particles solutions after two days being kept at room temperature. The excessive amount of plant extract can create a favourable environment for fungal growth due to the nutrient composition of plant extract (Singh et al., 2019). According to Younos &

Embaby (2023), the sugar content in plant extract can serve as a nutrient source for fungal growth as the microbes can adhere to the surface of extract, invade cellular space, and proliferate in the environment, leading to contamination issue. This can potentially affect the quality of the particles and indirectly impact the safety of the consumers (Stępień et al., 2015).

Another important parameter studied for both sol-gel and green synthesis methods were the coating cycle. The coating cycle affects the thickness and stability of the coatings as well as their morphology, porosity, crystallinity, and properties (Mahltig et al., 2010; Xu et al., 2021). However, the number of coating cycles did not significantly affect the antibacterial properties of the sol-gel and green synthesis-derived antibacterial particles (Soule et al., 2020). The antibacterial activity patterns of the 2× and 3× coating cycles were similar to that of the 1× coating cycle. Thus, the 1× coating cycle was selected as the optimal parameter to save time, energy, and overall production costs. Studies have shown that the antibacterial properties of the coated fabric are primarily influenced by the antibacterial compounds present in the particles, rather than the coating cycle itself (Gulati et al., 2022; Zada et al., 2020).

Lastly, sol-gel synthesis parameter that influences the bactericidal activity of the particles is pH. According to Iyer et al. (2021), the optimal pH levels for the growth of bacteria vary among species, and growth is hindered below or above optimal pH range (Wan et al., 2020). *B. linens*, for example, has an optimal pH range for growth of 6.5 – 8.5 (El Soda & Awad, 2014), thus it showed antibacterial activity at pH 5.5. Meanwhile, *C. acnes* and *S. epidermidis* have an optimal pH range of 5.0 – 7.4 and 4.5 – 7, respectively (Dréno et al., 2018; Iyer et al., 2021). Therefore, no antibacterial activity of particles was observed at pH 5.5 and pH 7 for these species. Antibacterial particles may exhibit higher contact killing efficiency at acidic pH due to the release of more metal ions, leading to increased oxidative stress on bacterial cells and their subsequent death (Mendes et al., 2022). In the formulations containing Cu and Mg, antibacterial activity was not detected at pH levels of 4 and 5. This is due to the insufficient quantity of precursor and the omission of an antibacterial agent in the solution, both of which are critical to the antibacterial mechanism's effectiveness (Abebe et al., 2020).

Table 4.3 The antibacterial activity of particles based on synthesis parameters against *B. linens*, *C. acnes*, and *S. epidermidis*.

Bacteria	Synthesis method	Formulation	Amount of PRE (mL)			pH			Number of coating cycles		
			5	10	15	4	5.5	7	1×	2×	3×
B. linens	Sol-gel	Cu	N/A	N/A	N/A	×	×	×	×	×	×
		Cu + PRE	×	a	×	a	a	×	a	a	a
		Mg	N/A	N/A	N/A	×	×	×	×	×	×
		Mg + PRE	×	a	×	a	a	×	a	a	a
	Green synthesis	Cu + PRE	×	a	×	N/A	N/A	N/A	a	a	a
		Mg + PRE	×	a	×	N/A	N/A	N/A	a	a	a
C. acnes	Sol-gel	Cu	N/A	N/A	N/A	×	×	×	×	×	×
		Cu + PRE	×	a	×	a	×	×	a	a	a
		Mg	N/A	N/A	N/A	×	×	×	×	×	×
		Mg + PRE	×	a	×	a	×	×	a	a	a
	Green synthesis	Cu + PRE	×	a	×	N/A	N/A	N/A	a	a	a
		Mg + PRE	×	a	×	N/A	N/A	N/A	a	a	a
S. epidermidis	Sol-gel	Cu	N/A	N/A	N/A	×	×	×	×	×	×
		Cu + PRE	×	a	×	a	×	×	a	a	a
		Mg	N/A	N/A	N/A	×	×	×	×	×	×

Table 4.3 Continued

Bacteria	Synthesis method	Formulation	Amount of PRE (mL)			pH			Number of coating cycles		
			5	10	15	5	10	15	1×	2×	3×
	Green synthesis	Mg + PRE	×	a	×	a	×	×	a	a	a
		Cu + PRE	×	a	×	N/A	N/A	N/A	a	a	a
		Mg + PRE	×	a	×	N/A	N/A	N/A	a	a	a

Note: The symbol indicates; no antibacterial activity observed (×), presences of antibacterial activity (a), antibacterial activity test did not conducted (N/A).

4.4 XRF Analysis

The XRF analysis was conducted to confirm the presence of CuO and MgO elements in each particle's solution. Based on the XRF spectra (Appendix C), CuO and MgO were identified in their respective particle formulations, confirming the reaction of copper nitrate and magnesium nitrate with PRE, resulting in the production of CuO and MgO. The components identified in CuO and MgO particles solutions are listed in Table 4.4

Table 4.4 The component identified in the formulations based on the synthesis methods.

Synthesis process	Formulation	Component identified	Mass (%)
Sol-gel	Cu + PRE	CuO	0.620
		P	0.0025
		K	0.0180
		H_2O	99.4
	Mg + PRE	MgO	0.236
		P	0.014
		K	0.0217
		Ca	0.0034
		H_2O	99.7
Green synthesis	Cu + PRE	CuO	0.685
		P	0.0046
		S	0.0017
		K	0.0201
		H2O	99.4
	Mg + PRE	MgO	0.216
		P	0.0018
		K	0.0188
		Fe	0.0019
		S	0.0098
		H_2O	99.7

Among these, H_2O was the major component found in all particle formulations, as it served as a solvent during both synthesis processes. Other elements identified included P, K, Ca, S, and Fe. However, these observed components are not considered as impurities or contaminants, as PRE was intentionally added as antibacterial agent during the synthesis process, and these components are naturally present in pomegranate rind (Ammulu et al., 2021). Additionally, only trace amounts of these elements were detected in CuO and MgO particles, making them insignificant in affecting the identity of both CuO and MgO particle solutions, respectively (Hirphaye et al., 2023).

4.5 Synthesis Mechanism

The synthesis of CuO particles and MgO particles through sol-gel method involves the use of Cu $(NO_3)_2$ and $Mg(NO_3)_2$ as precursors, PRE as antibacterial agent, water as the solvent, citric acid as the catalyst, and ethylene glycol and diethyl ether as reagents. The process includes hydrolysis and condensation of the sol-gel precursor, as well as condensation of the hydrolysed product with PRE. Initially, the addition of citric acid to the metal nitrate solution promotes the hydrolysis reactions of the metal ions (Muthuvel et al., 2020). The coordination of ethylene glycol with the metal ions prevents hydrolysis, and the condensation reaction begins with the addition of diethyl ether to form the gel (Rex & dos Santos, 2023). The addition of PRE, which acts as reductant and stabilizer for the metal ions, forms stable complexes in the sol and reduces them to metal oxides (Bao et al., 2021). The proposed mechanisms for the production of metal oxide using MgO as an example is shown in Figure 4.1. Meanwhile, the following figures (Figure 4.2, Figure 4.3, and Figure 4.4) shown the interactions of MgO with different types of fabrics.

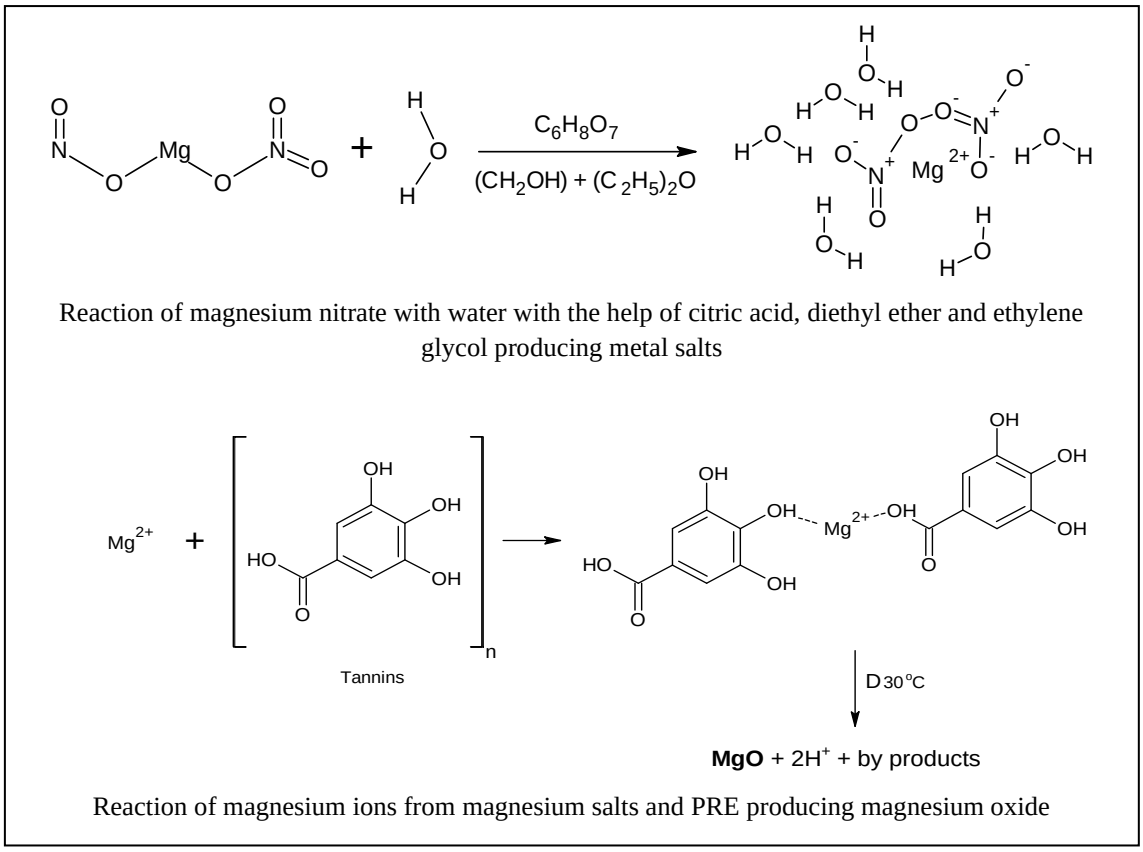


Figure 4.1 Possible reaction for the production of MgO particles.

Source: Correia et al. (2023); Fuku et al. (2020); Kaur et al. (2022).

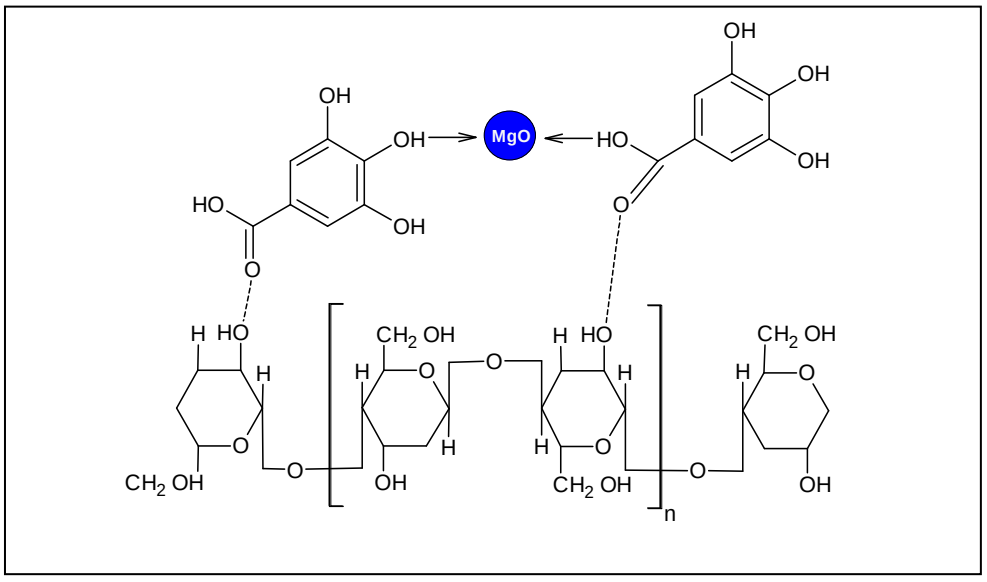


Figure 4.2 The interaction of MgO-PRE with cotton fabric.

Source: Granados et al. (2021)

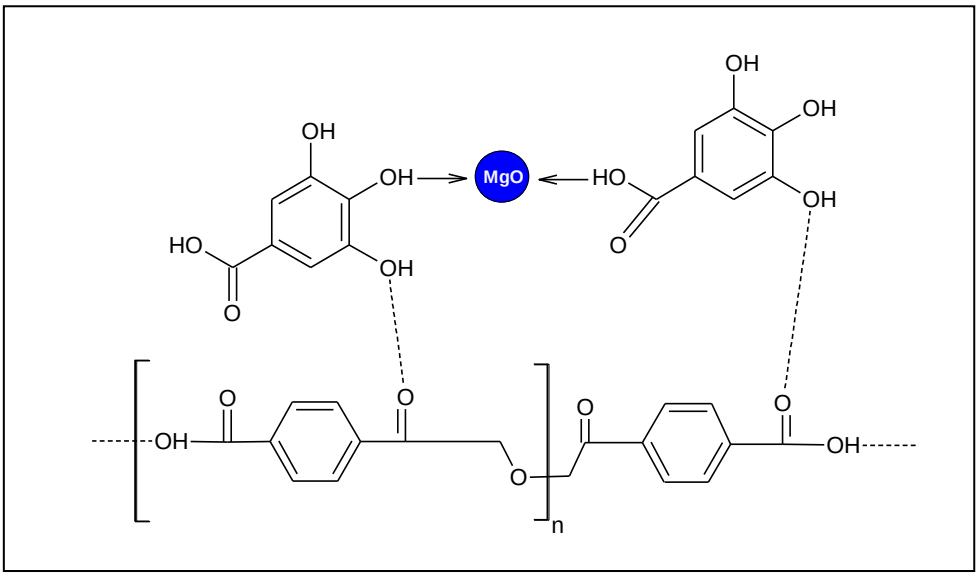


Figure 4.3 The interaction of MgO-PRE with polyester fabric.

Source: Pasichnyk et al. (2022)

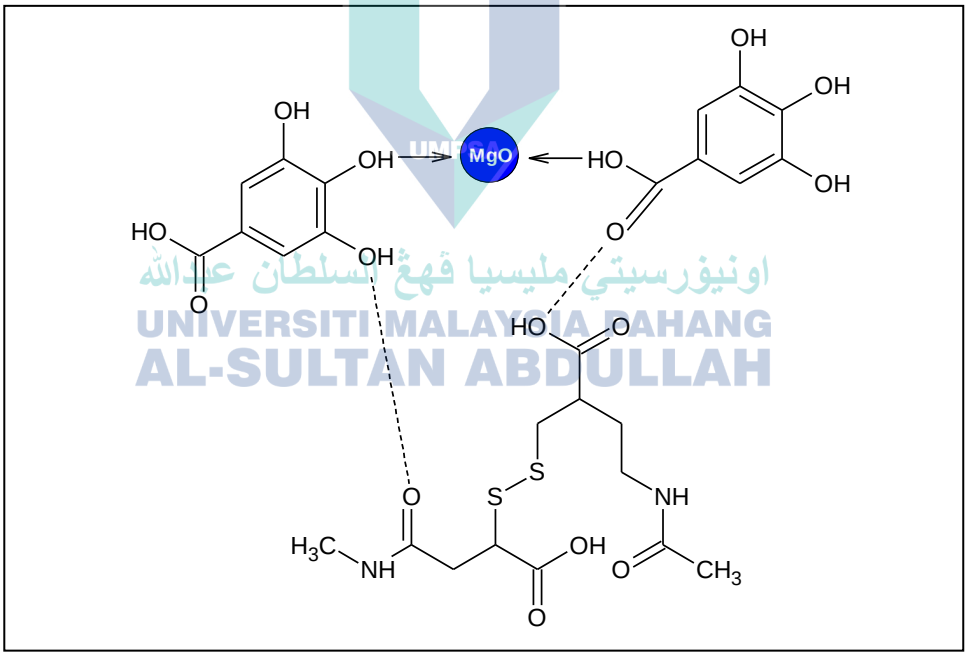


Figure 4.4 The interaction of MgO-PRE with blend wool fabric.

Source: Guo et al. (2022)

Green synthesis is a straightforward process that involves the use of plant extracts for the preparation of metal or metal oxide particles. The active compounds present in the PRE, such as phenolic acids, flavonoids, tannins and saponins, that act as antioxidants are responsible for the green synthesis process of CuO particles and MgO particles (Altemimi et al., 2017; Xu et al., 2017). These compounds also act as stabilizer to prevent the agglomeration and crystal growth (Singh et al., 2018). The antioxidant compounds donate electrons, leading to the reduction of copper salts and magnesium salts. The addition of PRE extract to copper and magnesium solutions results in observable colour changes, indicative of the formation of CuO and MgO particles, respectively. The emergence of a brownish-black colour suggests the formation of CuO particles (Vasantharaj et al., 2019), while a light brownish-orange colour signifies the formation of MgO particles (Ammulu et al., 2021) (Figure 4.5). While these studies provide initial validation, further supporting studies are crucial to confirm these observations and to understand the underlying mechanisms of particle formation in greater detail.

The synthesis methods and precursor types used resulted in variations of colour to the particle solutions. The MgO particle solutions synthesized via both sol-gel and green synthesis methods exhibited an orange-brownish colour, with the sol-gel synthesis produced a darker tone compared to the green synthesis method. On the other hand, the CuO particles synthesized via sol-gel method displayed a green-brownish colour, which appeared lighter than those produced via the green synthesis method. According to Khurana & Jaggi (2021), the observed colour variations in particle solutions can be attributed to localized surface plasmon resonance (LSPR), which is influenced by factors such as particle size, shape, and composition. Specifically, the LSPR frequency is affected by the dielectric permittivity of the particles and the medium surrounding them (D'Ambrosio et al., 2022). Smaller particles typically exhibit a blue shift, indicating a higher frequency, whereas larger particles demonstrate a red shift, corresponding to a lower frequency (Ringe et al., 2009). Additionally, particles with sharp edges and corners can intensify electric fields, thereby impacting the resonance frequency (Lee et al., 2023). Furthermore, the composition of the particles plays a role in modulating the conductivity of electrons in response to the

electromagnetic field, thus altering the LSPR frequency (Hamamoto & Yagyu, 2023). Therefore, the colour variation in particle solutions may depend on the synthesis method, and the composition of the particles.

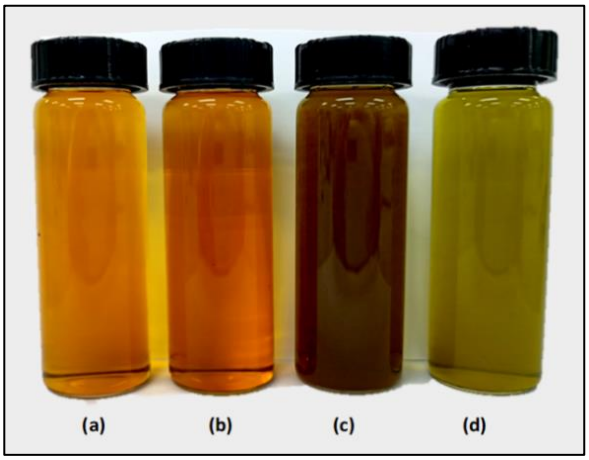


Figure 4.5 The colour of MgO particles synthesized via (a) green synthesis and (b) sol-gel methods; CuO particles synthesized via (c) green synthesis and (d) sol-gel methods.

4.6 Particles Size Analysis

The particles sizes of CuO and MgO synthesized using both the sol-gel and green synthesis methods were determined to compare the effect of synthesis methods and precursor types on the size of particles and their effectiveness in fabrics coating. The sizes of the produced particles are listed in Table 4.5, and the particle size distribution is provided in Appendix D. The results showed that both synthesis methods and precursor types slightly affect the particles size. The sol-gel method produced smaller particles than green synthesis method. Specifically, the average particle size for CuO particles synthesized via the sol-gel method was 325.9 nm, while for MgO particles, the average size was 317.5 nm. Conversely, CuO and MgO particles synthesized via the green synthesis method exhibited average sizes of 374.5 nm and 325 nm, respectively.

The differences in particle sizes between synthesis methods likely stem from distinct reaction mechanisms that affect nucleation, growth, and aggregation of particles (Hachem et al., 2022). Moreover, the choice of precursors influences these processes

during synthesis. In the nucleation phase, the type of precursor determines the rate at which monomers are formed and become available for nucleation (Wen et al., 2021). Once nucleation has commenced, particles begin to grow through the addition of monomers. The growth rate depends on the monomer concentration, which is controlled by the precursor. A precursor that leads to a high concentration of monomers can result in rapid growth, potentially yielding larger particles (Gao et al., 2019). Aggregation may occur during or after growth, and the chemical nature of the precursor can influence the surface properties of the particles, such as charge and hydrophobicity, affecting their tendency to aggregate (Harish et al., 2022). MgO, being more soluble and reactive in water compared to CuO, might limit the growth and aggregation of particles, resulting in the production of smaller particles (Rabea et al., 2023).

Table 4.5 An average particle sizes of CuO and MgO particles based on the synthesis method.

Synthesis method	Solution	Particles size (nm)
Sol-gel	CuO	325.9
	MgO	317.7
Green synthesis	CuO	374.5
	MgO	325

4.7 Appearance of the Fabrics

Coating fabrics with particles can alter their colour appearance due to the small size and large surface area of the particles, which may affect their optical properties (Munir et al., 2022). The absorption, reflection and scattering of light by particles vary depending on their shape, size, and surface modification (Ustin & Jacquemoud, 2020). Based on the observations, the particles coated onto fabrics resulted in variations of colour depending on the fabric types, particles used, and synthesis methods (Figure 4.6). The particles coated onto blend wool fabric contributed to a darker colour compared to cotton and polyester fabrics. There were slight changes in the colour of coated polyester fabrics, but they were not very noticeable. Fabrics coated with particles synthesized using sol-gel method had lighter colours compared to fabrics coated with

particles synthesized using green synthesis method. Cotton, polyester, and blend wool fabrics coated with CuO particles synthesized via the sol-gel method changed from white to light brown-greenish, light green and dark brown-greenish colour, respectively. Meanwhile, CuO particles synthesized using the green synthesis method changed the fabric colour to light brown (cotton and polyester) and dark brown (blend wool). The fabrics coated with MgO particles had lighter colours than fabrics coated with CuO particles. The MgO particles altered the fabric colour to a yellowish-brown for both sol-gel and green synthesis methods. However, the tone of the colour varied between the synthesis methods and fabric types.

The difference in the chemical and physical properties of the fabric materials influences the specific mechanism by which the colour is absorbed (Ali et al., 2023). Blend wool fabric contains protein fibres which can form covalent bonds with CuO particles and reduce them to metallic Cu particles, resulting in a dark brown-greenish (sol-gel) and dark brown (green synthesis) colour on the fabric (Román et al., 2020). According to El-Meligi et al. (2016), MgO particles are stable and resistant to the reduction by blend wool fabric, therefore, they do not form metallic colour. However, in this study, the alteration of the colour in MgO particles coated blend wool fabric occurred possibly due to the presence of bioactive compounds in plant extract which interact with MgO particles and modify their optical properties, giving the fabric a slightly yellowish-brown colour (Venkatachalam et al., 2021).

Cellulose, the main component of cotton fabric, can form hydrogen bonds with CuO particles, resulting in a light brown colour on the fabric coated with CuO particles synthesized via the green synthesis method (Dulta et al., 2022). The reaction of CuO partciles synthesized via the sol-gel method with carbon dioxide and water in the air may occur, resulting in the formation of a light brown-greenish and light greenish patina on cotton and polyester fabric, respectively (Turakhia et al., 2020). The formation of hydrogen bonds between cotton fabric and MgO particles can scatter light, giving the fabric a slightly yellowish-brown colour (Araújo et al., 2022).

Polyester, being a synthetic fabric, has a lower affinity to absorb CuO particles and MgO particles compared to the cotton and wool fabrics (Saade et al., 2021). This is

due to the different surface charges and functional groups of the fabric, which can affect the electrostatic and chemical interactions between polyester fabric and particles, causing significant colour changes in the fabric (Palacios-Mateo et al., 2021; Ketema & Worku, 2020). The difference in colour of the fabrics between the synthesis methods may be attributed to the distribution and shape of the particles. According to Baig et al. (2021), the green synthesis method produces particles with a broader size distribution and irregular shape compared to the sol-gel synthesis, resulting in a darker colour of the fabric due to the increased of light scattering and absorption.

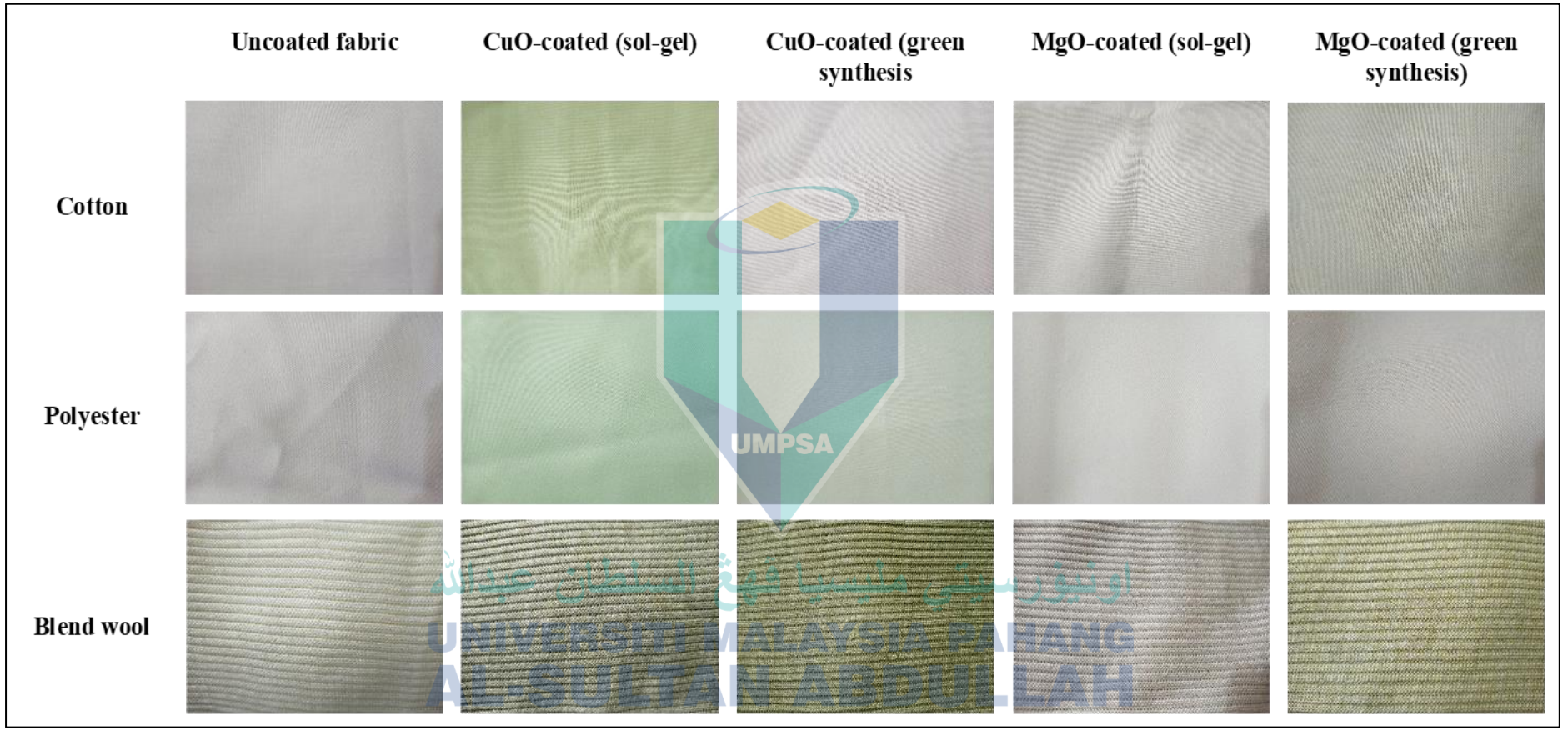


Figure 4.6 The appearance of the uncoated fabric, CuO particles coated fabric and MgO particles coated fabric synthesized using sol-gel and green synthesis method.

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4.8 SEM-EDX Analysis

The surface morphology of the coated and uncoated fabrics and their chemical compositions were studied using a combination of two effective systems called SEM-EDX. The SEM images of the fabric samples at 500× and 5K× magnifications are shown in Figure 4.7, Figure 4.8, and Figure 4.9. Based on the observations, the cotton fabric coated with MgO particles synthesized via sol-gel and green synthesis methods showed uniform and smooth coating surfaces without cracking, respectively. The MgO particles exhibited good adhesion strength to the cotton fabric due to the presence of cellulose, which forms hydrogen bonds with the particles, resulting in homogenous coatings (Permyakova et al., 2022). However, for the cotton fabric coated with CuO particles, small agglomerations of particles were observed. In the case of polyester and blend wool fabric samples, non-homogenous coatings with agglomerated and uneven depositions of amorphous particles were observed. The CuO particles and MgO particles were not fully coated on these fabrics and did not attach well, probably due to low concentration of particles precursor. This limits the available active sites for the particles deposition and weakened the hydrogen bonds formed between the fabric and particles, resulting in the particles agglomeration in certain areas of the fabric (Shaban et al., 2018). pH 4 for particles solution synthesized via sol-gel method, may also contribute to the non-homogenous coatings. According to Alias et al. (2010), the acidic particles solutions may lack sufficient vacant OH^- ions in the sol, allowing for nucleation, growth of particles, and particle formation.

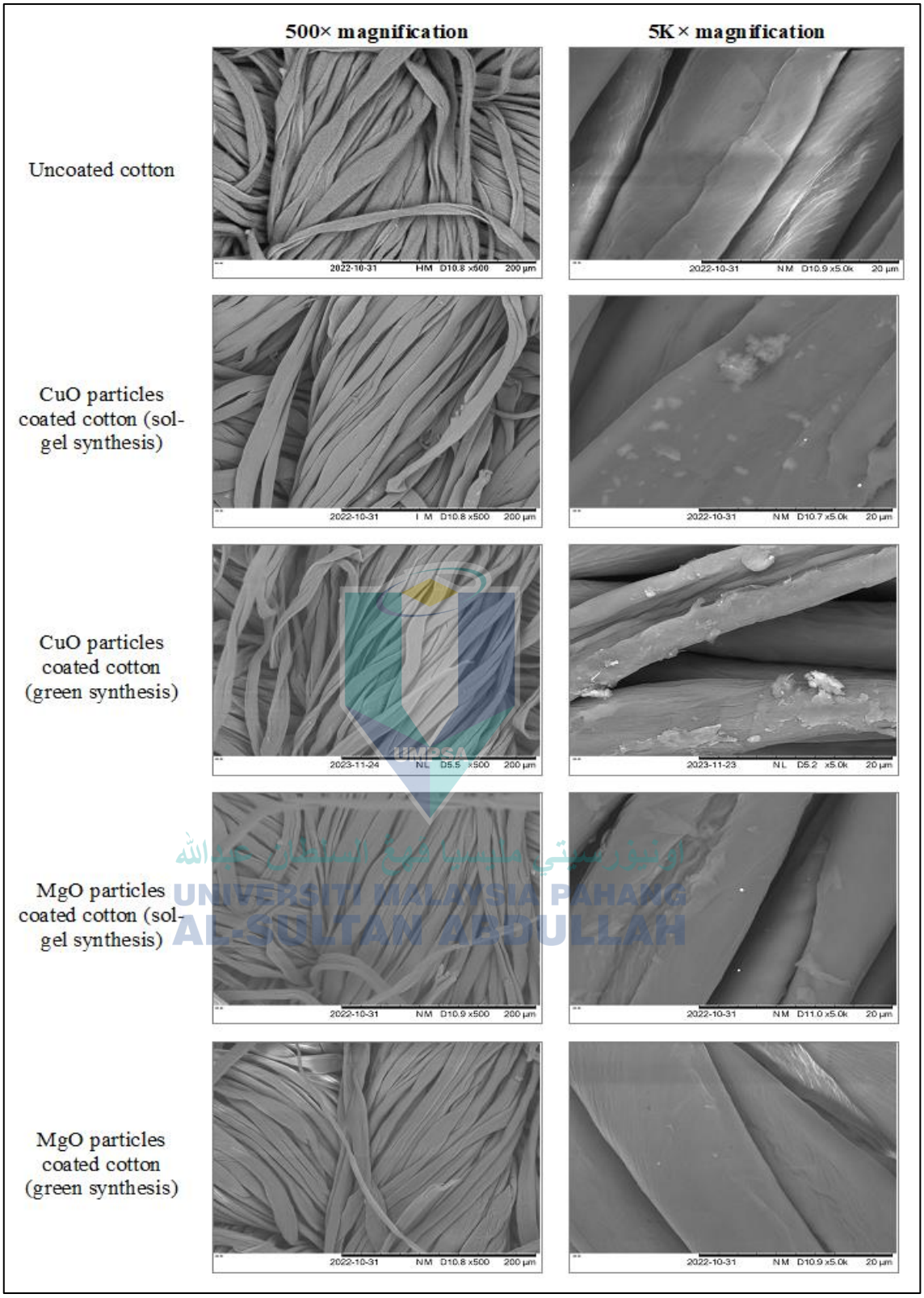

Figure 4.7 SEM images of uncoated and coated cotton fabrics at 500× and 5K× of magnifications.

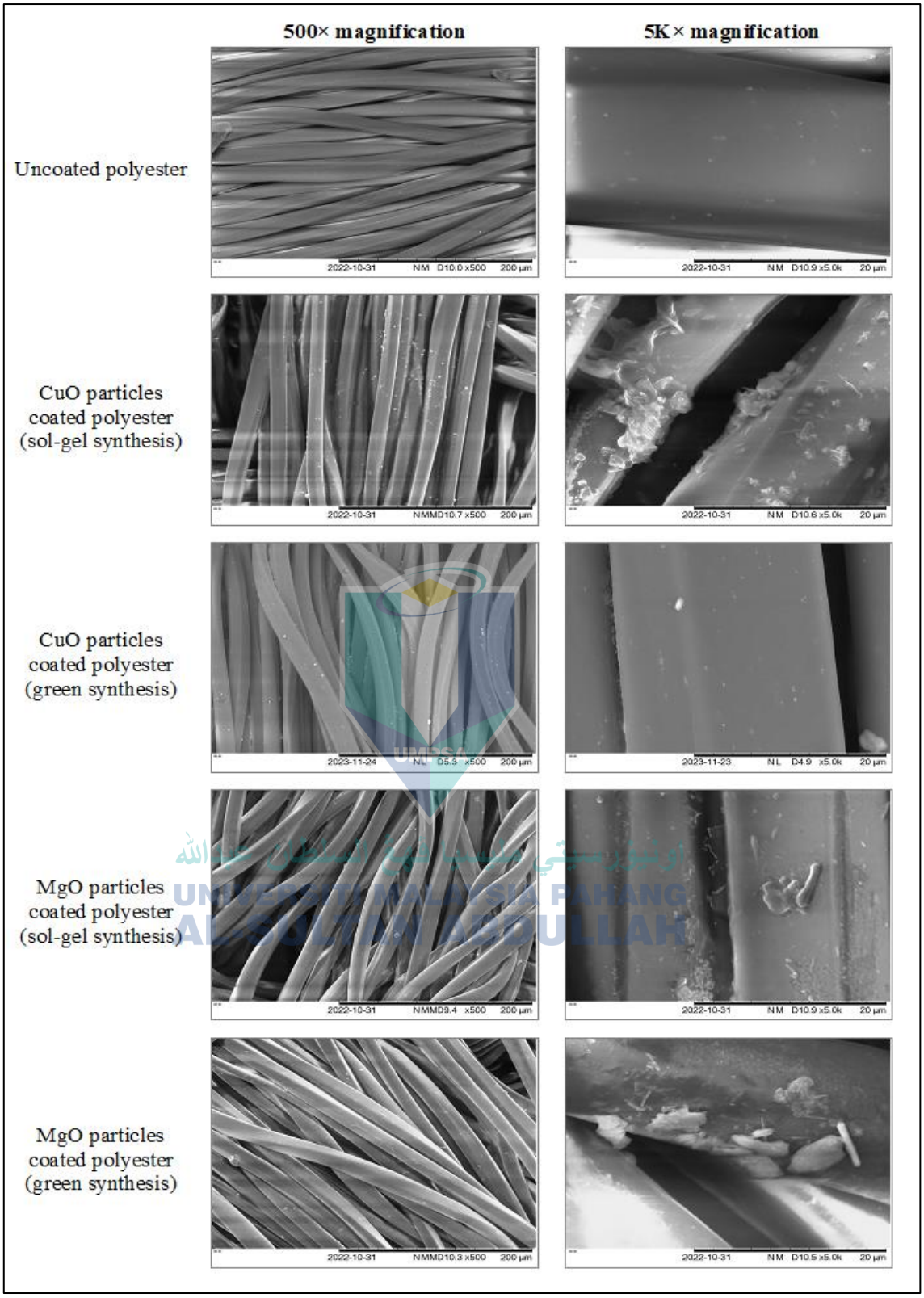

Figure 4.8 SEM images of uncoated and coated polyester fabrics at 500× and 5K× of magnifications.

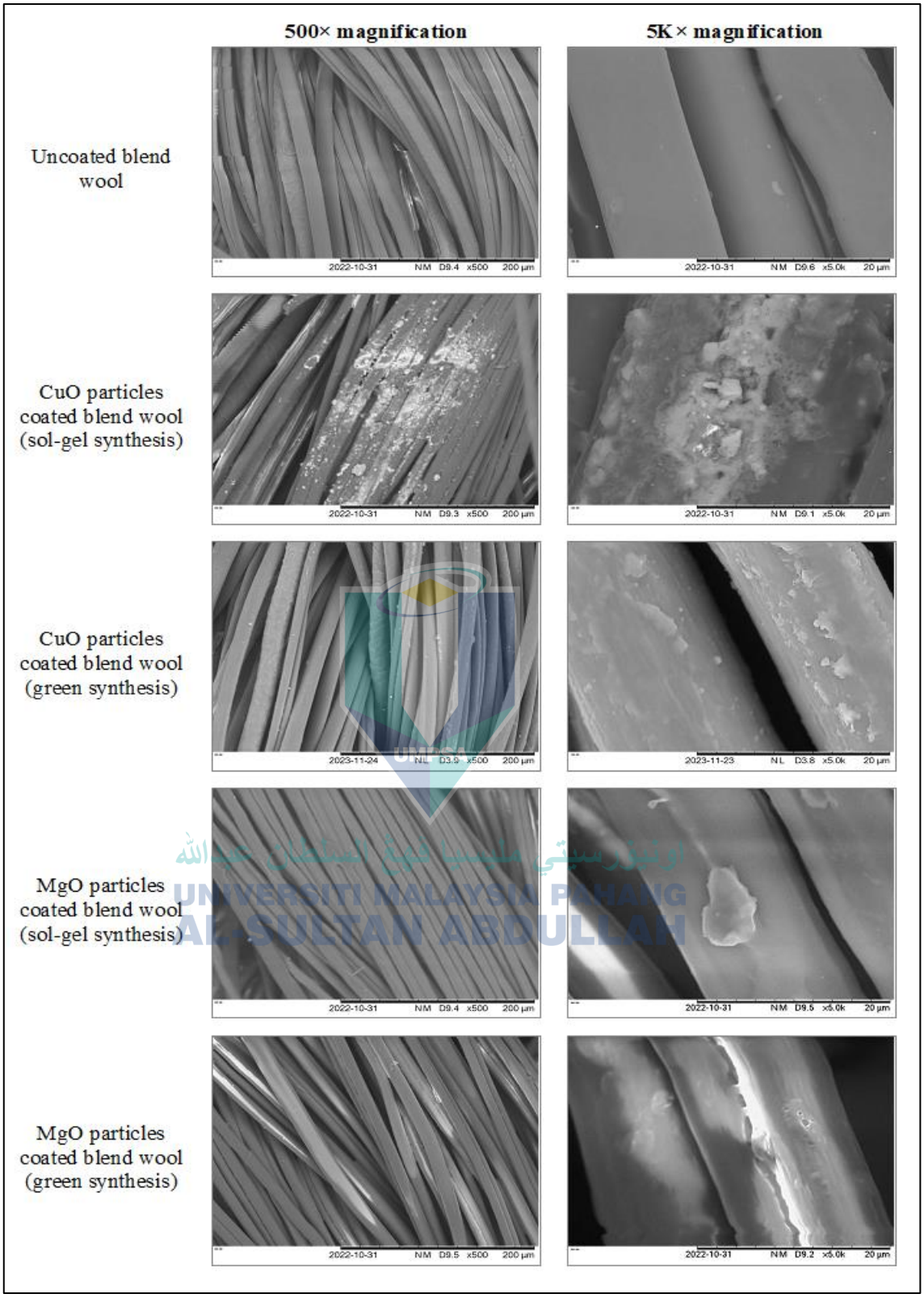

Figure 4.9 SEM images of uncoated and coated blend wool fabrics at 500× and 5K× of magnifications.

The elemental composition of the uncoated and coated cotton, polyester, and blend wool fabrics are shown in Figure 4.10, Figure 4.11, and Figure 4.12, respectively. Based on the EDX spectrum, only carbon and oxygen elements were present in the uncoated cotton and polyester fabrics. The uncoated blend wool fabric contained carbon, oxygen, silicon, and sulfur. The presence of silicon and sulfur elements can be attributed to the anti-pilling acrylic (El Gabry et al., 2021) and wool (Jose et al., 2022), correspondingly. The EDX spectrum confirmed the presence of copper element in all CuO particles coated fabrics and the presence of magnesium element in all MgO particles coated fabrics, proportionately. However, the presence of potassium, which is associated with the PRE, was not found in the coated cotton spectra. Okonkwo, (2019) suggested that potassium, serving as an activator for the reaction between cellulose in cotton fabric and cellulase enzyme in the plant extract, is likely consumed during the coating process, leading to its absence in the EDX spectrum analysis.

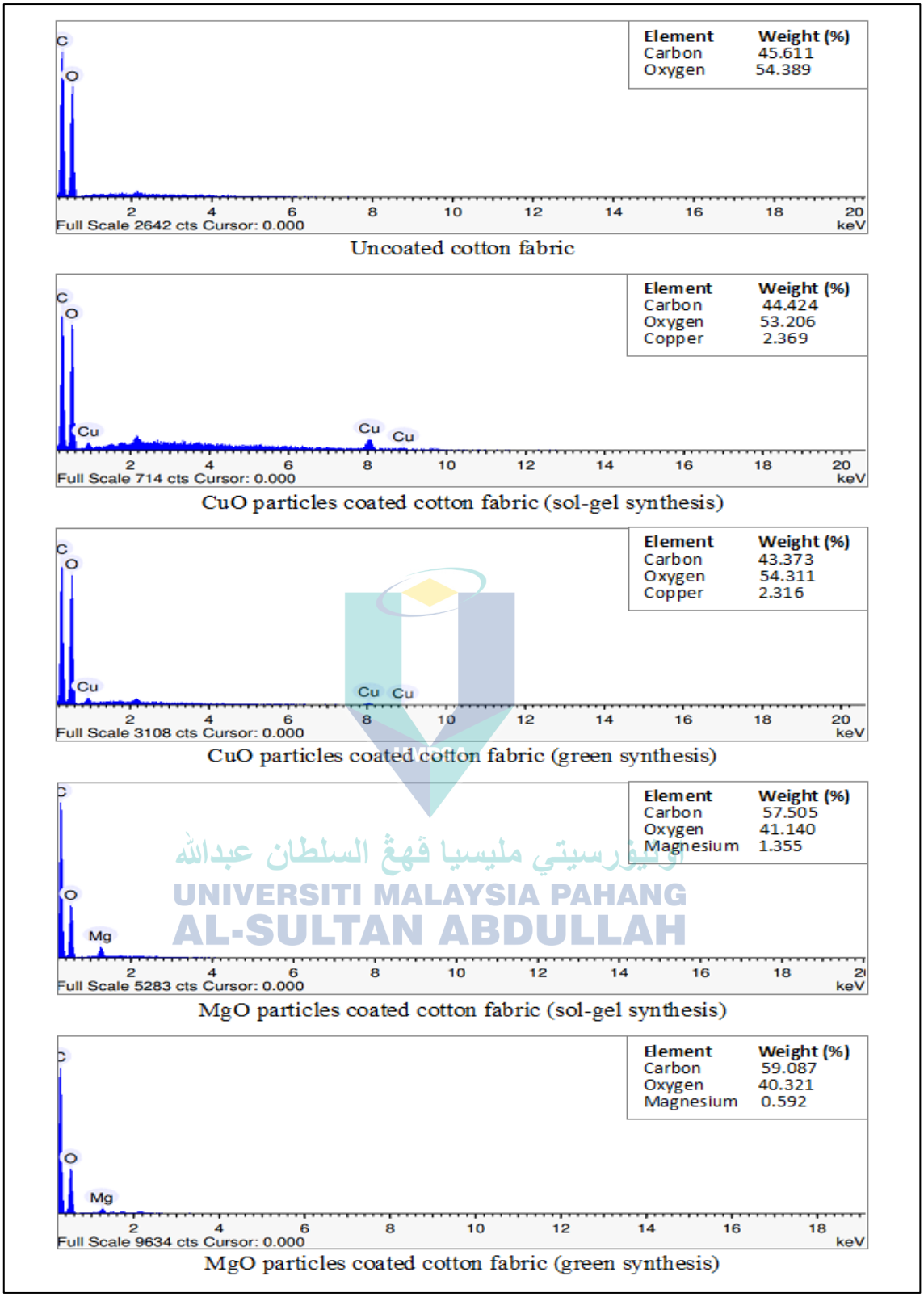

Figure 4.10 EDX spectra of uncoated cotton fabric, CuO and MgO particles coated cotton fabric using sol-gel and green synthesis process.

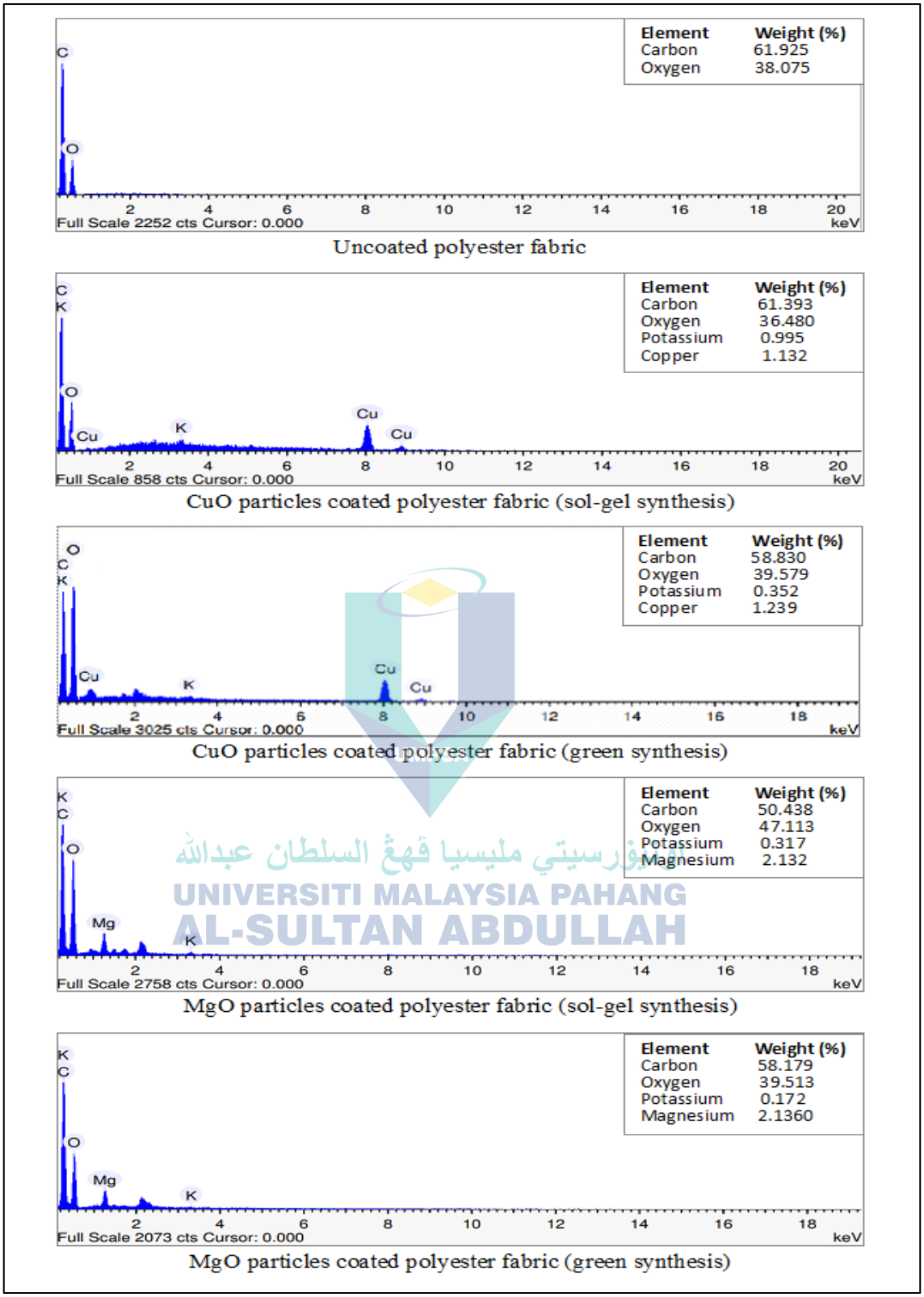

Figure 4.11 EDX spectra of uncoated polyester fabric, CuO MgO particles coated polyester fabric using sol-gel and green synthesis process.

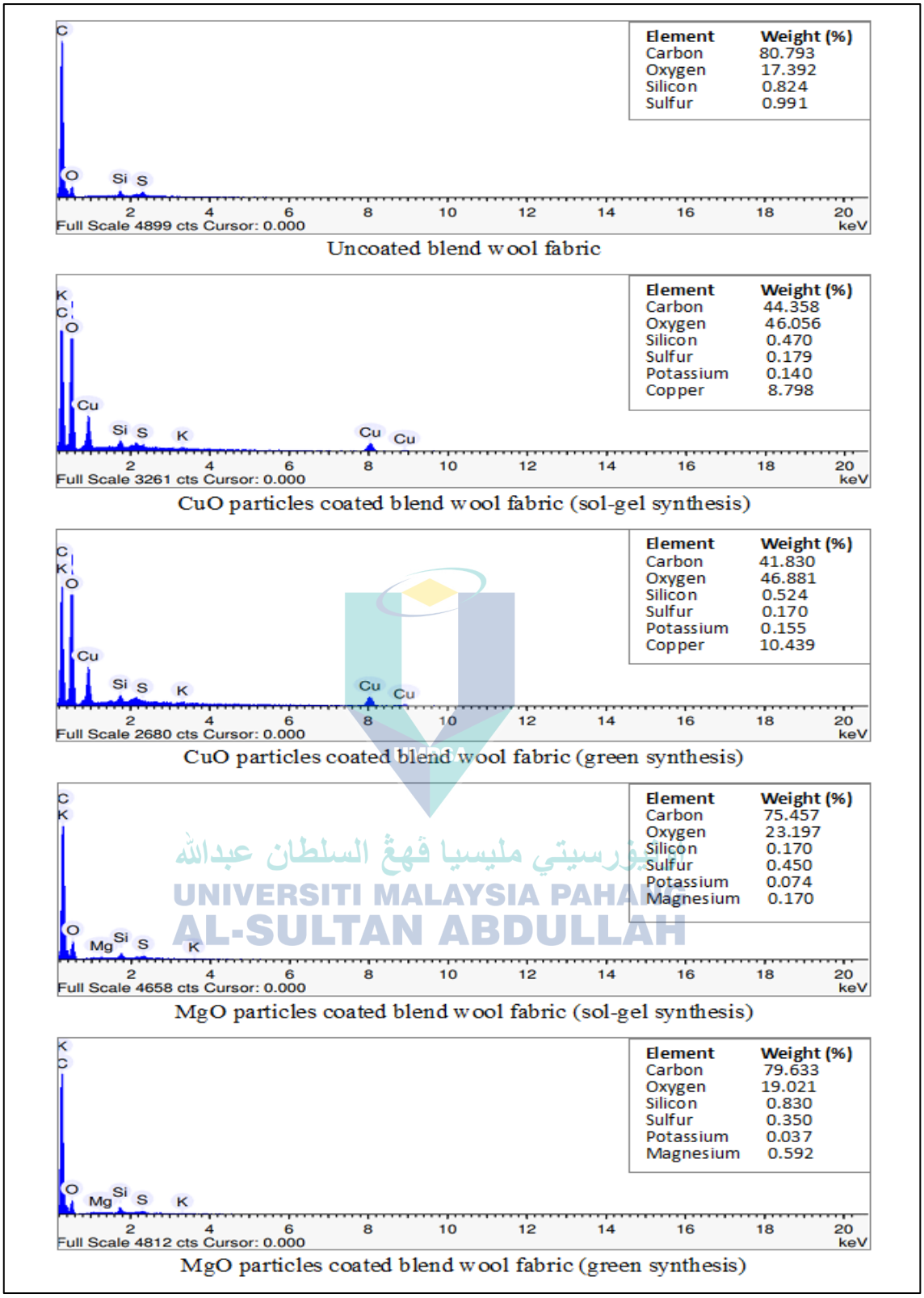

Figure 4.12 EDX spectra of uncoated blend wool fabric, CuO and MgO particles coated blend wool fabric using sol-gel and green synthesis process.

4.9 Antibacterial Activity Test

The CuO and MgO particles synthesized via optimized sol-gel and green synthesis methods were coated onto the cotton, polyester and blend wool fabrics and tested for their antibacterial properties against three species of gram-positive bacteria using the disc diffusion assay. The bacteria used in this study were *B. linens*, *C. acnes* and *S. epidermidis*, which are known to cause body odour as they produce odoriferous compounds when breaking down the amino acids (Lam et al., 2018). The inhibition zone values of the coated fabrics are listed in Table 4.6. Figure 4.13, Figure 4.14, Figure 4.15, and Figure 4.16 showed the antibacterial activities of the CuO and MgO coated fabrics.

Table 4.6 The inhibition zone value (mm) of coated cotton, coated polyester and coated blend wool fabric based on the synthesis process and solution against *B. linens*, *C. acnes*, *S. epidermidis* and antibiotic.

Bacteria Species	Synthesis Process	Formulation	Inhibition zone (mm)			
			Cotton	Polyester	Blend wool	Antibiotic
B. linens	Sol-gel	CuO	3	1	7	10
		MgO	2	1	4	10
	Green synthesis	CuO	1	1	3	10
		MgO	2	3	7	10
C. acnes	Sol-gel	CuO	1	1	2	12
		MgO	2	2	3	12
	Green synthesis	CuO	1	1	1	12
		MgO	2	3	6	12
S. epidermidis	Sol-gel	CuO	2	2	2	17
		MgO	4	4	4.5	17
	Green synthesis	CuO	1.5	2	2	17
		MgO	2	2	3	17

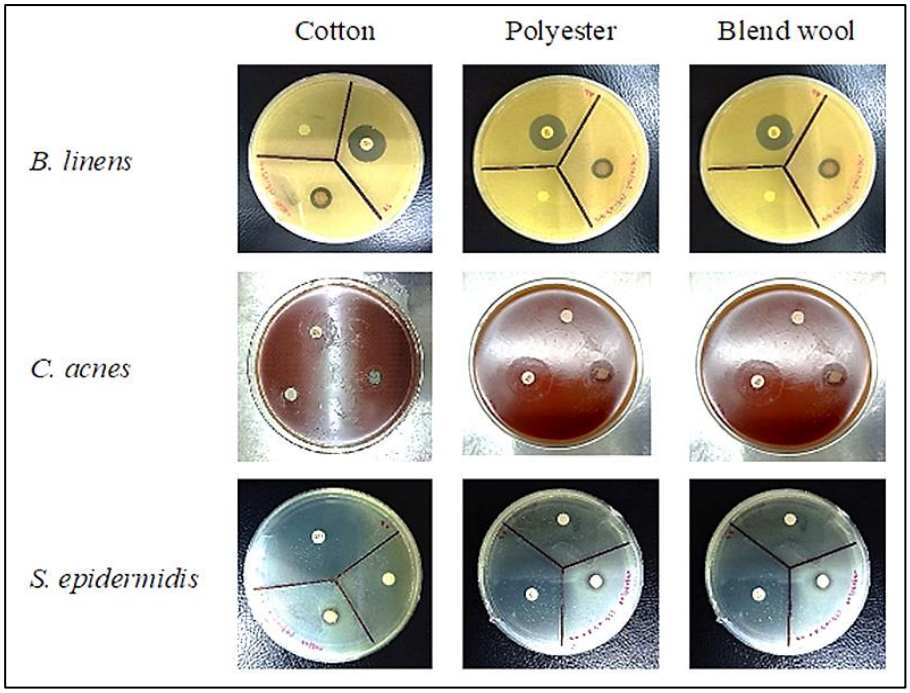

Figure 4.13 The antibacterial activities of the modified CuO particles coated fabrics using sol-gel synthesis against *B. linens*, *C. acnes* and *S. epidermidis*.

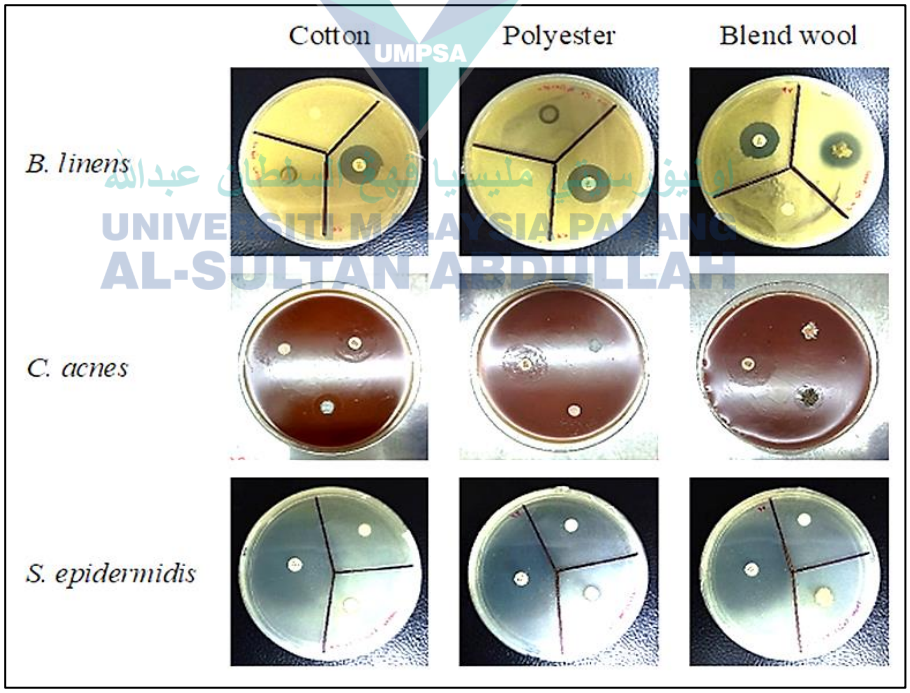

Figure 4.14 The antibacterial activities of the modified CuO particles coated fabrics using green synthesis against *B. linens*, *C. acnes* and *S. epidermidis*.

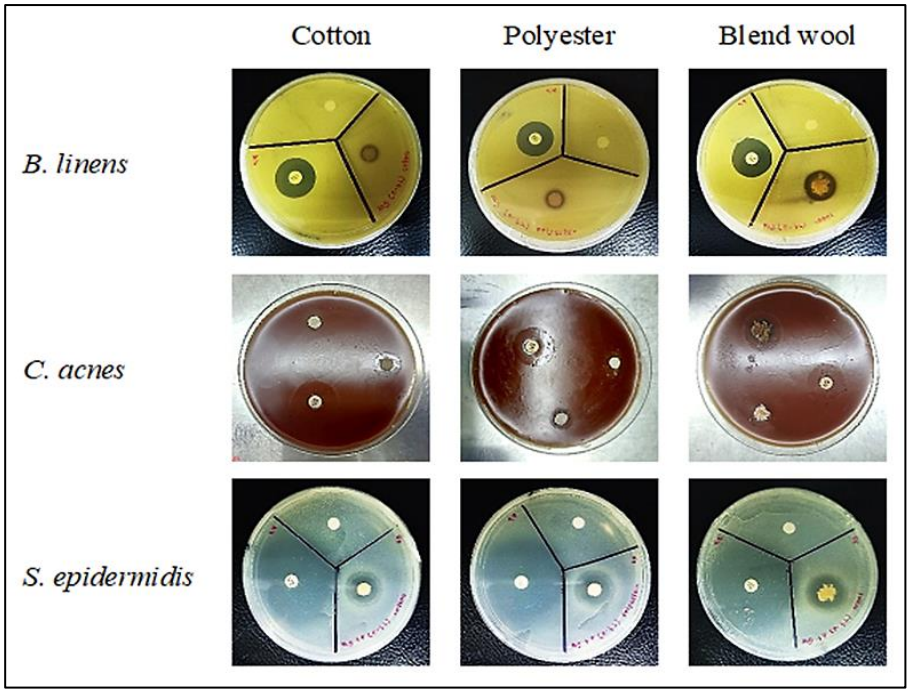

Figure 4.15 The antibacterial activities of the modified MgO particles coated fabrics using sol-gel synthesis against *B. linens*, *C. acnes* and *S. epidermidis*.

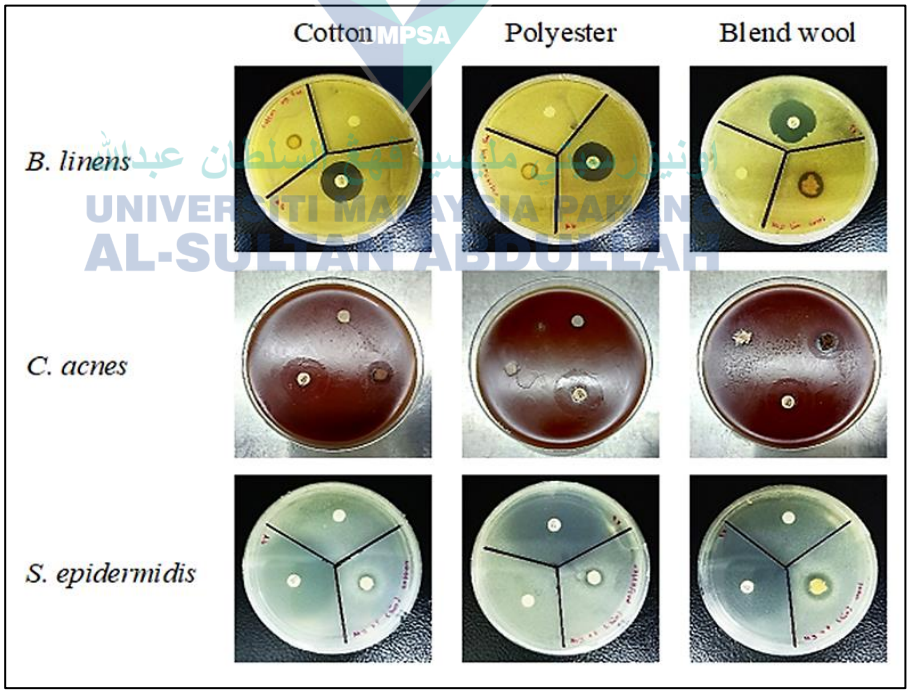

Figure 4.16 The antibacterial activities of the modified MgO particles coated fabrics using green synthesis against *B. linens*, *C. acnes* and *S. epidermidis*.

The CuO- and MgO-coated fabrics demonstrated antibacterial activity against all the tested species of the bacteria. However, the inhibition zones observed for all the coated fabrics were less than those observed for antibiotics. Among the fabric types, the blend wool fabric showed the highest antibacterial activity against all tested bacteria compared to cotton and polyester fabrics. This is probably due to the greater thickness of the blend wool fabric which provides more surface area for particles to adhere to, potentially enhancing the antibacterial effect (Ahmed et al., 2020). The maximum inhibition zone of 7 mm was observed for *B. linens* on blend wool fabric coated with CuO particles and MgO particles synthesized via sol-gel and green synthesis methods, respectively. The MgO particles produced via green synthesis method showed the strongest antibacterial activity against *C. acnes* (6 mm), while the MgO particles produced via sol-gel method exhibited the strongest antibacterial activity against *S. epidermidis* (4.5 mm). The cotton fabric coated with CuO particles synthesized via green synthesis method only showed 1 mm of inhibition zone against *B. linens.* Both cotton and polyester fabrics coated with CuO particles synthesized via sol-gel and green synthesis methods showed similar trends of antibacterial activity against *C. acnes*, with an inhibition zone of 1 mm. The minimum inhibition zone of *S. epidermidis* was observed on cotton fabric coated with CuO particles (green synthesis method), with an inhibition zone of 1.5 mm.

Based on the observations, the fabric types were found to affect the antibacterial activities of the particles. According to Abou Elmaaty et al. (2021), woven fabrics offer a larger surface area for particles penetration compared to knitted fabrics due to the pore size between the yarns. However, in this study, the knitted blend wool fabric showed higher antibacterial activity towards all tested species compared to the woven fabrics (cotton and polyester), probably due to the thickness of the fabric allowing for greater particles penetration into the yarn fibers (Shaker et al., 2022; Mohd Yusop et al., 2023). The presence of the phenylene group in the polymeric chain of the polyester fabric makes it hydrophobic, resulting in less penetration of particles (Gobikannan et al., 2023). Cotton fabric, on the other hand, contains cellulose which aids in moisture absorption. Nevertheless, the absence of potassium element from PRE in the particles, which is consumed during the coating process (Okonkwo, 2019), may contribute to the

lower antibacterial properties of cotton fabric compared to blend wool fabric. The antibacterial activities of CuO particles and MgO particles correlated with the synthesis method of the particles and the species of bacteria. The synthesis methods affected the size, surface area, and stability of particles, resulting in variations of the inhibition zone of the bacteria (Ferreira et al., 2022). The agglomeration of the particles as seen in SEM images due to differences in synthesis methods, may reduce the surface area and consequently affect the antibacterial activity (Valenti & Giacomelli, 2017). The differences in antibacterial activity of the particles against different bacteria species are likely due to variations in the bacteria cell structure, which influences the attachment and interaction between the particles and the bacterial cell membrane (Phan et al., 2020).

The interactions between the bacterial surface and particles primarily occur through electrostatic interactions, mediated by the neutralization of surface charge on the bacteria membrane (Behera et al., 2019; Arakha et al., 2015). This surface charge neutralization is a common biological process that can result in antibacterial activity (Vejzovic et al., 2022). The gram-positive bacteria have a thick layer of peptidoglycan (Zhang et al., 2023). The teichoic acid in the peptidoglycan layer and lipoteichoic acid in the bacterial membrane contribute to the negatively charged surface of the cell (Mendes et al., 2022). The interaction between positively charged MgO particles and CuO particles with the negatively charged bacterial cell wall alters the membrane permeability and damages the cell surface due to the strong bonds formed between the particles and membranes (Raj et al., 2021; Yusof et al., 2019). According to Abebe et al. (2020), the inhibitory action of metal oxide depends on the size of particles. The particle size analysis reveals that the MgO particles are smaller than the CuO particles, which gives them an advantage in antibacterial activity for most of the samples. Smaller particles have better electrostatic interactions due to the diffusion of metal ions generated by the movement of hydrogen ions across the cell membrane of the bacteria. The electrostatic interactions between CuO particles and MgO particles with the bacterial cell wall caused damages to the cell membrane and allow the particles to penetrate into the cells. This generates oxidative stress and leads to the loss of cell functionality due to the leakage of intracellular components (Alnehia et al., 2022;

Ahmed et al., 2020; Demissie et al., 2020). The oxidative stress also inhibits the antioxidant defence mechanisms of bacteria against reactive oxygen species (ROS) by oxidizing glutathione (Soheili et al., 2022). As a result, CuO particles and MgO particles can interact with cellular structures such as proteins and DNA, directly disrupting cell function (Rajagopalachar et al., 2022). The penetration of Cu^{2+} ions and Mg^{2+} ions into the cell can cause the death of bacteria by inhibiting enzyme functions, metabolisms, and transportation.

In addition, pomegranate rind extract contains phenolic compounds, flavonoids, and tannins, which contribute to its broad spectrum of antimicrobial effects against several highly pathogenic and drug-resistant bacteria strains (Chen et al., 2020). The phenolic compounds increase bacteria permeability (Alnehia et al., 2022) by inducing hyper-acidification at the plasma membrane interphase through phenolic acid dissociation (Chen et al., 2020). The sensitivity of bacteria to phenolic acids varies, with gram-positive bacteria being more susceptible due to the absence of an outer membrane which allows easier diffusion of phenolic acids through the cell wall (Lobiuc et al., 2023). The phenolic compounds exhibit anti-infective activity by forming complexes with proteins through hydrogen bonding, or covalent linkages (Jiang et al., 2022). The leakage of nucleic acids, proteins and inorganic ions from bacteria cells occurs due to the presence of membrane-active properties in phenolic acids. Flavonoids disrupt bacterial membranes by penetrating the lipid bilayer, reducing membrane fluidity, causing cell lysis through the formation of hydrogen bonds with lipids (Renzetti et al., 2020; Kumar & Pandey, 2013). Flavonoids also inhibit biofilm formation by reducing active nutrient uptake and finally causing the death of bacteria cells through membrane diffusion (Cushnie & Lamb, 2011). Tannins inhibit bacteria by chelating iron, which is essential for bacterial growth. The deficiency of iron leads to a decrease in the activity of metalloenzymes and inhibition of oxidative phosphorylation (Lobiuc et al., 2023). Tannins can also inactive bacterial enzymes such as protease, phospholipase, urease, neuraminidase, and collagenase and bind them to the peptidoglycan layer of cell wall, making the bacteria more susceptible to osmotic lysis (Tintino et al., 2017).

4.10 Tensile Strength Test

The effect of tongue tear strength and breaking load on the uncoated fabrics, CuO particles coated fabrics, and MgO particles coated fabrics synthesized using sol-gel and green synthesis method are shown in Table 4.7. The applied coating had both positive and negative effects on the coated fabrics, depending on the type of fabrics and synthesis methods. The CuO particles coated on cotton fabric and synthesized via the green synthesis method showed the highest percentage increment of tongue tear strength in the warp direction and breaking load in both warp and weft directions. The percentage of tearing strength increment in the warp direction was 33.73%, while for both warp and weft directions of breaking load, the percentage increment was 13.59% and 10.67%, respectively. However, negative results were obtained for all coated polyester fabrics, as there was a decrement of tongue tear strength and breaking load in both warp and weft directions. Among the coated polyester fabrics, the polyester fabric coated with CuO particles and synthesized via sol-gel method showed the highest percentage of decrement. The coating resulted in a 36.42% reduction in tongue tear strength of warp direction and a 28.27% reduction of weft direction. Meanwhile, the percentage decrease in breaking load was 6.16% for warp direction and 5.75% for weft direction. As for the coated blend wool fabric, positive results were observed only in the weft direction, with a 7.63% increase of breaking load for the fabric coated with MgO particles synthesized via the green synthesis method.

The study revealed that the fabric types, along with the synthesis methods, greatly affected the fabric's strength due to the reaction with acidic particles solutions. The use of citric acid in the sol-gel synthesis could contribute to the formation of more cross-linking of acid molecules with the fibre (Ji et al., 2016). The small size of citric acid molecules could penetrate deeply into the fibre (Dheyab et al., 2020), causing damage to the yarn fibres and consequently reducing the fabric strength. The high affinity of acid to blend wool fibre resulted in a decrement of fabric strength. The degradation of polyester fibre probably occurred due to the high affinity of acid to the fibre, resulting in the breakdown of ester bonds (Woodard & Grunlan, 2018). Cotton fabric, composed of cellulose, exhibited resistance to the yarn fibres (Lv et al., 2020), contributing to a slight increase in tensile strength in most of the coated cotton fabrics.

The acid treatment probably had a low affinity to cellulose, thus causing no damage to the glycoside bond (Ahmad et al., 2012). Furthermore, the adhesion of particles restricted yarn movement upon tearing, resulting in the increment of fabric strength (Aslam et al., 2019). In addition, the physical morphology of the yarn played a role in the differences of fabric strength between the warp and weft directions. According to Eltahan, (2018), twisted yarn could improve the strength and elasticity of the fabric, making it harder to break or stretch. In the case of cotton fabric, the weft direction had lower fabric density, allowing for the absorption of a higher number of particles compared to the warp direction and consequently causing the decrement in fabric strength. This is in contrast with polyester and blend wool fabrics as both fabrics have low fabric density in the warp direction than in the weft direction.

Table 4.7 The tongue tear strength and breaking load of the uncoated and coated cotton, polyester, and blend wool fabrics with different particles solution.

Fabric	Process	Solution	Tongue tear strength (N)		Breaking load (N)	
			Warp	Weft	Warp	Weft
Uncoated cotton			15.83 ± 1.852	15.58 ± 2.624	743 ± 9. 626	355.1 ± 10.764
Coated cotton	Sol-gel	CuO	14.5 ± 0.353	7.75 ± 0.353	747 ± 9.741	330.3 ± 27.118
		MgO	17.0 ± 0.353	9.5 ± 0.353	752 ± 17.913	367.3 ± 8.543
	Green synthesis	CuO	21.17 ± 0.824	8.83 ± 2.201	844 ± 18.457	393 ± 14.291
		MgO	16.67 ± 1.328	11.08 ± 0.471	720 ± 15.839	339.3 ± 12.221
Uncoated Polyester			73.8 ± 3.982	100.8 ± 0.000	1268 ± 3.091	1685 ± 10.498
Coated Polyester	Sol-gel	CuO	46.92 ± 0.824	72.3 ± 0.707	1190 ± 8.379	1588 ± 24.385
		MgO	55.00 ± 1.429	79.8 ± 2.160	1224 ± 8.164	1633 ± 20.677
	Green synthesis	CuO	57.8 ± 0.707	91.1 ± 2.094	1199 ± 14.522	1615 ± 3.399
		MgO	60.8 ± 0.736	90.4 ± 0.849	1211 ± 14.522	1578 ± 16.573
Uncoated Blend Wool			N/A	N/A	366.7 ± 67.633	120.5 ± 3.001
Coated Blend Wool	Sol-gel	CuO	N/A	N/A	236.5 ± 23.700	91.3 ± 6.789
		MgO	N/A	N/A	238.4 ± 6.086	107.8 ± 5.493

Table 4.7 Continued

Fabric	Process	Solution	Tongue tear strength (N)		Breaking load (N)	
			Warp	Weft	Warp	Weft
	Green synthesis	CuO	N/A	N/A	293.9 ± 15. 596	119.8 ± 1.761
		MgO	N/A	N/A	258.1 ± 21.559	129.7 ± 3.456

Notes: Values (mean ± standard deviation) are significantly different at $P < 0.05$ based on LSD Multiple Range Test.

N/A indicates the breaking load test was not conducted.

4.11 Air Permeability Test

The air permeability is an important property to the fabric as it can affect the breathability and comfort of the wearer (Agrawal et al., 2019). Additionally, it can provide protection to the wearer by facilitating the evaporation of sweat, removal of heat from the body, acting as an insulator to keep body warm during cold or windy conditions, and serving as a protective layer to reduce the impact or penetration of external forces and substances (Mandal et al., 2022; Shen et al., 2020). There are a few factors that influence the air permeability of a fabric, such as fabric density, warp and weft linear density, weaving structure, and fibre content (Buzaite & Mikucioniene, 2022). In this study, it was found that applying CuO particles synthesized via the sol-gel and green synthesis methods to polyester fabrics has significantly increased their air permeability by 12.01% and 6.73%, respectively (Table 4.8). The MgO particles synthesized via sol-gel method showed a slight increase in air permeability (~0.48%), while a slight reduction (1.92%) was observed for MgO particles synthesized via green synthesis method. On the other hand, the coating of CuO particles and MgO particles on cotton and blend wool fabrics resulted in a reduction of the air permeability, possibly due to the blockage of gaps structures (Nazarov & Dedov, 2020). The results obtained for the coated polyester and blend wool fabrics with MgO particles synthesized via green synthesis and sol-gel methods, respectively, did not align with the tensile strength results, probably due to the fabric density variations. The type, concentration, synthesis method of coating materials, coating method, fabric structure, and properties can all influence the fabric density (Jhanji et al., 2015).

The thickness of the fabric also plays a role in the air permeability of the fabric (Ma et al., 2020). Based on the results, the application of MgO particles did not significantly affect the thickness of cotton fabric, as the coating was thin, uniform, and well-adhered to the fabric surface, as observed in the SEM image. However, there were differences in thickness between the warp and weft directions of cotton fabric coated with CuO particles, probably due to small agglomeration of particles. The filling of spaces between yarn fibres with particles materials resulted in decreased air permeability in all coated cotton fabrics (Shahid et al., 2021). As shown in Table 4.8, the thickness of the polyester fabric, both coated and uncoated, showed no difference

due to the hydrophobic nature of the fabric, which limits the particles penetration (Gobikannan et al., 2023). Nevertheless, the acidic particles solution caused degradation of the fibre, hence affecting the air permeability of the polyester fabric. The decrease in thickness was observed in all coated blend wool fabrics, likely due to the wool fibre degradation, resulting in increased porosity of the fibre (Liu & Lv, 2022). However, the agglomerations and uneven depositions of particles, as observed in the SEM image, blocked the loop structure of the fabric, and consequently reduced the air permeability of blend wool fabric.

Table 4.8 The thickness and air permeability of the coated and uncoated cotton, polyester, and blend wool fabrics with different particles solution.

Fabric	Process	Solution	Thickness (mm)		Air Permeability (cfm)
			Warp	Weft	
Uncoated cotton			0.26 ± 0.00	0.26 ± 0.00	40.4 ± 2.612
Cotton	Sol-gel	CuO	0.27 ± 0.00	0.27 ± 0.00	25.5 ± 1.411
		MgO	0.26 ± 0.00	0.26 ± 0.00	23.1 ± 1.506
	Green	CuO	0.27 ± 0.00	0.26 ± 0.00	24.5 ± 1.808
	synthesis	MgO	0.26 ± 0.00	0.26 ± 0.00	37.9 ± 1.408
Uncoated Polyester			0.35 ± 0.00	0.35 ± 0.00	20.8 ± 0.679
Polyester	Sol-gel	CuO	0.35 ± 0.00	0.35 ± 0.00	23.3 ± 1.240
		MgO	0.35 ± 0.00	0.35 ± 0.00	20.9 ± 0.440
	Green	CuO	0.35 ± 0.00	0.35 ± 0.00	22.2 ± 0.458
	synthesis	MgO	0.35 ± 0.00	0.35 ± 0.00	20.4 ± 0.839
Uncoated Blend Wool			1.32 ± 0.00	1.30 ± 0.00	522.8 ± 9.520
Blend Wool	Sol-gel	CuO	1.26 ± 0.00	1.24 ± 0.00	502.6 ± 7.761
		MgO	1.26 ± 0.00	1.26 ± 0.00	509.2 ± 10.186
	Green	CuO	1.26 ± 0.00	1.24 ± 0.00	495.8 ± 18.712
	synthesis	MgO	1.26 ± 0.00	1.24 ± 0.00	507 ± 7.924

Notes: Values (mean + standard deviation) are significantly different at $P < 0.05$ based on LSD Multiple Range Test.

4.12 Durability Test of the Coated Fabrics

Durability testing is important in the development of any product in order to meet the industry standards. This test evaluates the quality, reliability and fitness of the product and indirectly identifies any weaknesses. It allows for product improvement to enhance customer satisfaction and retention. In this study, the durability of the coated cotton, coated polyester, and coated blend wool fabrics against bacteria after several washing cycles was determined. The antibacterial activities of the coated fabrics after one to five washing cycles are presented in Table 4.9. The results showed that the fabrics coated with CuO particles and MgO particles synthesized using the sol-gel method exhibited antibacterial properties for up to three washing cycles. However, the fabrics coated with CuO particles and MgO particles synthesized via the green synthesis method did not show antibacterial properties even after one time washing.

Based on the results, the synthesis method of the particles was found to have a significant effect on the washing durability of the coated fabrics. The particles synthesized using the sol-gel method exhibited better adhesion to the fabric after the washing process compared to the particles synthesized via green synthesis method. This can be attributed to the presence of ethylene glycol in sol-gel synthesis, which acts as a structure-directing agent (SDA) or crosslinking agent, forming covalent or ester bonds between the particles and the fabric. Ethylene glycol contributes to the polymerization and network formation in metal oxides, resulting in a uniform and stable coating (Mao et al., 2022). Its interaction with metal precursors is key to stabilizing particles during synthesis, which is vital for a consistent antibacterial effect. Additionally, combining ethylene glycol with citric acid enhances particle attachment to fabric surfaces, significantly improving adhesion properties (Gorbunova et al., 2014). Moreover, the particles produced through sol-gel synthesis are more stable when coated onto the fabric surfaces owing to the controlled hydrolysis and condensation reactions of the precursors (Bokov et al., 2021). The weak adhesion of the particles synthesized via green synthesis method is probably due to their irregular shapes and sizes, which affect the surface contact and bonding between the particles and fabric (Baig et al., 2021b). The low stability and uniformity of these particles can also impact the quality of the coating and

the performance of the fabric surface, resulting in low adhesion of particles to the fabric after the washing process (Mohd Yusop & Wan Ismail, 2021; Liu et al., 2020). Furthermore, according to Ahmed et al. (2023), the types and number of particles, such as magnesium or copper, embedded in plant extract may influence the stability of the particles after washing cycles and directly affect their antibacterial properties. The plant extract also affects the grafting efficiency of the fabric composite, thereby influencing the formation of a durable bond between the particles and the fabric surface (Akter et al., 2024). The solvent types used can also distress the washing durability of the fabric, as the dissolution of particles may occur, reducing their adhesion and performance (Zeng et al., 2015).

Table 4.9 The antibacterial activity of the coated cotton, coated polyester and coated blend wool fabric after 1, 2, 3, 4 and 5 times of washing cycles against *B. linens*, *C. acnes*, and *S. epidermidis*.

Bacteria	**Fabric**	**Method**	**Solution**	**Washing cycles**				
				1	**2**	**3**	**4**	**5**
B. linens	Cotton	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
	Polyester	Sol- gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
	Blend wool	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
C. acnes	Cotton	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×

Table 4.9 Continued

Bacteria	Fabric	Method	Solution	Washing cycles				
				1	2	3	4	5
C. acnes	Polyester	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
	Blend wool	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
S. epidermidis	Cotton	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
	Polyester	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×
	Blend wool	Sol-gel	CuO	a	a	a	×	×
			MgO	a	a	a	×	×
		Green synthesis	CuO	×	×	×	×	×
			MgO	×	×	×	×	×

Note: The symbol indicates; no antibacterial activity observed (×), presences of antibacterial activity (a).

4.13 GC-MS Analysis

Fabric can develop unpleasant odours due to both internal and external factors related to the human body. Odour issues in fabrics arise from the presence of volatile organic compounds (VOCs) produced by bacterial metabolism of sweat compounds and fabric fibres. Secondary odours, which develop from either biotic or abiotic processes

within the fabric, are often more intense than primary odours originating from the adjacent axilla (Broadhead et al., 2021). In order to confirm the success of this research in preventing secondary odours, GC-MS analysis was conducted to characterize the volatile profile of both uncoated and coated fabrics. The GC-MS results revealed the identification of a total of 5 VOCs, including short-chain fatty acids (SCFAs), medium-chain fatty acids (MCFAs), and an ester in the uncoated fabric. In contrast, none of these VOCs were detected in the coated fabrics (Table 4.10). Acetic acid, a SCFA, was detected in uncoated blend wool fabric, while ethyl carbamate (ester) was detected in uncoated polyester fabric (Appendix E, and Appendix F). Additionally, three VOCs detected in uncoated cotton fabric comprises of phosphoric acid (fatty acid), hexanoic acid (SCFA), and octanoic acid (MCFA) (Appendix G). These findings confirmed that the use of CuO and MgO particles as coating solutions for fabrics effectively prevents body odour. This efficacy is evident from their ability to inhibit the growth of gram-positive bacteria, as demonstrated in the antibacterial test.

The breakdown of branched amino acids, such as leucine, isoleucine, and valine, by *S. epidermidis* could generate volatile fatty acids (VFAs) (Wang et al., 2022). The oxidation of saturated aliphatic alcohols to VFAs occurs through the formation of saturated aliphatic aldehydes in the presence of Nicotinamide adenine dinucleotide (NAD+) (Kaskow et al., 2020). *S. epidermidis*, *C. acnes*, and *B. linens* are responsible for the production of SCFAs, the most abundant compounds responsible for body odour, as they metabolize skin lipids into long-chain fatty acids, which are then converted into highly volatile SCFAs (Rankin-Turner & McMeniman, 2022). Notably, acetic acid has an intense odour with vinegar-like aroma, while hexanoic acid emits a goat-like aroma (Tian et al., 2020; Cha et al., 2019). Octanoic acid has a mild odour with a sweat-like aroma (Carunchiawhetstine et al., 2003). Phosphoric acid, despite being a fatty acid, does not contribute to any specific aroma or in other word it is odourless (Li et al., 2006). According to Roslund (2023), SCFAs and MCFAs are cytotoxic by-products released by bacterial cells during their metabolism. Volatile esters produced by lactic acid bacteria (LAB) such as *B. linens* could impart a sweet and fruity odour when present in low concentrations. However, at high concentrations, an off-odour may arise (Kruis et al., 2019). As reported by Park et al. (2009), most esters

have a low odour threshold, typically found in the range of part per million (ppm). Ethyl carbamate, which was identified in uncoated polyester fabric has almost no odour (Zou et al., 2022). Esters are produced through the oxidation of hemiacetal hydroxyl groups by alcohol and aldehyde mixtures, requiring Nicotinamide adenine dinucleotide phosphate ($NADP^+$) as a hydrogen acceptor (Lai et al., 2022). In addition, bacteria can utilize ketones as a carbon source to form esters and the presence of ethyl carbamate in polyester fabric probably generated from the degradation of arge by *B. linens* (Natsch & Emter, (2020); Tian et al., (2022).

The types of fabric and the presence of bacteria have a significant influence on the formation of malodour. The surface properties and functional groups of the yarn fibres has impacted the attachment and growth of bacteria, which indirectly affects the adsorption and retention of VOCs (Van Herreweghen et al., 2020). Cotton and blend wool fibers consist of cellulose and keratin, respectively, and these compounds promote the growth of bacteria. *S. epidermidis*, *C. acnes*, and *B. linens* have a high affinity for both cotton and blend wool fabrics (Yan et al., 2022; Syafiuddin, 2019), while *B. linens* and *C. acnes* prefer to grow on polyester, a petroleum-based synthetic fibre (Dehari et al., 2023). The presence of reactive sites such as hydroxyl groups in cellulose (cotton fabric), amino acid chains (blend wool fabric) and esters (polyester fabric) provide adsorption sites for volatile compounds and affect the odour profile of the fabric (Van Herreweghen et al., 2020). The polarity and hydrophobicity of the fabric also play important roles in the formation of odorant compounds. Fabrics with higher polarity can adsorb more moisture, while fabrics with lower polarity adsorb less moisture (Yu et al., 2022). Both cotton and blend wool fabrics have higher polarity but less hydrophobic than polyester fabric, resulting in more intense odours due to increased absorption of odorous volatiles (Siddique et al., 2021; Wang et al., 2019). Despite its hydrophobicity feature, polyester fabric has a strong adherence to fatty acids and aromatic compounds, as reported by Prada et al. (2011).

Table 4.10 The volatile organic compounds detected in coated and uncoated fabrics.

Fabric	Method	Solution	VOCs
Uncoated cotton			Phosphoric acid
			Hexanoic acid
			Octanoic acid
Coated cotton	Sol-gel	CuO	None
		MgO	None
	Green synthesis	CuO	None
		MgO	None
Uncoated polyester			Ethyl carbamate
Coated polyester	Sol-gel	CuO	None
		MgO	None
	Green synthesis	CuO	None
		MgO	None
Uncoated blend wool			Acetic acid
Coated blend wool	Sol-gel	CuO	None
		MgO	None
	Green synthesis	CuO	None
		MgO	None

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Introduction

This chapter wraps up the study by presenting the key findings and consequences of the study on antibacterial coating of fabrics. The objective was to fabricate antibacterial coatings capable of effectively inhibiting the growth of gram-positive bacteria across various types of fabric using CuO and MgO particles as coating materials, thereby minimizing, or eliminating body odour. The chapter begins with a summary of the research objectives and methodologies, followed by a discussion of the key results, and highlights the main conclusions and contributions of the study. Finally, it provides recommendations for future research in the field of antibacterial coating for fabrics.

5.2 Conclusion

The present study successfully fabricated environmentally friendly and low-cost antibacterial coatings for cotton, polyester, and blend wool fabrics using CuO particles and MgO particles as coating materials. The particles were synthesized via sol-gel synthesis and green synthesis methods, with PRE serving as antibacterial agent. The extraction parameters of PRE and synthesis parameters of particles solutions were optimized and characterized prior to coating the fabric samples to ensure their antibacterial properties. Various parameters of the coated and uncoated fabrics, including morphology and elemental compositions, antibacterial activity, tensile strength, air permeability, and odorous compounds were analysed in order to validate the success of this research. The durability of the coated fabrics was also assessed to evaluate the quality and suitability of the coating materials.

The UPLC-QTOF-MS analysis confirmed the presence of wide variety of natural compounds in the optimized PRE, including alkaloids, phenolics and

polyphenols compounds. These compounds contribute to the antibacterial properties of the particle solutions. Moreover, the XRF analysis confirmed the formation of CuO and MgO particles with the presence of both elements in their respective particle solutions. The average particle sizes observed for both CuO and MgO particles were less than 400 nm. The EDX analysis verified the deposition of the coatings by detecting the presence of copper and magnesium elements in the respective coated fabrics. The SEM images revealed that only MgO particles coated onto the cotton fabric produced a uniform and smooth coating surface without cracking. Despite the agglomeration and non-uniform deposition of particles coatings, the coated blend wool fabric showed higher antibacterial efficiency against all tested species of gram-positive bacteria compared to the coated cotton and polyester fabrics. The cotton fabric coated with CuO particles synthesized via the green synthesis method showed the highest percentage increments in tongue tear strength (warp direction) and breaking load (warp and weft directions). However, all the coated cotton and coated blend wool fabrics showed a reduction in air permeability compared to the uncoated fabrics. The highest increment of air permeability was observed in the polyester fabric coated with CuO particles synthesized through sol-gel method. Crucially, GC-MS analysis revealed that none of the VOCs were identified in any of the coated fabrics, confirming that the use of CuO particles and MgO particles effectively inhibited the growth of gram-positive bacteria responsible for body odour. The durability analysis showed that fabrics coated with CuO particles and MgO particles synthesized using sol-gel method maintained their antibacterial properties for up to three washing cycles, while fabrics coated with particles synthesized via the green synthesis method did not exhibit durable antibacterial properties. Although all the coated fabrics demonstrated good antibacterial efficiency, the cotton fabric coated with MgO particles synthesized via sol-gel method was selected as the best antibacterial coated fabric due to its uniformity in coating, washing durability and the increase in air permeability and tensile strength. Meanwhile, the best method and formulation selected in this study was MgO particles synthesized via so-gel, while the least effective method was green synthesis method used to synthesis CuO particles. The selections were made based on the antibacterial properties, and the characterization of the particles.

In conclusion, this research has effectively showcased the feasibility and efficiency of utilizing plant extracts (PRE) in the synthesis of antibacterial particles for fabric coatings. Key insights from this study include the effectiveness of PRE in the synthesis process, the crucial role of the synthesis method in determining the antibacterial properties and durability of the coatings, and the influence of fabric types on the effectiveness of the antibacterial coating. These findings have significant real-world applications, particularly in the textile industry, paving the way for the manufacture of antibacterial fabrics. The eco-friendly and cost-efficient nature of these antibacterial coatings makes them a promising solution for sustainable textile production. Moreover, this study provides a guide for further enhancement of antibacterial coatings, potentially leading to the creation of more potent, sustainable, and economically viable antibacterial fabrics. This research could add substantial knowledge to the domain of antibacterial fabric production and hold the potential to stimulate innovation in this field.

5.3 Recommendations

The findings of this study can be used to improve the synthesis methods of CuO particles and MgO particles in order to produce stable and durable coating materials with high antibacterial efficiency. Future research could explore the use of coupling agents, such as silane, in sol-gel synthesis to modify the surface of particles and increase the durability of the coated fabric (Aziz et al., 2021). Additionally, combining coating methods, such as padding and treating with microwave irradiation or ultrasound, is recommended to enhance the washing durability by achieving a uniform distribution and high stability of particles (Zhang et al., 2016). The microwave or ultrasound treatment generates heat or cavitation, which enhances the penetration and fixation of particles into the fabric (Vernès et al., 2020). Substituting water with ethanol as a solvent is also suggested, as it possesses different dielectric constants, polarity, viscosity, and solubility, which may influence the nucleation, growth, and aggregations of particles during synthesis process, thereby affecting the morphology, size, adhesion of particles to the fabric, and resistance to washing (Bari et al., 2020). Study on the dispersion of particles also could be considered as it affects their reactivity, toxicity, transport, and bioavailability (Mourdikoudis et al., 2018). Further studies could

investigate the effects of MgO particles and CuO particles coatings on gram-negative bacteria, fungi, and viruses to develop antimicrobial fabrics. The investigation of the mechanisms underlying the anti-inflammatory properties of MgO particles and CuO particles, which contribute to accelerated wound healing and enhanced its antimicrobial activity, could give positive impact to the healthcare industry. Moreover, it is crucial to determine the effects of MgO particles and CuO particles coatings on human skin, as both precursors exhibit beneficial effects that could lead to the production of cosmo-textiles.

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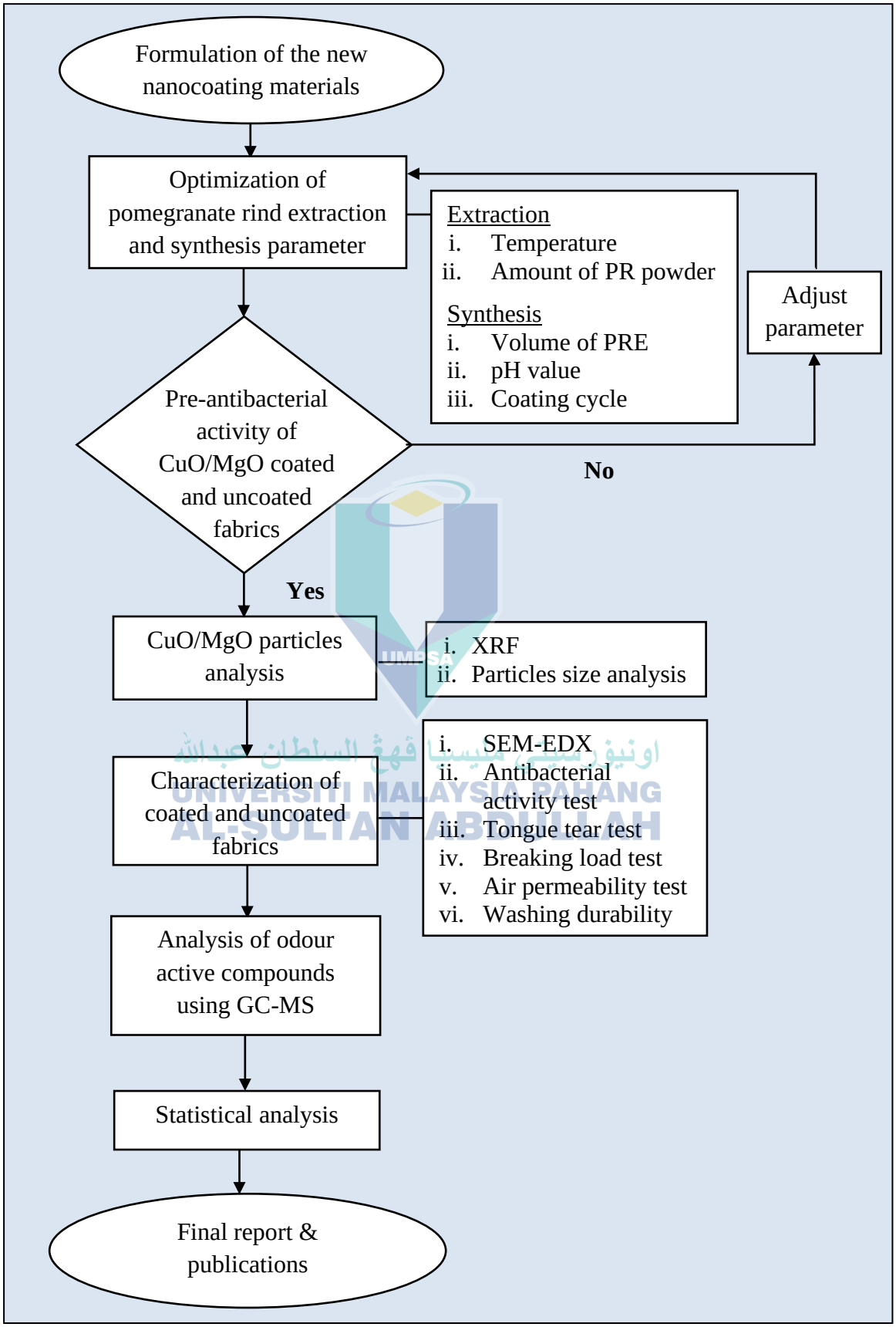
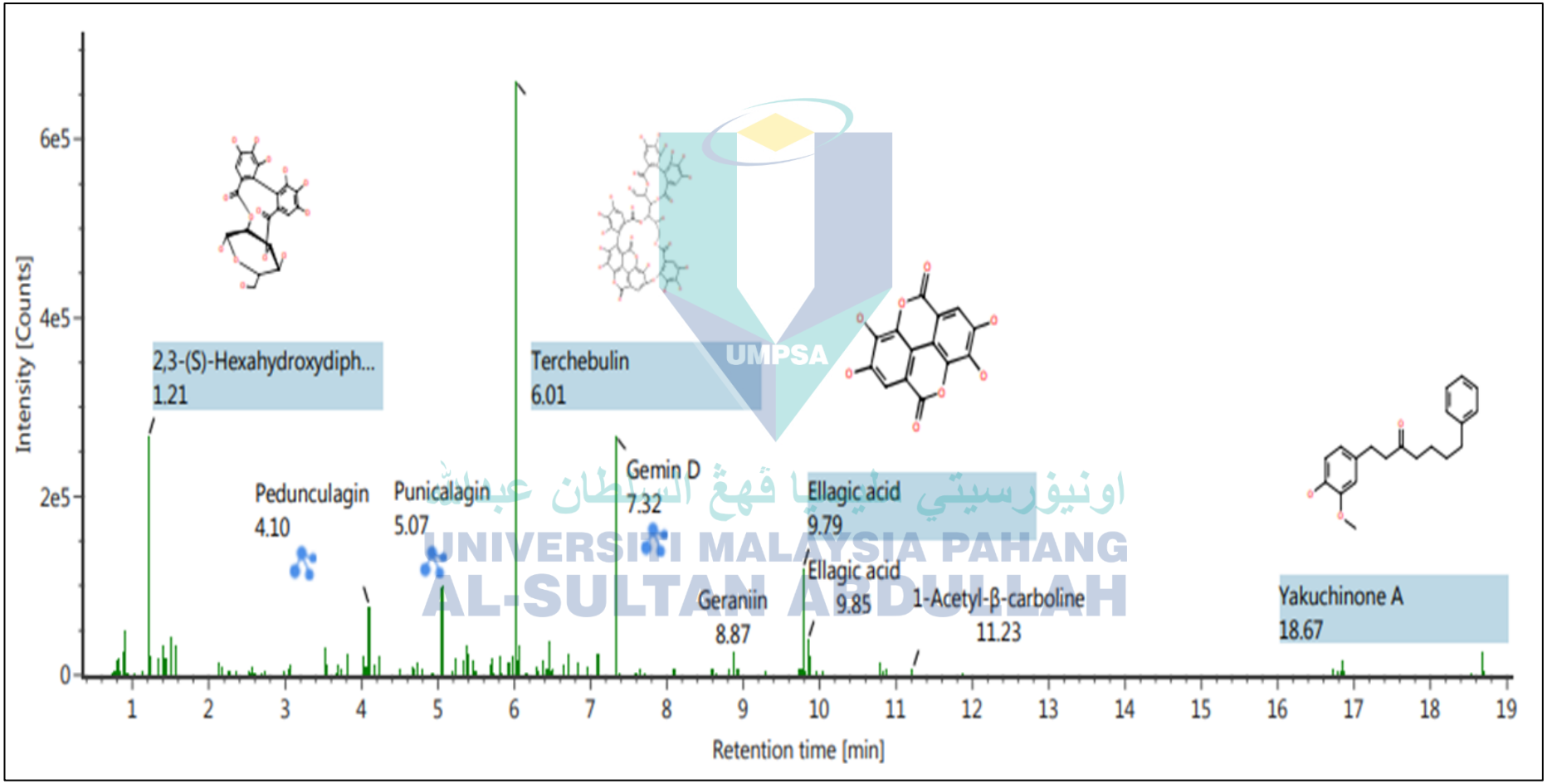
APPENDICES

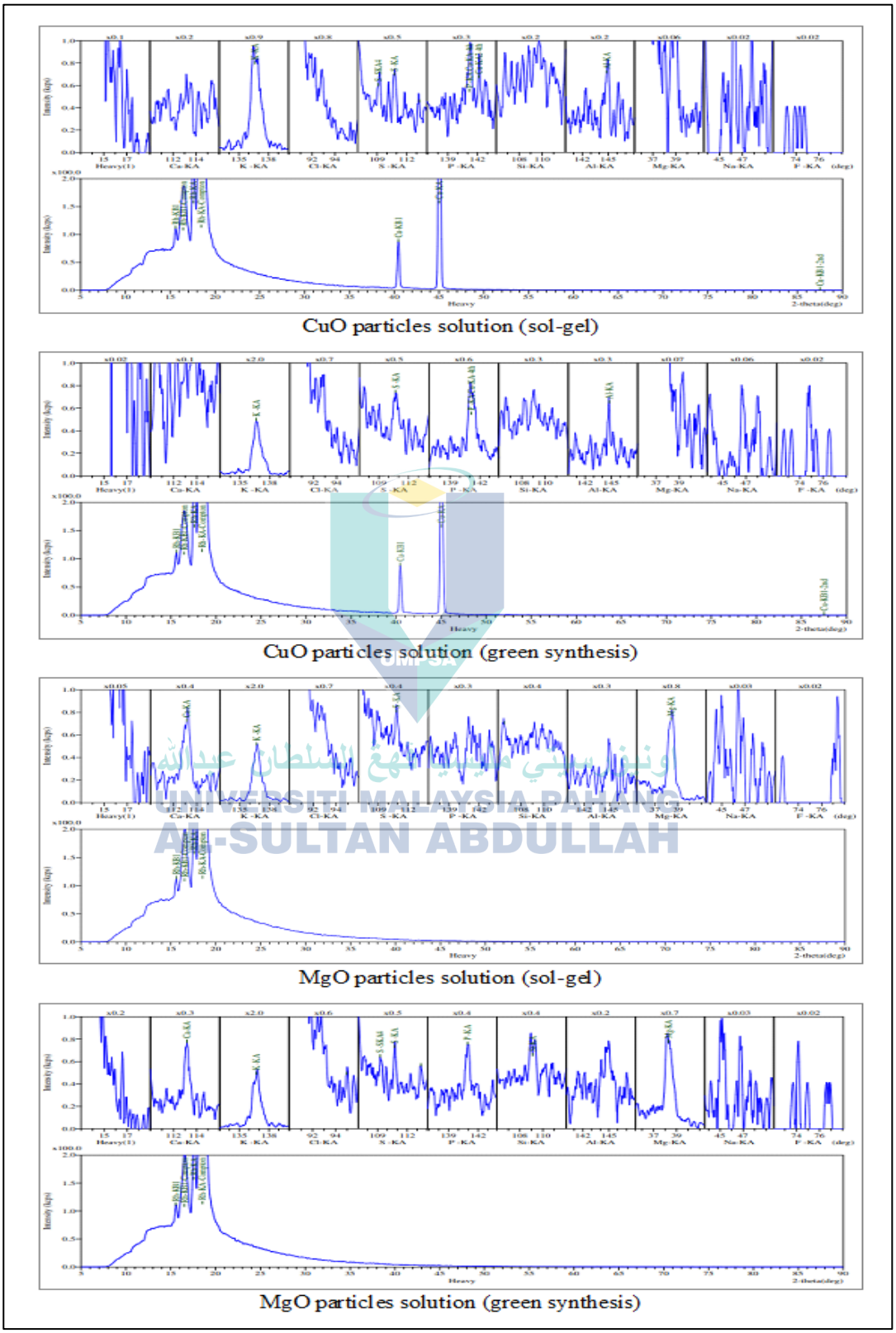
Appendix A: The flowchart of the experimental design.



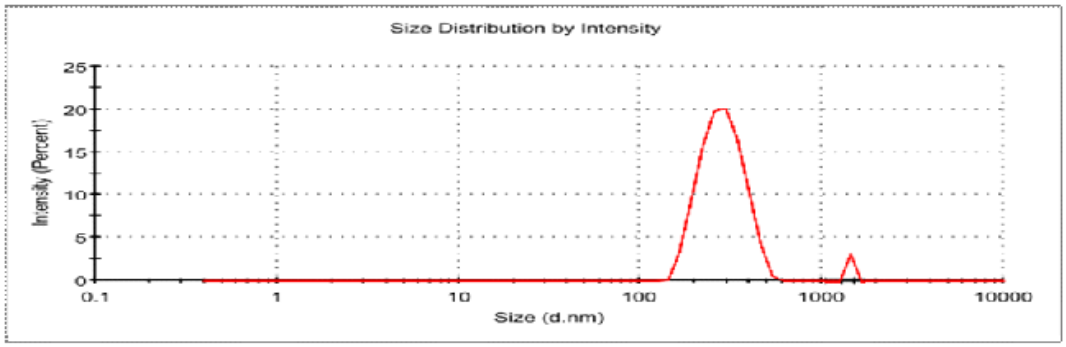
Appendix B: The UPLC spectra of pomegranate rind extract.



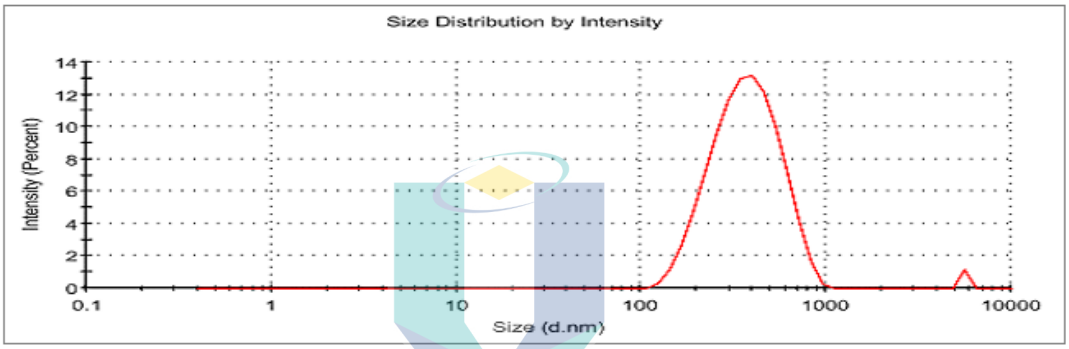
Appendix C: The XRF spectra of CuO and MgO particles synthesized via sol-gel and green synthesis method.



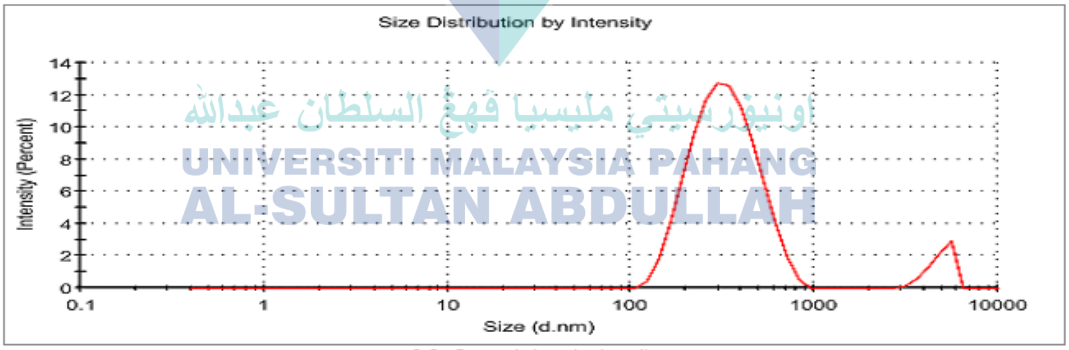
Appendix D: Size distribution of CuO and MgO particles synthesized via sol-gel and green synthesis method.



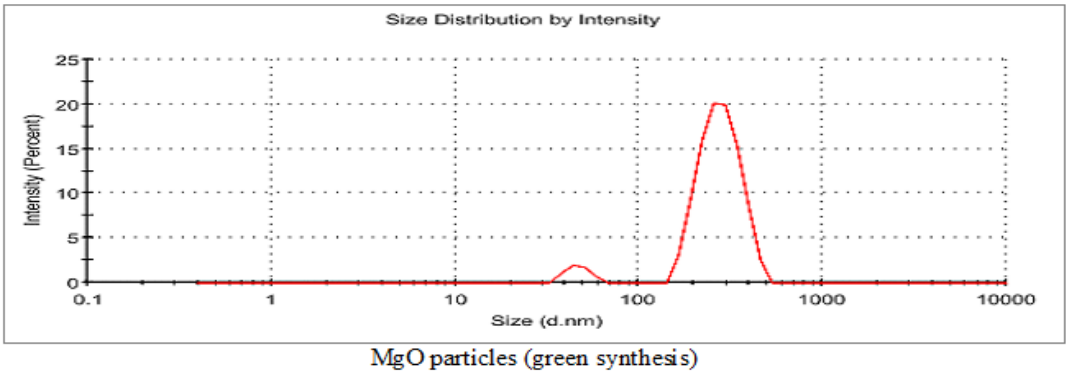
CuO particles (sol-gel)



CuO particles (green synthesis)

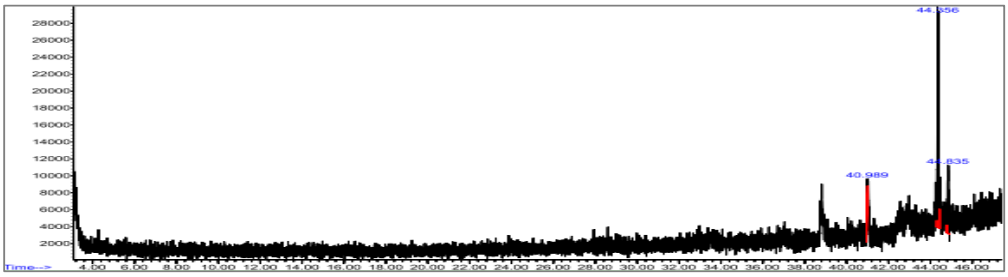


MgO particles (sol-gel)

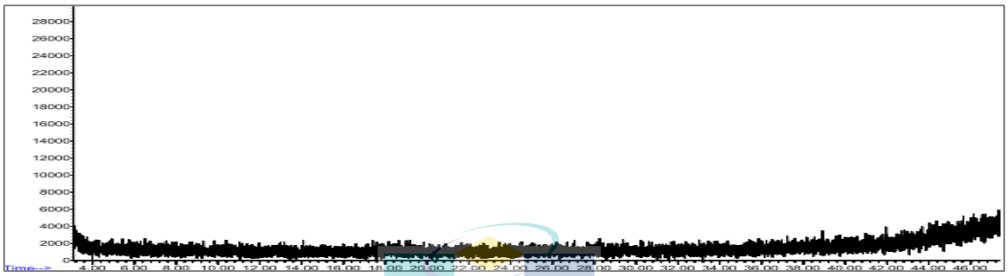


MgO particles (green synthesis)

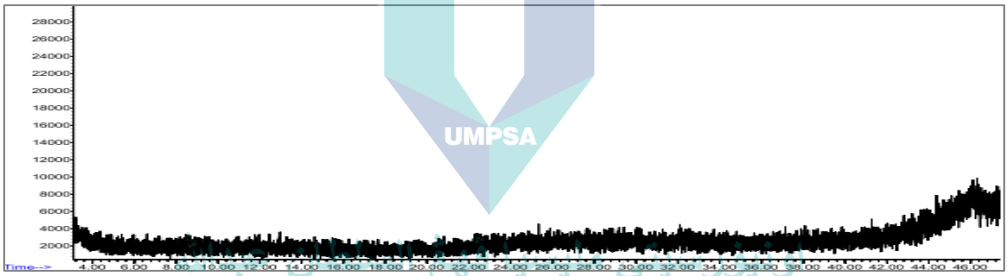
Appendix E: The GC-MS spectra of uncoated cotton fabric, CuO particles and MgO particles coated cotton fabric using sol-gel and green synthesis process.



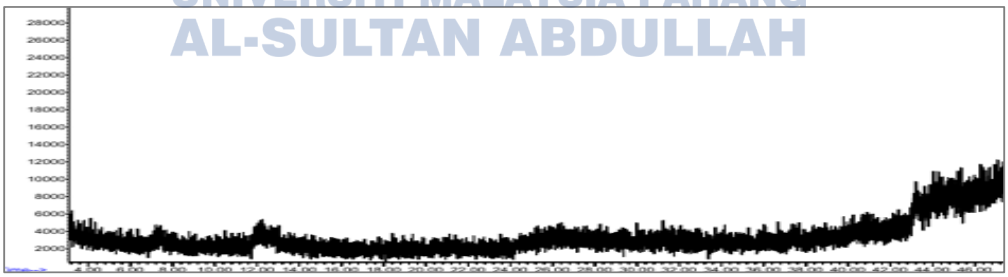
Uncoated cotton fabric



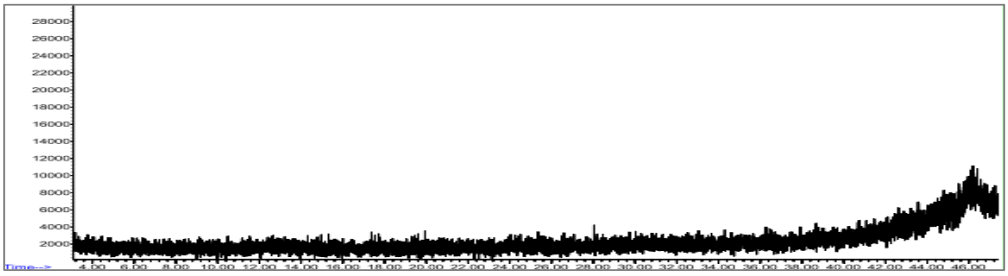
CuO particles coated cotton fabric (sol-gel)



CuO particles coated cotton fabric (green synthesis)

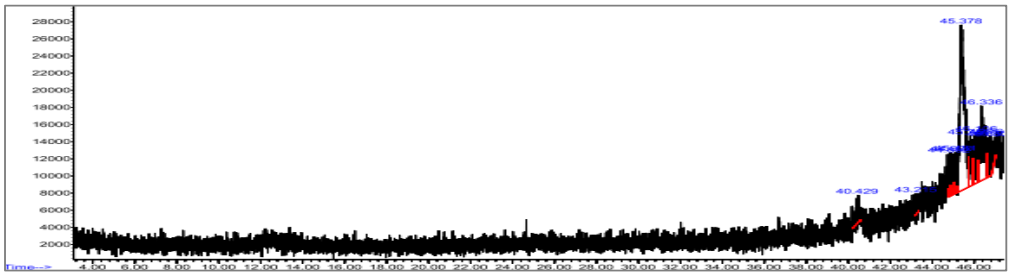


MgO particles coated cotton fabric (sol-gel)

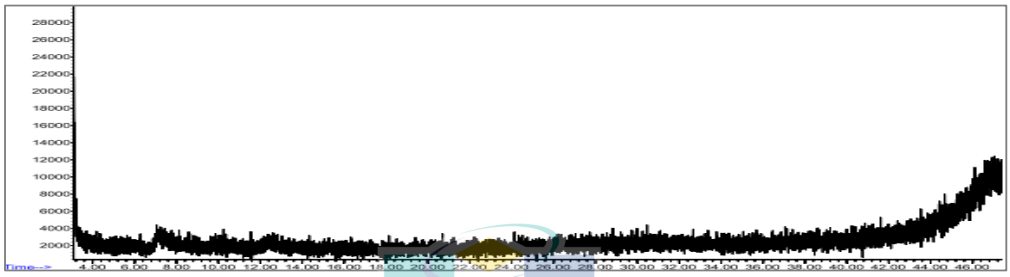


MgO particles coated cotton fabric (green synthesis)

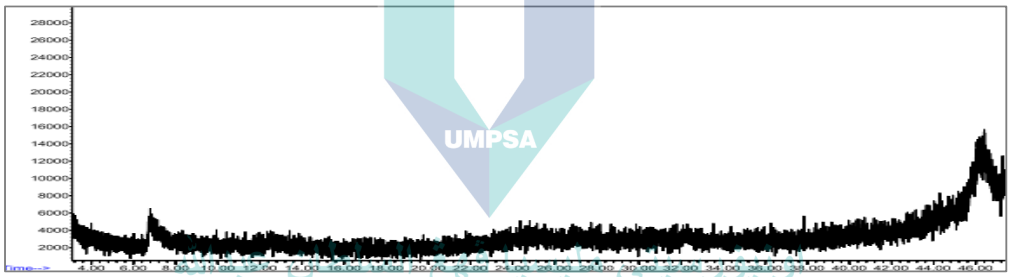
Appendix F: The GC-MS spectra of uncoated polyester fabric, CuO particles and MgO particles coated polyester fabric using sol-gel and green synthesis process.



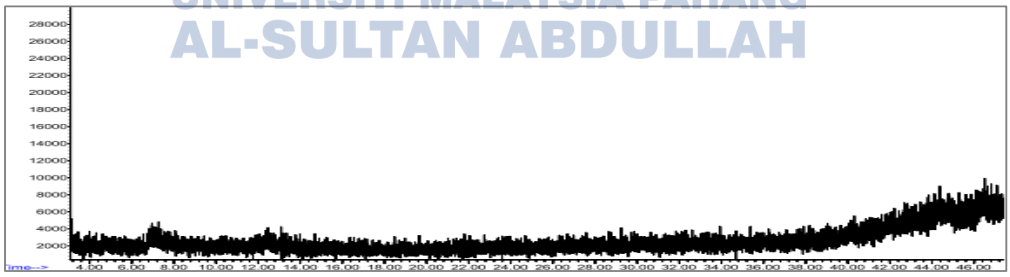
Uncoated polyester fabric



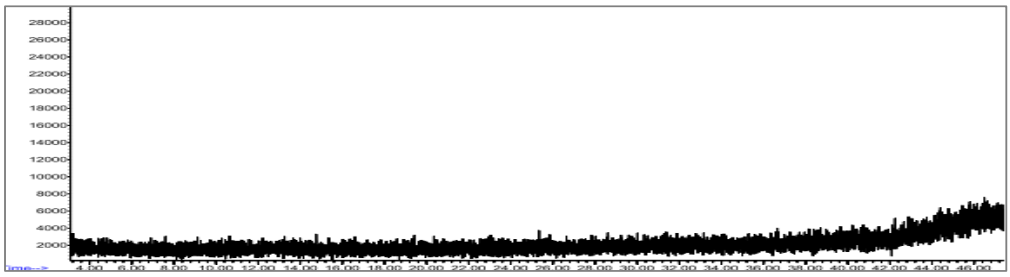
CuO particles coated polyester fabric (sol-gel)



CuO particles coated polyester fabric (green synthesis)

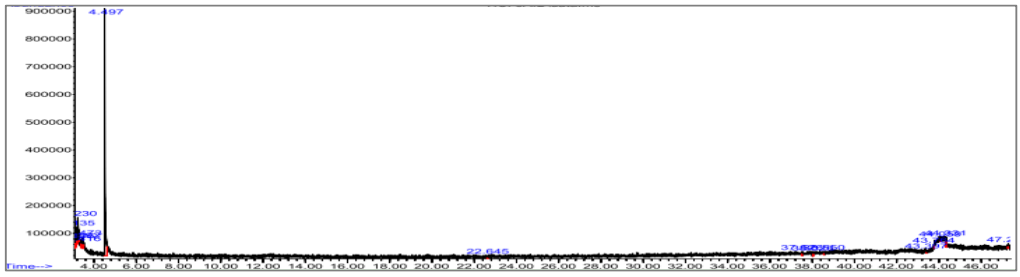


MgO particles coated polyester fabric (sol-gel)

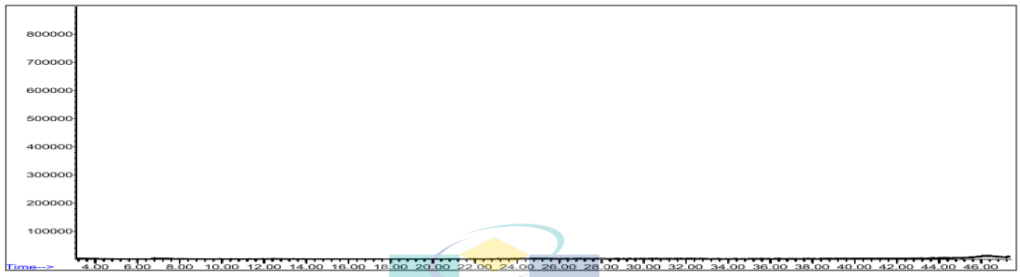


MgO particles coated polyester fabric (green synthesis)

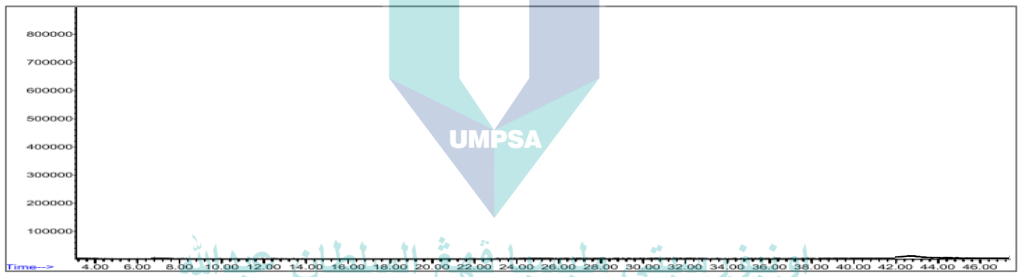
Appendix G: The GC-MS spectra of uncoated blend wool fabric, CuO particles and MgO particles coated blend wool fabric using sol-gel and green synthesis process.



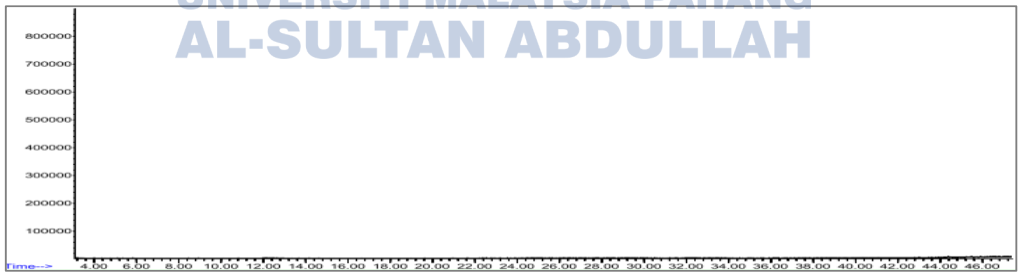
Uncoated blend wool fabric



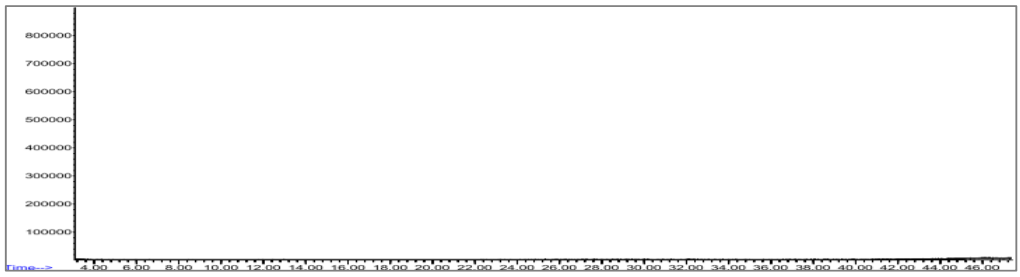
CuO particles coated blend wool fabric (sol-gel)



CuO particles coated blend wool fabric (green synthesis)



MgO particles coated blend wool fabric (sol-gel)



MgO particles coated blend wool fabric (green synthesis)

Appendix H: List of publications.

1. Bakar, N. H. A., Wan Ismail, W. N., Mohd Yusop, H., & Mohd Zulkifli, N.F. (2024). Synthesis of a water-based TEOS-PDMS sol-gel coating for hydrophobic cotton and polyester fabric. *New Journal of Chemistry.* https://doi.org/10.1039/D3NJ03206J.
   UMPIR REGISTRATION NO: 39677

2. Mohd Yusop, H., Wan Ismail, W. N., Mohd Zulkifli, N. F., & Tajuddin, S. N. (2023). Effect of CuO Antibacterial Coating on Cotton, Polyester, and Blend Wool Fabrics. *Biointerface Research in Applied Chemistry*, 13(6), 591. https://doi.org/10.33263/BRIAC136.591.
   UMPIR REGISTRATION NO: 39208

3. Bakar, N. H. A., Yusop, H. M., Ismail, W. N. W., & Zulkifli, N. F. (2023). Sol-Gel Finishing for Protective Fabrics. *Biointerface Research in Applied Chemistry*, 13 (3), 283. https://doi.org/10.33263/BRIAC133.283.
   UMPIR REGISTRATION NO: 35096

4. Wan Ismail, W. N., Syah, M. I. A. I., Abd Muhet, N. H., Bakar, N. H., & Mohd Yusop, H. (2022). Adsorption behavior of heavy metal ions by hybrid inulin-TEOS for water treatment. *Civil Engineering Journal*, 8 (9), 1787-1798. http://dx.doi.org/10.28991/CEJ-2022-08-09-03.
   UMPIR REGISTRATION NO: 36967

5. Mohd Yusop, H., & Norfazilah Wan Ismail, W. (2021). A Review on Synthesis of Plant-Mediated Metal Nanoparticles for Fabric Coating. *Malaysian Journal of Chemistry*, 23 (2), 40-54.
   UMPIR REGISTRATION NO: 34559

6. Za'im, N. N. M., Yusop, H. M., & Wan Ismail, W. N. (2021). Synthesis of water-repellent coating for polyester fabric. *Emerging Science Journal*, 5 (5), 747–754. https://doi.org/10.28991/esj-2021-01309.
UMPIR REGISTRATION NO: 32581

7. Mohd Yusop, H., Mohd Ismail, A. I. H., & Wan Ismail, W. N. (2021). Preparation and Characterization of New Sol–gel Hybrid Inulin–TEOS Adsorbent. *Polymers* 13 (8), 1295. https://doi.org/10.3390/polym13081295.
UMPIR REGISTRATION NO: 31492

Appendix I: List of awards from this study.

1. Bronze medal at 13th Creation. Innovation. Technology & Research Exposition (CITREX, 2023), Universiti Malaysia Pahang, 12th -14th March 2023. Invention: "Antibacterial Coating for Fabric". Inventors: Wan Norfazilah Wan Ismail, Hartina Mohd Yusop, Nurul Hidayah Abu Bakar.

2. Post-Graduate Research Scheme (PGRS) for PhD Study (2021). Grant amount: RM4200.00 for invention entitled "Synthesis and Fabrication of Antibacterial Nanoparticle for Fabrics to Prevent Odour". Awarded by Universiti Malaysia Pahang.

3. Malaysia Toray Science Foundation (MTSF) Science & Technology Research Grant (2020). Grant amount RM 20,000.00 for invention entitled "Antimicrobial Encapsulated Nanoparticles for Socks to Prevent Bromodosis (Foot Odour)". Awarded by Toray Science Foundation, Japan.

Appendix J: List of conferences.

1. *IKM Pahang Branch Online Symposium 2021: Chemistry for Sustainable World*. Malaysia, January 2021. Paper presented: A review on biosynthesis of nanoparticles for fabric coatings.

2. *4th Symposium on Industrial Science and Technology (SISTEC 2022)*. Malaysia, November 2022. Paper presented: Synthesis of Magnesium Oxide Nanoparticles for Fabric Coating and Its Antibacterial Activities.