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THE EVALUATION OF THE EFFECTIVENESS OF SELECTED ANTIMICROBIAL PLANT EXTRACTS COATING ON TEXTILES

by

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DECLARATION

I, **Elvino Nortjie,** declare that the contents of this dissertation/thesis represent my unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Elvino Nortjie 01 May 2023

Signed **Date**

ABSTRACT

Antimicrobial textile development has increased due to microbial growth which is a global health threat facing humanity. Microbial growth can be inhibited by employing an antimicrobial agent or coating application to prevent and treat infections from humans to plants. This study aims to provide information on the effectiveness of the selected plant extracts from South African biomass (*Eucalyptus globulus* and *Artemisia afra*) coated on textiles for application as biomedical textiles. The extracts were prepared by pulsed ultrasound-assisted solvent extraction method. A simple immersion method was used to coat the polyester and cotton fabrics. The extracts have also been analysed for their yields, antimicrobial activity, phytochemical analysis (phytochemical screening) minimum inhibitory concentration, zone of inhibition and washing durability. The methanolic extractions show the highest yields compared to the hexanoic *Artemisia afra* and *Eucalyptus globulus* extractions.

Phytochemical screening of *Artemisia afra* and *Eucalyptus globulus* crude leaf extract was determined. The results showed that all extracts contain the bioactive compounds phenolic compounds, quinones, and sterols.

The *Eucalyptus globulus* hexanoic extracts showed the highest antibacterial activity which has high inhibition zones against Gram-positive bacteria *S. aureus* ATCC 33591 followed by the methanolic *Eucalyptus globulus* extract. *Artemisia afra* methanol extracts showed higher antibacterial activity than that of the hexanoic extract against *S. aureus* ATCC 33591. The methanol extracts had higher inhibition zones against Gram-positive and Gram-negative bacteria.

Among the crude extracts, the highest zone of inhibition (258.4 mm^2) was recorded against *S. aureus* ATCC 33591 when the *E. globulus* methanolic extract was applied to the cotton fabric. On the other hand, the lowest inhibition was exhibited on the *E. globulus* methanolic extract coated on polyester fabric (65.97 mm^2). Bioactivity was only detected against the *S. aureus* strain. MIC values of the different extracts showed varied results. The MIC for the samples tested is therefore all >500 μg/ml. The MIC values obtained from antimicrobial tests ranged from 5-500 μg/ml (Table 5). The results showed that the bacterial strain S. *aureus* ATCC33591 was the most sensitive to *A. afra* methanol extract with a MIC value of 5 μg/ml to 25 μg/ml.

Furthermore, the inhibition zone was determined after laundering and no activity was found in the samples. This might be due to the preparation of fabric, inactivation of active compounds during the treatment/preparation of the material samples, or improper binding of the active compounds to the textile material.

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Dedication

This thesis is dedicated to my family.
My late mom- Mrs Elizabeth Caroline Nortjie
My dad Johannes Nortjie,
My brother Heino Nortjie
My friend Rainer Absenger
The Mannel Family
My Friends
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Rest in peace!

"Life is a surf. Love and live life to the fullest."
Elvino Nortjie

Thank you!

RESEARCH OUTPUTS

Published review paper.

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The layout of the thesis

Chapter 1

Chapter one covers the background and motivation of this study, it describes the problem statement, hypothesis, research aim and objectives and further outlines the delineation of the study.

Chapter 2

This chapter covers the literature review for this study, it focuses on the analysis of the various aspects of producing antimicrobial finishings, the microorganisms, their mechanisms of attachment on natural and synthetic fibres, the effect of microbial growth, and the principal and mechanism of microbial activity of the medicinal plants. Furthermore, the extraction methods, qualitative and quantitative phytochemical evaluations and antimicrobial efficacy and developments of antimicrobial-treated textiles using various agents are covered in the literature review. The chapter was published in the MDPI Plant Journal for 2022.

Chapter 3

This chapter presents the methodology and experimental results of the study. The chapter also discusses the – results, problems faced during the laboratory work and any observations made that could have impacted the outcome of the results.

Chapter 4

This chapter gives recommendations and conclusions based on, emphasizing what can be improved for the experimental work to achieve better results.

Chapter 5

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LIST OF ABBREVIATIONS AND ACRONYMS

PPE	Personal Protective Equipment
A. afra	*Artemisia afra*
E. globulus	*Eucalyptus globulus*
S. aureus	*Staphylococcus aureus*
E. coli	*Escherichia coli*
A. eupatoria	*Agrimona eupatoria*
H. alpestre	*Hypericum alpestre*
R. obrusifollus	*Rumex obrusifollus*
S. officinalis	*Sanguisorba officinalis*
PPE	Personal Protective Equipment
WHO	World Health Organization
FTIR	Fourier-transform infrared spectroscopy
HCAIs	Healthcare-related Infections
CDC	Centres for Disease Control and Prevention
AATCC	American Association of Textile Chemists and Colorists
rpm	Revolutions per minute
ml	Millilitre
mg	Milligram
nm	Nanometer
µg	Microgram
ISO	International Standards Organization
AATCC	American Association of Textile Chemists and Colourists
JIS	Japanese Industrial Standards
PC	Paper Chromatography
TLC	Thin Layer Chromatography
GC	Gas Chromatography
HPLC	High-performance liquid chromatography
QAC	Quaternary Ammonium Compounds
mRNA	messenger Ribonucleic acid
HAI	Health Associated Infections
RNA	Ribonucleic acid

DNA	Deoxyribonucleic acid
UTI	Urinary Tract Infection
ESKAPE	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanni, Pseudomonas aeruginosa, Acinetobacter aerogenes.*
PUAE	Pulsed Ultrasound-Assisted Extraction
MIC	Minimum Inhibitory Concentration
ZOI	Zone of Inhibition
UV	Ultraviolet
COVID-19	Coronavirus Disease of 2019
W/V	Weight-to-volume ratio
FTIR	Fourier transform infrared spectrometry
MTT	(3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
S. pyogenes	*Streptococcus pyogenes*
H. influenzae	*Hemophilus influenzae*
PPL	Priority Pathogen List
GRAS	Generally Recognized as Safe

GLOSSARY

a) AATCC147 Method	The AATCC 147 technique evaluates the antibacterial action of diffusible antimicrobial agents on treated fabric and assesses the capability of the treated fabric to inhibit the multiplication of microorganisms.
b) Antibacterial:	Can be defined as an agent that inhibits bacterial growth.
c) Antimicrobial activity:	Can be defined as a collective term for all active agents that inhibit the growth of bacteria, prevent the formation of microbial colonies
d) Antimicrobials:	Is a subclass of antibacterial that kills or inhibits microbial growth of microorganisms including bacteria, viruses, fungi, mould, and protozoans.
e) Pathogens:	Can be defined as an organism causing disease to its host. Every living organism is affected by pathogens, including bacteria.
f) Phytochemicals:	Can be defined as chemical compounds that are biologically active compounds found in plants.
g) Sonicator Apparatus:	Is an apparatus used to extract several types of material with volatile solvents. The sonicator applies sound energy to agitate particles in liquid media reaching ultrasonic frequencies of more than 20 kHz.
h) Zone of Inhibition:	It is the area of media where bacteria do not grow.

CHAPTER 1

INTRODUCTION

CHAPTER 1: INTRODUCTION

1.1 Introduction

The development of antimicrobial textiles has become of utmost importance for enhanced durability of the fabrics' intended use. Antimicrobial textiles are defined as functional textiles that may kill or inhibit the growth of pathogens (Gulati et al., 2022). Cotton and polyester are commonly used textiles being used in everyday life due to their properties that include comfort, strength, handling, and durability. They are also prone to microbial attacks. Cotton is a natural fabric that bears an environment for microbial growth depending on the growth of microorganisms e.g., pH, temperature, time, oxygen, and moisture (Gao et al., 2008). Bacterial growth leads to a bad odour and a reduction mechanical strength of fibres. The microbial growth on polyester fabric is slower than that of cotton due to its polymer backbone. Pathogens responsible for cross-contamination and infections include *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.

The ideal antimicrobial coating on textiles against pathogens should satisfy the following requirements (Chauhan & Kumar, 2020):

1. be effective against a broad spectrum of pathogens but exhibit low toxicity to the user.
2. be durable to laundering.
3. It should not negatively affect the quality or appearance of the textile.
4. be cost-effective

Antimicrobial textiles are required in the apparel, commercial, healthcare and household sector (Gulati et al., 2022), namely:

- Caps, jackets, sanitary pads, sportswear, undergarment, and winter wear in the apparel sector.
- Carpets, covering for seats, windows, vehicles, dusting cloths, military fabrics, tents, and uniforms in the commercial sector.
- Bandages, earbuds, scrubs, masks, lab coats and protective kits in the healthcare sector.
- Bedding, carpets, covers, curtains, mops, pillows, and towels in the household sector.

1.2 Problem Statement

Textiles are regarded as the only barrier between humans and pathogens. Antimicrobial-resistant pathogens are a threat to human health, especially healthcare-associated infections. The COVID-19 pandemic crisis proved the importance of personal hygiene and PPE. The World Health Organization suggested and implemented the use of Personal Protective

Equipment (triple-layered face masks, bodysuits), social distancing (Chauhan & Kumar, 2020) and disinfectant recipes to sanitize surface areas etc.

Large surface areas and moisture content favour the growth of microbes and lead to odour. In textiles, microbial growth is also led by moisture, pigmentation, and deterioration in quality, which affect the products and the wearer (Reshma et al., 2022). The attachment of the bacteria onto the fabric is dependent on the characteristics of the fabric and the contact time of the microbe onto the fabric, surface roughness and moisture retention. Natural and synthetic fibres react differently to microbial growth. Natural fibres are more prone to microbial attack because they retain water easily. Microbial growth on synthetic fibres like polyester is slower due to their polymer backbone (Gupta & Bhaumik, 2007). Cotton and polyester fabrics were chosen for this study due to their high absorbency and being the most widely used available fabrics in the world.

Antimicrobial textile agents and coatings allow the re-use of face masks, clothing, and textiles, reducing domestic laundering that results in a reduction in water consumption, and curtailing the worldwide pandemic, global warming, and environmental degradation.

This study focused on natural plant-based antimicrobial agents because it is less poisonous, less irritant, environmentally safe, and therefore used as antimicrobial finishes on textiles. Consumers have become aware of the harmful antimicrobial agents and the exposure of harmful microorganisms to humans. There are limitations in extracting from natural plants e.g., the bioactive compounds could be destroyed when subjected to high temperatures and extraction times and might influence the yield. The Pulsed Ultrasound-Assisted Extraction (PUAE) method was used in this study.

This research aimed at developing an eco-friendly plant-based antimicrobial coating from *Artemisia afra* and *Eucalyptus globulus* plant extracts coated on textile surfaces to combat the growth of pathogens. The extracts were applied to the polyester and cotton fabric. The yield, phytochemical screening, antibacterial analysis, minimum inhibition concentration and zone of inhibition of the extracts and washing durability were studied. Due to the nature of the extraction and application process of the plant extracts onto fabric and testing against Gram-negative and Gram-positive bacteria, this study qualifies under bioengineering. Overall, studies show there is a need for more effective ways to produce antimicrobial agents and coatings. This study is aligned with the same vision of providing a greener approach to antimicrobial plant extractions.

1.3 Research Questions

1.3.1 Do the drying conditions, extraction method, time and temperature affect the antimicrobial activity of the extracts?

1.3.2 Which extraction method that uses polar or non-polar solvents is more effective?

1.3.3 Which medicinal plant extract from the *Artemisia afra* and *Eucalyptus globulus* plant shows the best antimicrobial activity?

1.3.4 How effective are the antimicrobial coatings against the *S. aureus* and *E. coli* bacteria?

1.3.5 How effective is the application of the coating on polyester and cotton fabric for laundering?

1.4 Hypotheses

The plant extracts (*A. afra* and *E. globulus*) will inhibit microbial growth on textiles (*E. coli* and *S. aureus).*

1.5 Aim and Objectives

1.5.1 Aim

The aim is to evaluate the effectiveness of the selected plant extracts from South African biomass (*Eucalyptus globulus* and *Artemisia afra*) for possible textile coating or antimicrobial agents for surfaces.

1.5.2 Objectives

The objectives of this study are:

1.5.2.1 Prepare extracts containing antimicrobial compounds by pulsed ultrasound-assisted extraction using polar and non-polar solvents separately.

1.5.2.2 To conduct qualitative phytochemical screening analysis on *Eucalyptus globulus* and *Artemisia afra* to identify the bioactive compounds from the selected plants.

1.5.2.3 Apply the extracts onto cotton and polyester fabrics and assess the antimicrobial activity before and after laundering. The coated samples will be analysed using Fourier-transform infrared spectroscopy (FTIR).

1.6 Delineation of the study

This study will not look at:

- An economic evaluation of the process
- Composition of the compounds
- The toxicity of the compounds
- Quantitative analysis of the extracts

1.7 Significance and contribution of the study

The development of antimicrobial agents and coating for textiles is necessary to combat pathogens. Researchers are focusing on the development of natural antimicrobial agents and enhancement of the coating for washing durability of natural antimicrobial coated fabrics after washing (Reshma et al., 2022).

Antimicrobial coatings have become a highly active field of research, strongly stimulated by the increasing urgency of identifying alternatives to the traditional administration of antibiotics. This study plays a pivotal role in advancing green extraction techniques and shedding light on the antimicrobial effects when employing the pulsed ultrasound-assisted extraction approach, in contrast to conventional extraction methods.

1.8 General outcomes and contribution of the research

The purpose of the study was to evaluate the efficacy of the antimicrobial coating using selected medicinal plant extracts coated on the fabric. This study investigated the antimicrobial activity and the laundering effects of the antimicrobial coating in clothing and textiles. The research findings were written up as a master's thesis and published in scientific journals.

CHAPTER 2

LITERATURE REVIEW

Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review

Nortjie, E.; Basitere, M.; Moyo, D.; Nyamukamba, P. Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. ***Plants*** 2022, ***11***, **2011.**
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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Antibiotics play a vital role in fighting bacterial infections, but antibacterial resistance has caused havoc in the healthcare and pharmaceutical sector that accelerates socio-economic losses (O'Neill, 2016). Multidrug Resistance is said to increase by 10 million deaths per year by 2050 (O'Neill, 2016; Kraker et al., 2016). Biological screening, separation of the phytochemicals, and clinical trials of the medicinal plants have advanced over the years unfolding the secrets of ancient herbal remedies (Bibi et al., 2017). Traditional medicine is effective in dealing with diseases caused by bacteria or oxidative stress (Baydoun et al., 2015; Singh et al., 2017; Restuati & Diningrat, 2018).

Natural compounds have been extensively explored for new drug discoveries (Chandra et al., 2017). Humanity has always been fascinated by natural compounds from pre-biotic, microbial, plant, and animal sources. Extracts of different parts of plants contain bioactive compounds that fight against diseases such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, terpenoids, and flavonoids (Kutama, 2018) .The phenolic phytochemicals from plants play a key role as antimicrobial agents (Restuati & Diningrat, 2018; Smeriglio et al., 2017). Antimicrobial agents decay the protein components of the cell wall, disrupting the work of enzymes and DNA and RNA replication (Bakal et al., 2017) . Table 2.1 shows a selection of plants, their phytochemicals responsible for antimicrobial activity, and their applications.

Table 2.1: Representation of medicinal plant extracts and their applications

Plant Name	Phytochemicals	Applications
Sutherlandia fructecens	Saponins, pinitols, flavonoids, triterpenoids, canavanine, cycloartane glycosides, flavanols glycosides, and aminobutyric acid (Dube et al., 2017).	Wound treatment, cancer treatment, diabetes, skin diseases, rheumatism, urinary tract infection, fever, gonorrhea, kidney, and liver problems (Dube et al., 2017).
Eucomis autumnalis	Homoisoflavanones, terpenoids, and dibenzo-α-pyrones (Alaribe et al., 2018).	Reducing fever, urinary diseases, stomach, lower backaches, and syphilis. *Eucomis autumnalis* is sometimes used to induce labour (Alaribe et al., 2018).
Plumbago auriculata	Tannins, phenols, alkaloids, saponins, flavonoids, plumbagin, α-amyrin, capensisone, and diomuscinone (Lediga et al., 2018)	Treating headaches, warts, skin infections, wounds, and fractures (Lediga et al., 2018)
Catharanthus roseus	Vinblastine, deoxyvinblastin, vincoline, cathanranthamine, rosicine, leurosine, vindoline and vincristine (Gajalakshmi et al., 2011).	Treating rheumatism, venereal diseases, skin infections, high blood pressure, and diabetes (Oguntibeju et al., 2019) .
Aspalathus linearis	Spalathin, orientin, isoquercitrin, and luteolin hyperoside (Oguntibeju et al., 2019).	Treat insomnia, stomach cramps, allergies, and digestive problems as well as improve appetite (Mordeniz, 2019b).
Centella asiatica	Triterpenoids, centellose, medacassoside, triaponosides, flavonoid quercetin, rutin,	Treating fever, leprosy, syphilis, tuberculosis, leprosy, asthma, epilepsy, mental

	kaempferol, patuletin, apigenin, polyacetylenes, phenolic acids, sterols (Rout et al., 2013).	disorder, and minor wounds. Consumed as a vegetable and used as a spice (Rout et al., 2013).
Sclerocarya birrea	Steroids, glycosides, flavonoids, fatty oils, alkaloids, phenols, resins, calcium, and phosphorus (Joubert et al., 2011).	Treating dysentery, rheumatism, malaria, and diarrhoea (Joubert et al., 2011).
Hypoxis hemerocallidea	Rooperol, β-sitosterol **(Owira & Ojewole, 2009)**.	Immune booster, purgative, and laxative tonic. Treat tuberculosis, urinary tract infection, infertility, cancer, diabetes, and wounds **(Owira & Ojewole, 2009)**.
Galenia africana	Trihydroxyflavanone, trihydroxychalcone, dihydroxychalcone, and trihydroxy-3-methoxychalcone (Elbagory et al., 2017).	Treat venereal sores, eye infections, asthma, tuberculosis, cough, wounds, and skin infections and relieve toothache (Elbagory et al., 2017).

2.1.1 *Eucalyptus globulus*

The Eucalyptus species is a well-known plant species for its bioactive and pharmacological properties. It is the most represented species in the international pharmacopoeia. The essential oil compounds of the genus Eucalyptus (Myrtaceae) have been well characterized (Bakkali et al., 2008). *Eucalyptus globulus* is known to be the main furnisher of essential oils and is high in demand in the soap and cosmetic industries (Bajaj, 1995). It is a tree that grows to a height of 60-80 m with smooth bark and v yellow-brownish in colour and sheds in long strips leaving a white-greyish colour on the surface of the bark (Pereira et al., 2014; Araújo et al., 2010). Flowering occurs between May and January and the flower buds appear to be in leaf axils. The fruits are woody circular capsules that close at the edge in Figure 2.1 below (Mason et al., 2000; Mulyaningsih et al., 2010).

Figure 2.1: *Eucalyptus globulus*

The Eucalyptus oil extracted from the leaf contains volatile terpenes and aromatic compounds and the most abundant monoterpenoid 1,8-cineole (Pino et al., 2006; Brophy et al., 2009; Luís et al., 2016). British and European pharmacopoeias guide to using eucalyptus oil for medicinal purposes is that it must least contain at least 70% 1,8-cineole. The medicinal use of the *Eucalyptus globulus* is based on its biological activities shown by the oils e.g., antioxidant (González-Burgos et al., 2018), anti-inflammatory, analgesic (Silva et al., 2003), and antimicrobial activities (O. Oyedeji et al., 1999; Takahashi, Kokubo and Sakaino, 2004; Gilles et al., 2010; Sidana et al., 2010; Djenane et al., 2011; González-Burgos et al., 2018). Clinical trials with eucalyptus oil and the major component 1,8-cineole (eucalyptol) have been conducted to evaluate their efficacy in the treatment of a diverse range of conditions and diseases, including respiratory disorders (Worth, Schacher and Dethlefsen, 2009), and head lice infestation (Barker and Altman, 2011) The antimicrobial capabilities of the essential oils have been reported by the different species (Mulyaningsih et al., 2011; Ghaffar et al., 2015). The *Eucalyptus globulus* leaf extract shows antibacterial activity against bacteria responsible for respiratory tract infections such as *Staphylococcus aureus* ATCC 33591, *Streptococcus pyogenes* (*S. pyogenes*), and *Hemophilus influenzae* (*H. influenzae*).

2.1.2 *Artemisia afra Jacq.*

Artemisia afra is known as the Wild wormwood, African wormwood (Eng.); Wilde-Als (Afr.); umhlonyane (isiXhosa); mhlonyane (isiZulu), a soft aromatic shrub that grows up to 0.6-2 m (Figure 2.2). It is one of the most popular medicinal plants in South Africa. *A. afra* is a common species in South Africa with a wide distribution from the Cederberg Mountains in the Cape, northwards to tropical East Africa and stretching as far north as Ethiopia (van Wyk, 2008).

Figure 2.2*: Artemisia afra* Jacq.

Artemisia afra covers a wide range of ailments such as coughs, colds, fever, loss of appetite, colic, headache, earache, intestinal worms to malaria (van Wyk, van Oudtshoorn and Gericke, 1997). The *Artemisia afra* species can be administered in a variety of ways for personal medicinal use like enemas, infusions to cosmetics (Patil et al., 2011). It's also used as a preservative in food (Muyima et al., 2002) as well as an insecticide (Patil et al., 2011) The uses of the *Artemisia afra* have been studied for its antimicrobial activities (More et al., 2012; Martini et al., 2020) and its antifungal properties, It was found that it possesses antifungal activity (José Abad et al., 2000) as well as inhibitory activity against a broad spectrum of Gram-positive and Gram-negative bacteria (Jager, 2003; More et al., 2012; Martini et al., 2020; Graven et al., 1992; Muyima et al., 2002).

2.2 Textiles and Microorganisms

Textiles are carriers of microorganisms and are subjected to the growth of microorganisms, such as bacteria and fungi, depending on the food, acidic Ph, temperature, time, oxygen, and moisture (Dube et al., 2017). Bacteria interrelates with fibres in phases, from initial attachment onto fibres to the growth and damage to the fibres (Alaribe et al., 2018). Cotton is one of the ideal natural fibre fabrics for the growth of pathogens than polyester. Neely & Maley (2000) has shown the survival of several Gram-positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*) on standard hospital fabrics made of 100% cotton clothing, 100% cotton terry towels, 60:40 cotton/polyester-scrub suits and lab coats, and 100% polyester drape.

A study by Neely & Maley (2000) showed the growth of bacteria within 48 hours, most bacterial growth survived at least a day, and some survived more than 90 days. Natural fibre textiles are more prone to microbial growth and could lead to the spread of infections (Gajalakshmi et al., 2011). A study by Gupta & Bhaumik (2007) reports that the attachment of the bacteria onto the fabric is dependent on the characteristics of the fabric, the contact time of the microbe onto the fabric, surface roughness, and moisture retention for natural and synthetic fibres reacting differently to microbial growth (Kraker et al., 2016). Natural fibres are more prone to microbial attack because they retain water easily. Microbial growth on synthetic fibres like polyester is slower due to their polymer backbone (Alaribe et al., 2018).

2.3 Requirements, Modes of Antimicrobial Action of Antimicrobial Agents

The ideal antimicrobial treatment for textiles must be effective against a broad spectrum of pathogens but exhibit low toxicity to the user. It must be cost-effective, durable to launder, and not alter the quality or appearance of the textile (Chauhan & Kumar, 2020).

A study by Gao (2008) reported that microbes are microscopic organisms that exist as unicellular, multicellular, or cell clusters. They consist of an outermost cell wall that constitutes polysaccharides. The cell wall maintains the integrity of cellular components and shields the cell from the extracellular environment. Beneath the cell wall is a semi-permeable membrane that encloses intracellular organelles and multiple enzymes and nucleic acids. The enzymes are responsible for the chemical reactions within the cell, followed by the storage of nucleic acid genetic information of the organism. The purpose of antimicrobial agents is to destroy the cell wall or alter cell membrane permeability, denature proteins, inhibit enzyme activity, or inhibit lipid synthesis so that the cell does not survive.

Various classes of phytochemicals and antimicrobial agents possess different mechanisms of action against microbes.

As for phytochemicals tested in this study, their activity and mechanism of action are described below (Tiwari et al., 2011):

- Quinones: possess antimicrobial activity which binds to adhesins and inactivates enzymes.
- Flavonoids: possess antimicrobial activity which is and binds to adhesins
- Phenol and tannins: possess antimicrobial activity which binds to adhesins, enzyme inhibition, substrate deprivation, membrane disruption, and metal ion complexation.
- Terpenoids: disrupts the membrane of microorganisms.
- Saponins: inhibits Gram-positive and Gram-negative bacteria.

Table 2.2 shows the different antimicrobial classes, the mechanisms of action, and the activity spectrum, respectively (Tille, 2014). Adapted with permission from Ref. (Tille, 2014). 2014, Dr Patricia Tille.

Table 2.2: Representation of mechanisms of action of antimicrobial agents (Tille, 2014)**. Adapted with permission from Ref.** (Tille, 2014)**. 2014, Dr Patricia Tille.**

Antimicrobial Class	Mechanism of Action	Activity Spectrum
β-lactams	They inhibit cell wall synthesis by binding enzymes in peptidoglycan production	Gram-negative bacteria and Gram-positive bacteria could differ with individual antibiotic
Aminoglycosides	Hinders the protein synthesis by binding 30S ribosomal subunits	Gram-negative bacteria and Gram-positive bacteria
Chloramphenicol	Inhibits the protein synthesis by binding 50S ribosomal subunits	Gram-negative bacteria and Gram-positive bacteria
Fluoroquinolones	Inhibits DNA synthesis by binding the DNA gyrase topoisomerase IV	Gram-negative bacteria and Gram-positive bacteria, but it could differ with individual antibiotic
Glycylglycines	Inhibits the protein synthesis by binding 50S ribosomal units	A wide spectrum of Gram-negative bacteria and Gram-positive species
Ketolides	Inhibits protein synthesis by binding 50S ribosomal subunits	Gram-positive cocci including certain macrolide resistance strains and Gram-negative strains
Lipopeptides	Binding and disruption of cell membrane	Gram-positive bacteria including β-lactams and glycopeptides
Nitrofurantoin	The mechanism is unknown and may have bacterial enzyme targets and damaging DNA.	Gram-negative bacteria and Gram-positive bacteria

Oxazolidinones	Hinders the initiation of protein synthesis by binding 50S ribosomal subunits	Wide variety of Gram-positive bacteria including those resistant antimicrobial classes
Polymyxins	Disrupts cell membrane	Poor activity against most Gram-positive bacteria. Gram-negative bacteria
Rifampin	Hinders RNA synthesis by binding DNA dependent, RNA polymerase.	Gram-positive and certain Gram-negative bacteria
Streptogramins	Hinders the protein synthesis by binding two separate sites on the 50S ribosomal subunit	Gram-positive bacteria
Tetracycline	Inhibits protein synthesis by binding of 30S ribosomal subunit	Gram-negative bacteria and Gram-positive bacteria and several intracellular bacterial pathogens
Sulfonamides	Hinders the folic acid pathway, binding the enzyme dihydropteroate synthase	Gram-negative bacteria and Gram-positive bacteria
Trimethoprim	Hinders with the folic acid pathway by binding the enzyme dihydrofolate reductase	Gram-negative bacteria and Gram-positive bacteria

Textiles are regarded as a barrier between humans and pathogens. Plant-based extracts and materials provide efficient and natural microbial resistivity. Antimicrobial textiles are essential in the apparel, commercial, and healthcare sector (Gulati et al., 2022). A study by Vastrad et al., (2016) reported on the evaluation of total phenolic content and flavonoid content using leaf extracts (eucalyptus and lemon grass) with methanol, ethanol, chloroform, and distilled water extract indicated the potential of antimicrobial application of textiles. The antimicrobial agents and finishing on textiles may allow the re-use of face masks, and clothing, reducing PPE kits in health care, and reducing domestic laundering that may lead to a reduction in water consumption.

2.4 Pre-Treatment and Processing of Biomass

2.4.1 Drying of Biomass

The selection of pre-treatment and processing methods may influence the reduction in extraction time, an increase in extraction yield, the quality of the biological compounds, and a reduction in input energy (Safarzadeh Markhali, 2021). The drying of any biomass inhibits microbial growth (Joubert et al., 2011), and it aids in the longer shelf life and transportation costs due to the weight and space of dry products (Pham et al., 2015; Saifullah et al., 2019;

Shrestha et al., 2007). Drying can affect the phytochemical components of the thermally sensitive components (Pham et al., 2015; Nóbrega et al., 2015; Nadi, 2017; Nguyen et al., 2015), and the process can also contribute to improved conservation of the bioactive compounds against oxidative (Ahmad-Qasem et al., 2013) and enzymatic activities (Babu et al., 2018) and spoilage bacteria (Saifullah et al., 2019; Afaneh et al., 2015; Orphanides et al., 2013), enabling cellular destruction (Saifullah et al., 2019; Ahmad-Qasem et al., 2013; Safarzadeh Markhali, 2021). There are many different drying methods, e.g., thermal through natural convection (shade and open sun drying), forced convection (oven drying, solar drying, and heat pump drying), freeze-drying, greenhouse drying, microwave drying, and infrared drying (Safarzadeh Markhali, 2021).

The freeze-drying method retains the bioactive compounds of the dried product due to minimal thermal damage to the cell tissue, thermolabile compounds, and its porous surface, enabling increased penetration of solvents (Saifullah et al., 2019; Harnkarnsujarit et al., 2016; Ahmad-Qasem et al., 2013). Olive leaf extracts pre-treated with a hot air drier at 120 °C showed higher phenolic recovery compared to freeze-drying (loss of polyphenols reached up to 39% in dry weight). Freeze drying shows great potential in the extraction of the total phenolic content (Ahmad-Qasem et al., 2013; Ghelichkhani et al., 2019).

Ahmad-Qasem (Ahmad-Qasem et al., 2013) reported that temperature plays a key role in the drying process as it may be beneficial or unfavourable to the microstructure of the biomass and the use of hot air drying at a high temperature. The study by Ahmad-Qasem (Ahmad-Qasem et al., 2013) also reported better extraction efficacy of some phenolic compounds in olive leaves when compared to samples dried at lower temperatures and by drying at a moderate to low temperature may need a longer drying time to reach the desired moisture content of the biomass.

2.4.2 Choice of Solvents

The solvent selection is crucial in determining the bioactive compounds of plants used for extractions. Ideal extraction solvent properties include low toxicity, evaporating easily at low temperatures, having good solubility of the target compound, and being sufficiently volatile. The factors affecting the selection of solvents are the rate of extraction, diversity of compounds extracted, ease of handling of extracts, and the cost-effectiveness of the extraction solvents and targeted compounds. Plants contain diverse bioactive compounds with different polarities. A variety of techniques have been devised and employed to isolate pure compounds, thereby identifying their structures and biological activities (Sasidharan et al., 2011).

Many solvent extractions have been done to obtain phytochemical compounds for their activity against pathogens. Various phytochemicals possess distinct structural characteristics and comprise diverse compounds, each exhibiting unique action mechanisms, as elaborated below:

- Phenols and polyphenols are obtained from acetone and ethanol solvent extractions which consist of a C_3 sidechain, hydroxyl groups and a phenol ring e.g., catechol, epicatechin, cinnamic acid that has antimicrobial, anthelmintic, and antidiarrheal activity (Cowan, 1999; Tiwari et al., 2011).
- Chloroform, methanol, and ethanol solvents extract mainly quinones. They consist of aromatic rings, and two ketone substitutions e.g., hypericin which has antimicrobial activity (Cowan, 1999; Tiwari et al., 2011).
- Ethanol and water mainly extract tannins which consist of polyphenols e.g., ellagitannin which has antimicrobial anthelmintic and antidiarrheal activities (Cowan, 1999; Tiwari et al., 2011).
- Chloroform solvents extract mainly flavonoids which consist of a phenolic structure, a carbonyl group, hydroxylated phenols C_3 -C_5 unit linked to an aromatic ring, flavones and a +3-hydroxyl group that has antimicrobial, anthelmintic and antidiarrheal activity (Cowan, 1999; Tiwari et al., 2011).
- Ether solvent extracts mainly coumarins and it consists of phenols made up of fused benzenes e.g., warfarin with antimicrobial activity (Cowan, 1999; Tiwari et al., 2011).
- Water, ethanol, chloroform, and ether solvents extract mainly terpenoids which consist of fatty acids and acetate units with antimicrobial activity (Cowan, 1999; Tiwari et al., 2011).
- Lectins and polypeptides can be extracted by water which consists of mainly extracts proteins e.g., mannose-specific agglutinin, and fabatin that has antimicrobial activity (Cowan, 1999; Tiwari et al., 2011).
- Alkaloids can be extracted by ethanol and ether solvents which consist of heterocyclic nitrogen compounds e.g., berberine, piperine, palmatine and tetrahydropalmatine which has antimicrobial, anthelmintic and antidiarrheal activity (Cowan, 1999; Tiwari et al., 2011).

- Saponins can be extracted by methanol, water, and hydro-alcoholic 70 % methanol which consists of amphipathic glycosides e.g., vina-ginsenosides R5-R6 with antidiarrheal activity. (Cowan, 1999; Tiwari et al., 2011).

During extraction, solvents diffuse in the plant material and dissolve compounds with similar polarity. The different extraction methods will also affect the composition of the secondary metabolites of the extracts namely, type of extraction, time of extraction, temperature and nature of the solvent, solvent concentration, and polarity.

The determination of biologically active compounds from plant materials is crucial and dependent on the type of solvent used (Velavan, 2015). Solvents are selected based on their availability, low toxicity, boiling point and solvent polarity (Das et al., 2010; Kumar, 2015).

The FAO/WHO Expert Committee reported seventeen solvents that are generally recognized as safe (GRAS) to use for food and personal-care products.

2.5 Microorganisms

Resistance to antibiotics has become a serious problem globally. ESKAPE are multidrug-resistant pathogens such as *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species that are responsible for Hospital-Acquired Infections (HAI). New antibiotics have been produced over the years. The resistance by the ESKAPE pathogens to the drugs has accelerated tremendously (FAO/WHO, 2018). A Priority Pathogen List (PPL) was released by the WHO in 2016 as a guide to research, discovery, and development of new antibiotics globally (Bhatia et al., 2021). Pathogens occupy the surfaces of fabrics depending on the contact time, moisture retention, and surface roughness. *Staphylococcus aureus and Escherichia coli* pathogens cause hospital infections leading to pneumonia and sepsis. It is, therefore, important to keep track of the availability of alternative medicinal plants and herbs to conquer this challenge (World Health Organisation., 2017). The discovery of new drugs that can be mastered with the use of plant extracts is a hoard of a spectrum of secondary metabolites (Ghareeb et al., 2015; Ugwoke et al., 2020; Cragg & Newman, 2013; Madubuonu et al., 2019; Aisida et al., 2020; Kandemir et al., 2020; Ginting et al., 2020).

2.5.1 *Enterococcus faecium*

Enterococcus faecium Enterococcus faecium is a Gram-positive bacterium that causes infections; it is increasingly resistant compared to *Vancomycin-resistant Enterococci faecium* (Aisida et al., 2019; Lebreton et al., 2013). *E. faecium* lives in the gut microbiome of animals (Pendleton et al., 2013; Marques et al., 2018). Food is an excellent hideout for the strains to remain dormant (Fiore et al., 2019). Treatment is dependent on second-line antibiotics (Ubeda et al., 2010). Urinary tract infections, bacteraemia, and endocarditis are caused by this bacterium (Marques et al., 2018).

2.5.2 *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive bacterium prevalent on the human skin, particularly in immune-compromised individuals. This bacterium causes infections on medical implants and forms biofilms that make it extremely difficult to treat with antibiotics. The Methicillin-resistant *Staphylococcus aureus* developed resistance against β-lactam antibiotics (Lebreton et al., 2013). Community-associated MRSA lineages are associated with skin and soft tissue infections. The strains are associated with pneumonia and bloodstream infections (Prasad et al., 2012).

2.5.3 *Klebsiella pneumonaie*

Klebsiella pneumonaie is a Gram-negative bacterium that causes urinary tract infections septicaemia, surgical wound infections, pneumonia, endocarditis, pyogenic liver abscess cystitis, and endogenous endophthalmitis (DeLeo et al., 2010). Cephalosporin- and carbapenem-class antibiotics have been the base treatment for *Enterobacterales* infections, such as *Klebsiella pneumoniae.* The efficacy of the antibiotics is compromised by the widespread acquisition of genes and encoding enzymes that aid in the respective resistance to these critical drugs (Navon-Venezia et al., 2017).

2.5.4 *Acinetobacter baumannii*

Acinetobacter baumannii is a Gram-negative bacterium that is more common in hospital settings (Paterson & Bonomo, 2005). It is aerobic and non-fermenting pleomorphic. This bacterium can resist dehydration. It forms biofilms, surface adhesins, that help this bacterium thrive in its environment (Munoz-Price & Weinstein, 2008). The infection rates of *A. baumannii* are low compared to the ESKAPE pathogens (Magill et al., 2014).

2.5.5 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative bacterium associated with respiratory infections and displays resistance to multiple classes of antibiotics (Magill et al., 2014). *P. aeruginosa* grows and colonizes in moist environments, especially in healthcare settings in the context of chronic wounds, respiratory support, or urinary tract devices, immune evasion, and antimicrobial resistance (Yayan et al., 2015).

2.5.6 *Enterobacter* spp.

It's a Gram-negative bacterium, anaerobic in nature. *Enterobacter aerogenes, known as Klebsiella aerogenes* are responsible for the increasing number of hospital-acquired infections [79]. Immunocompromised individuals are more susceptible to urinary and respiratory tract infections of this bacterium (Malek et al., 2019).

2.5.7 *Escherichia coli*

Escherichia coli, which is not part of the ESKAPE pathogens is the major cause of bloodstream and urinary tract infection (UTI) in both community and healthcare settings globally. Sepsis is one of the most common manifestations of *E. coli* urinary tract infection. *E. coli* is the most common Gram-negative bacterial species isolated from blood and urine cultures (Reza et al., 2019).

2.6 Brief Description of the Biocide Agents on the Market

Many biocide agents already exist on the market. They are classified into the following compounds (Morais et al., 2016):

Quaternary Ammonium Compounds: These compounds represent a group of compounds. They consist of a subgroup of alkyl linear ammonium compounds, composed of hydrophobic alkyl chain and hydrophilic-counterpart. Quaternary Ammonium Compounds damage cell membranes, modify proteins and inhibit DNA production. They are applied in cotton, polyester, nylon, and wool fibres. These compounds are active against a wide range of pathogens but lack physical bonding in textiles.

Triclosan: These compounds are odourless chlorinated bisphenol and improve the durability of laundering. They are active against a wide range of pathogens. They block lipid biosynthesis and hinder the integrity of the cell membranes. They are applied to polyester, nylon, cellulose acetate, and polypropylene.

Metals and metallic salts: At low concentrations, they are exceptionally active against pathogens. They generate reactive oxygen species, damaging cellular proteins lipids and DNA. Silver, copper, zinc, and cobalt are used has been widely used as antimicrobial agents and applied to cotton, wool, nylon, and polyester.

Chitosan: is a natural hydrophilic copolymer. It's a linear polysaccharide that is biocompatible, non-toxic, non-carcinogenic, and antimicrobial. They are applied to cotton, wool, polyester, and nylon fibres. The low molecular weight results in inhibiting the synthesis of mRNA, preventing protein synthesis, and the high molecular weight cause leakage of intracellular substances or blocks the transport of essential solutes.

Poly (Hexamethylene Biguanide): These agents are polycationic amines biguanide repeat units separated by aliphatic chains. They interact with membrane phospholipids, resulting in disturbance and the fatal leakage of cytoplasmic materials. They are applied to cotton, nylon, and polyester fibres.

N-halamines: They are heterocyclic organic compounds. N-halamines prevent the cell enzymatic and metabolic processes, causing the consequent microorganism destruction. They are applied in cotton, nylon, polyester, and wool fibres and are active against a wide range of pathogens.

Many plant-based compounds with a wide range of antimicrobial activity spectrum have been identified and are commercially available. Table 2.3 shows the wide range of commercially available antimicrobial agents on the market (Morais et al., 2016).

Table 2.3: Representation of commercially available antimicrobial agents on the market

Product Name	Company	Description
Agion®	Sciessent, Massachusetts , United States	Silver and zeolite-based additive
AlphaSan®	Milliken Chemical, Spartanburg, South Carolina	Silver-based additive
BioGaurd®	AEGI Microbe Shield, Huntersville, North Carolina	Finishing agent based on 3-trimethoxysilylpropyldimethyloctadecylammonium chloride
Biozac ZS	Zschimmer & Schwarz Mohsdorf GmbH, Burgstadt, Germany	PHMB-based finishing agent
Cosmocil CQ™	Lonza, Basel, Germany	Polyaminopropyl biguanide- based additive
Eosy®	Unitika, Osaka, Japan	Finishing agent based on chitosan
Irgaurd ® 1000	BASF, Ludwigshafen, Germany	Finishing agent based on triclosan
Irgasan	Sigma Aldrich, Missouri, United States	Finishing agent based on triclosan
Microban®	Microban International, North Carolina, United States	Triclosan-based agent
Reputex™	Lonza, Basel, Germany	PHMB-based finishing agent
Sanigard KC	L. N. Chemical Industries, Maharashtra, India	Finishing agent belonging to the QAC group
Saniguard Nano-ZN	L. N. Chemical Industries, Maharashtra, India	Finishing solution based on aqueous nano-dispersion of zinc oxide
Sanitised®	SANITIZED AG, Burgdorf, Germany	Finishing agent based on 3-trimethoxysilylpropyldimethyloctadecylammonium chloride
Silpure®	Thomson Research Associates, Toronto, Canada	Silver particles-based finishing agent
Silvadur™	The Dow Chemical Company, Michigan, United States	Interpenetrating polymer network with silver ions
SmartSilver/;®	Nanohorizon Inc. Philadelphia, United States	Silver nanoparticles-based agent
Silverion 2400	Pure Bioscience, Inc.California, United States	Stabilised silver complex-based agent

2.7 Extraction Methods for Studying Phytochemicals

2.7.1 Introduction

There are various extraction methods, e.g., solvent extraction, distillation method, pressing, and sublimation. Solvent extraction is the most widely used method where the natural products undergo a process where the solvent penetrates through the plant cell wall and the solute

dissolves in the solvents the solute followed by collecting the extract. It has been reported that the plant material, properties of the solid-to-solvent ratio, extraction temperature, and extraction time will affect the extraction efficiency (Li et al., 2008; Mordeniz, 2019a). The selectivity of the solvents, solubility, cost and safety play a crucial role in solvent extraction. Solvents with similar polarity of the solute will result in a greater yield. High temperature affects dispersion and solubility. High temperatures may result in solvents lost and the degradation of thermolabile compounds. The extraction yield increases with extraction time. The greater the solvent-to-solid ratio, the greater the extraction yield (Zhang et al., 2018). Solvent extraction is the most widely used extraction method from plant material.

2.7.1.1. Cold Extraction

In this extraction process, the plant parts are dried in a controlled environment at low temperatures and milled into a powder and weighed. The powder is added to a beaker with solvents and kept at room temperature for thirty minutes. The contents are shaken every twenty-four hours for seven days. The extract is filtered using Whatman filter paper under vacuum and dried at room temperature in a watch glass dish. The weight of the powder is recorded before and after drying (Harborne JB, 1998).

2.7.1.2 Plant Tissue Homogenization

Fresh plant parts are grounded in a blender. The solvent is added and shaken vigorously for 5–10 min or left for 24 h followed by filtration of the extract. The filtrate can be dried under reduced pressure and redissolved in the solvent to determine the concentration, or it can be centrifuged for clarification for further studies (Velavan, 2015).

2.7.1.3 Serial Exhaustive Extraction

In this extraction method, the solvent of increasing polarity from a non-polar solvent (hexane) to a polar solvent (methanol) is used to ensure a broad polarity range of compounds being extracted and to prepare crude extracts (Velavan, 2015).

2.7.1.4 Soxhlet Extraction

In this extraction method, solid material is placed in a thimble in the extractor. The solvent is heated until reflux. The vapour rises, and the solvent is condensed and fills up the thimble. The extraction is repeated (Handa et al., 2008; Patel et al., 2019).

2.7.1.5. Maceration

A whole or coarsely powdered plant is soaked in the solvent in a container for a period of continuous mixing until agitation until the biomass matter is dissolved (Velavan, 2015).

2.7.1.6 Decoction

In this extraction method, the plant parts are brought to a boil in water followed by cooling, straining, and passing sufficient cold water through the drug to produce the required volume (Handa et al., 2008).

2.7.1.7 Infusion

In this extraction method, the plant parts are macerated with either cold or boiling water (Handa et al., 2008).

2.7.1.8 Digestion

In this extraction method, the plant parts are macerated under gentle heating (Handa et al., 2008).

2.7.1.9 Percolation

In this extraction method, the raw material is placed in an appropriate amount of solvent for approximately 4 hours in a closed container. Additional solvent is added to the top of the raw material and macerated in a closed container for 24 hours. The percolator is opened, and the extract is poured out drip-wise. Additional solvent is added until the percolate measures about three-quarters of the required volume of the finished product. The marc is pressed, and the pressed liquid is added to the percolate. Additional solvent is added to produce the required volume, and the mixed liquid is clarified by filtration or by decanting (Handa et al., 2008).

2.7.1.10 Sonication

This method uses ultrasound technology to assist in the extraction of bioactive compounds under frequencies ranging from 20 kHz to 2000 kHz. The ultrasound increases the permeability of cell walls and produces cavitation and ruptures the plant cell wall (Handa et al., 2008).

2.7.1.11 Enzymatic Extraction

In this extraction method, enzymes are used to increase the yields during the extraction. Enzymes are used to soften the tissues of biomass and facilitate the degradation of the cells (Lu et al., 2017).

2.7.1.12 Microwave-Assisted Extraction

This extraction method uses microwave radiation and solvents to extract bioactive compounds. Microwave energy is generated through microwave radiation that heats the solvents whilst enhancing the kinetics of the extraction. Moisture occurs in the plant cells when heat is applied and evaporates. The pressure on the cell wall results in cell rupture. Exudation occurs and leads to an increase in extraction yield (Lu et al., 2017).

2.7.1.13 Pulsed Ultrasonic-Assisted Extraction

This is an extraction method using ultrasonic sound waves that pass through the solvent, producing energy by enhancing the diffusion of the solvent into the sample array. Pulsed Ultrasonic-Assisted Extraction is cost-effective in terms of the quantity of solvent used, temperature, and time (Lu et al., 2017). Increasing the intensity of ultrasound in a liquid lead to the breaking down of molecular structures and the formation of bubbles through a process called cavitation (Baig et al., 2010). The collapse of these bubbles produces physical, chemical, and mechanical effects, facilitating the disruption of biological membranes. This disruption aids in the release of extractable compounds, improves solvent penetration into cellular materials, and enhances mass transfer (Cares et al., 2010; Metherel et al., 2009) thus, this extraction method was used for this study.

2.7.1.14 Supercritical Fluid Extraction

In this extraction method, supercritical fluids at temperatures and pressures above the critical values are applied to the extraction and the supercritical fluids return to their gas phase and evaporate without leaving solvent residues (Lu et al., 2017).

2.7.1.15 Pressurised Liquid Extraction

This extraction method is conducted under high pressures and temperatures that aid in the high solubility of the compounds in the solvent and result in high diffusion of the solvent into the sample array (Lu et al., 2017).

Table 2.4 shows the various extraction methods used when extracting (Zhang et al., 2018).

Table 2.4: Extraction methods used in plant extractions (Zhang et al., 2018)

Method	Solvent	Temperature	Pressure	Time	Volume Consumed	The Polarity of Natural Products
Maceration	Water, Aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Percolation	Water, Aqueous and non-aqueous solvents	Room temperature, occasional heat	Atmospheric	Long	Large	Dependent on extracting solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on the extracting solvents
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Moderate	Dependent on extracting solvent
Pressurised liquid extraction	Water, aqueous and non-aqueous solvents	Under heat	High	Short	Small	Dependent on extracting solvent
Supercritical fluid extraction	CO_2	Near room temperatures	High	Short	None or small	Non-polar to moderate compounds
Ultrasound-assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Microwave-assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Short	Moderate	Dependent on extracting solvent
Pulsed electric field extraction	Water, aqueous and non-aqueous solvents	Room temperature or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Enzyme assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature or heated after enzyme treatment	Atmospheric	Moderate	Moderate	Dependent on extracting solvent

2.7.2. Chromatographic Techniques

2.7.2.1. Introduction

Chromatography is a technique used to separate molecules based on their size, shape, and charge. The analyte in the solvent passes through a molecular sieve which leads to its separation. Paper and thin-layer chromatography readily provides qualitative information.

Paper Chromatography (PC)

In this technique, a sheet of paper is used to carry out separations which act as both support as well a medium for separation. The sample is placed near the bottom of the filter paper and the filter paper is placed in the chromatographic chamber with solvent. The solvent moves forward by capillary action carrying soluble molecules along with it. Low-porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity (Coskun, 2016).

2.7.2.1.1. Thin Layer Chromatography (TLC)

This technique is used to separate the samples based on the interaction between a thin layer of adsorbent attached to the plate with low molecular weight compounds. Different adsorbents are used to separate various compounds (Coskun, 2016).

2.7.2.1.2. Gas Chromatography (GC)

This technique is used to separate volatile compounds. The rate of kinetics for the chemical species is determined through its distribution in the gas phase. Gas chromatography involves a sample being vaporized and injected into the head of the chromatographic column. The sample is transported through the column by the flow of the inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid (Coskun, 2016).

2.7.2.1.3. High-Performance Liquid Chromatography (HPLC)

This technique separates compounds based on their interactions with solid particles of a tightly packed column and the solvent of the mobile phase. The Diode Array Detector measures the absorption spectra of the analytes to aid in their identification of the compounds (Coskun, 2016).

2.7.3 Qualitative and Quantitative Phytochemical Screening

2.7.3.1 Introduction

The study of bioactive compounds encompasses phytochemical and pharmacological approaches (Foye et al., 2008). Many plant parts contain bioactive components, e.g., bark, leaves, stems, fruits, and seeds (Cragg & Newman, 2001). Phytochemicals are chemicals produced by the various parts of the plants namely, alkaloids, flavonoids, terpenoids, steroids, tannins, glycosides, etc. The bioactive compounds have various antimicrobial and antibacterial properties (Santhi & Sengottuvel, 2016). Qualitative phytochemical screening plays a crucial role in identifying various biochemical compounds produced by plants. The quantification of those metabolites may assist in the extraction, purification, and identification of the bioactive compounds for human use (Santhi & Sengottuvel, 2016).

Qualitative Phytochemical Analysis

The preliminary qualitative phytochemical screening is carried out as per standard methods described by Trease & Evans 1989:

Detection of Alkaloids

The extracts are dissolved in dilute hydrochloric acid and filtered individually and tested for the presence of alkaloids.

Mayers test: The extraction is added to the Mayers reagent. A yellow-cream precipitate formation indicates the presence of alkaloids.

Wagner's test: Wagner's reagent is added to the extraction of a brown-reddish brown formation is observed, and it indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test: A few drops of lead acetate solution are added to the extracts. A yellow-colour precipitate indicates the presence of flavonoids.

Sulfuric acid test: A few drops of sulfuric acid are added to the extracts, and the formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

A few drops of acetic anhydride are added to the extracts and the formation of violet to blue to green in some samples indicates the presence of steroids.

Detection of Terpenoids

Salkowski's Test: Extract 5 mg of the selected plant part is mixed it with 2 ml chloroform and 3 ml concentrated sulfuric acid added carefully to form a layer. A reddish-brown colour indicates the presence of terpenoids**.**

Detection of Anthraquinones

Bontrager's Test: About 5 mg of the extract is boiled with 10% HCl for a few minutes in a water bath. It's filtered and allowed to cool. An equal volume of $CHCl_3$ is added to the filtrate. A few drops of 10% NH_3 are added to the mixture and heated. The formation of pink colour indicates the presence of anthraquinones.

Detection of Phenols

Ferric chloride test: A few drops of ferric chloride are added to the 10 ml extract. A bluish-black colour indicates the presence of phenol**.**

Lead acetate test: A few drops of lead acetate solution are mixed with 10 mg extract. A yellow colour indicates the presence of phenol.

Detection of Saponins

0.5 mg of the extract is mixed vigorously with 5 ml of distilled water. The formation of frothing indicates the presence of saponins.

Detection of Tannins

A few millilitres of the extract are mixed with a few millilitres of water and heated in a water bath. The mixture is filtered. Ferric chloride is added to the filtrate. The dark green colour indicates the presence of tannins.

Detection of Carbohydrates

A 0.5 mg of the extract is dissolved individually in five ml of distilled water and filtered. The filtrate is used to test the presence of carbohydrates (Trease & Evans, 1989).

2.7.3.2 Quantitative Phytochemical Analysis

2.7.3.2.1 Estimation of Total Alkaloids

One gram of extract sample is added to a 250 ml beaker, and 200 ml of 10% acetic acid in ethanol is added, covered, and left for settling for 4 hours. The extract is filtered and concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide is added dropwise to the extract until the precipitation is complete. The solution is

allowed to settle, and the precipitate is collected and washed with dilute ammonium hydroxide, followed by filtration. The residue is dried and weighed (Harborne, 1984).

2.7.3.2.2 Estimation of Total Flavonoids

A gram of sample is extracted repeatedly with 100 ml of 80% aqueous methanol. The mixture is filtered through Whatman no.1 filter paper into a pre-weighed 250 ml beaker. The filtrate is transferred to a water bath and allowed for evaporation to dryness and followed by weighing off the sample (Santhi & Sengottuvel, 2016).

2.7.3.2.3 Estimation of Total Phenols

The sample is placed in a beaker and boiled for 15 minutes with 50 ml of ether for the extraction of phenolic compounds. Five ml of the extract is pipetted out into a 50 ml flask followed by the addition of 10 ml of distilled water, 2 ml of ammonium hydroxide solution, and 5 ml of concentrated amyl alcohol. The samples are left to react for 30 minutes for colour development and measured by spectrophotometry, reading the absorbance at 505 nm (Santhi & Sengottuvel, 2016).

2.7.4 Textiles Analysis

2.7.4.1 Biocidal Analysis

The biocidal analysis evaluates the effectiveness of antimicrobial textiles. Several test methods have been established through quantitative antimicrobial tests. The number of microbes present on the finished fabrics can be counted and expressed as a percentage or as a log reduction. The test methods for quantitative determination are ATCC TM100, JIS L1902, AATCC90 percentage reduction, and ISO 20743 shake flask reduction methods (Behary et al., 2020).

The Parallel Streak Method (AATCC TM147) is a qualitative method used to determine the antibacterial activity of diffusible antimicrobial agents on treated textile materials. The Parallel Streak Method has proven to be effective. This method shows antibacterial activity against both Gram-positive and Gram-negative bacteria. The sterilized agar is dispensed (cooled to 47 °C (117 °F) by pouring 15 ml into each standard (15 × 100 mm) flat-bottomed petri dish. Allow agar to gel firmly before inoculating. The inoculum is prepared by transferring 1.0 ml of a 24-hour broth culture into 9.0 ml of sterile distilled water and containing it in a test tube or small flask. A 4 mm inoculating loop is used, loaded with one loopful of the diluted inoculum and transferred to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart by covering the central area of a standard petri-dish without refilling the loop. The specimen is pressed onto the agar surface with a sterile spatula. After 18 to 24 hours of incubation at 37 °C, the plates are examined for bacterial growth directly

underneath the textiles and around the edges of the textiles. If the antimicrobial substance diffuses into the agar, an inhibition area is formed, and its size indicates the effectiveness of the antimicrobial effect or the rate at which the active agent is released (Neely & Maley, 2000; Gao et al., 2008). AATCC 100 (Suspension Test) is a quantitative antimicrobial test method used to determine the antibacterial activity of textiles and fabrics against bacteria. The bacterial counts are recorded, and a percent reduction is measured using the initial count and remaining count data (Gao et al., 2008).

2.7.3.2 Durability Analysis

Durability by washing method (ASTM E3162-18 or AATCC61-2A) is used to determine the durability of laundering. This test method is an accelerated laundering test method to measure the durability of antibacterial agents applied to textiles under simulated home laundering conditions. Ten grams of the coated fabric for laundering is prepared, followed by adding a 500 ml defined detergent solution. Set the washing machine at a temperature of 50 °C under abrasive action using stainless steel balls to simulate five home launderings for a 45-minute laundering cycle at 40 revolutions per minute. After each cycle, remove the fabric and rinse it with water thoroughly by hand. Repeat, depending on the total number of washes required.

Plants offer a unique source of bioactive compounds with medicinal properties and biological activities. The selection of solvents during extraction plays a crucial role in obtaining these bioactive chemicals. While synthetic antimicrobial agents have proven effective against pathogens, they also pose risks to the environment and human health. Therefore, there is a growing interest in exploring plant-based antimicrobial agents and textile finishes. By researching and developing these natural alternatives, we can enhance their longevity and durability on textile substrates, providing sustainable solutions for combating harmful microbes.

The emergence of antibiotic-resistant "super germs" presents a significant global health concern. To address this challenge, there is a need for extensive research on medicinal plants as potential sources of alternative medicines. By harnessing the bioactive properties of unexplored medicinal plants and using generally regarded as safe (GRAS) solvents during extraction, we can develop safer and eco-friendly therapeutic options. Moreover, it is essential to conduct in-depth studies to identify the most cost-effective pre-treatment, drying, and extraction methods for future therapeutics. By optimizing these processes, we can make bioactive compounds more accessible and contribute to the development of effective treatments.

In conclusion, delving deeper into the potential of medicinal plants, exploring eco-friendly extraction methods, and improving antimicrobial agents and textile finishes will have a positive impact on global health and the environment.

Chapter 3

Assessing the Efficiency of Antimicrobial Plant Extracts from *Artemisia afra* and *Eucalyptus globulus* as Coatings for Textiles.

Manuscript to be submitted for publication related to this publication.

Nortjie, E.; Basitere, M.; Moyo, D.; Nyamukamba, P. Assessing the Efficiency of Antimicrobial Plant Extracts from *Artemisia afra* and *Eucalyptus globulus* as Coatings for Textiles

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Introduction

Textiles serve as a barrier between the human skin and pathogens. The COVID-19 pandemic crisis made us aware of the importance of personal hygiene and personal protective equipment (PPE). The use of PPE, disinfectants and social distancing was important for our safety as championed by the World Health Organization (Chauhan & Kumar, 2020). Textile coatings improve the functional properties of textiles, creating a value-added product that offers comfort, strength, durability, and better handling. Other functional finishes on textiles may include flame retardancy, water repellency, anti-dirt, antibacterial, and antiviral properties (Ibrahim et al., 2017).

Many synthetic fibers are more resistant to microbial attack than natural fibers. Natural fiber-based fabrics, such as cotton, contain keratinous fibers and carbohydrates that serve as nutrients under optimal conditions, making them prone to microbial attack (Hipler & Elsner, 2006). According to a report by Fortune Business Insights (2020), the global antimicrobial textile market size was valued at USD 9.04 billion in 2020 and is expected to reach USD 13.63 billion by 2028, with a compound annual growth rate of 5.3% from 2021 to 2028. The report attributes the growth of this market to the increasing demand for antimicrobial textiles in medical and apparel applications (Fortune Business Insights, 2021).

There is a wide range of natural to synthetic antimicrobial products on the market. Synthetic antimicrobial agents pose a great concern due to side effects and water pollution hence the great demand for antimicrobial agents that fulfil the requirements of the regulating authorities (Ramachandra & Rao, 2008). While herbal extracts have demonstrated effective antibacterial properties, the longevity of the coating when subjected to laundering is inadequate (Bouchekrit et al., 2016).

Natural compounds have been extensively explored for new drug discoveries (Chandra et al., 2017). Extracts of different parts of plants contain bioactive compounds, such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols, terpenoids, and flavonoids that fight against diseases (Kutama, 2018). The phenolic phytochemicals from plants play a key role as antimicrobial agents (Restuati & Diningrat, 2018; Smeriglio et al., 2017).

Artemisia afra and *Eucalyptus globulus* plant species have been selected for this study as they may possess antimicrobial properties. Studies have shown that the mode of action of the antimicrobial agents applied to textiles can result in various effects, including the agglomeration of cell proteins, harm to the cell membrane, interference with its function, inhibition of protein synthesis, and suppression of nucleic acid synthesis (Kamel & Hassabo, 2021).

Antibiotic resistance to pathogenic bacteria has become a global problem, leading to natural-based antimicrobial compounds being studied extensively as an alternative therapeutic strategy to fight against microbial growth (Savoia, 2012; Upadhyay et al., 2014).
Antibiotics play a vital role in fighting bacterial infections. They also cause havoc in the healthcare and pharmaceutical sector, accelerating socio-economic losses (O'Neill, 2016). Multidrug resistance is expected to increase by ten million deaths annually by 2050 (O'Neill, 2016; Kraker et al., 2016). Biological screening, separation of the phytochemicals, and clinical trials of medicinal plants have advanced over the years, unfolding the secrets of ancient herbal remedies (Bibi et al., 2017). Traditional medicine is effective in dealing with diseases caused by bacteria or oxidative stress (Baydoun et al., 2015; Singh et al., 2017; Restuati & Diningrat, 2018). Despite the announcement by the World Health Organization in the seventies of *Artemisia afra* being unfit for human consumption. Till today, *Artemisia afra* is used for ethnomedicinal purposes in South Africa (Oyedeji et al., 2009). Eucalyptus species are the most represented species in the international pharmacopoeia in which *E. globulus* are the main supply of essential oils due to their medicinal properties (Bachir & Benali, 2012).

Therefore, the purpose of this study was to investigate whether plant extracts derived from South African biomass, namely *Eucalyptus globulus* and *Artemisia afra*, could be effective as antimicrobial agents or textile coatings. The work involved the preparation of extracts containing antimicrobial compounds by pulsed ultrasound-assisted extraction using polar and non-polar solvents separately, qualitative phytochemical screening analysis on *Eucalyptus globulus* and *Artemisia afra* to identify the bioactive compounds from the selected plants, and the application the extracts onto cotton and polyester fabrics and assess the antimicrobial activity before and after laundering and the characterisation of the coated samples using Fourier-transform infrared spectroscopy (FTIR).

3.2 Materials and Methods

3.2.1 Sources of plant material and fabric

The leaves of the medicinal plant *Artemisia afra*, except for *Eucalyptus globulus*, were acquired from the South African National Biodiversity Institute located at Kirstenbosch Gardens. The biomass of *Eucalyptus globulus* was collected from Deer Park in Vredehoek, Cape Town. Both *Artemisia afra* and *Eucalyptus globulus* plant specimens were identified by Mr. Pieter Winter at the South African National Botanical Institute (SANBI), with the assistance of Mr. Tielmann Haumann. The commercially available woven cotton fabric was obtained from SA Interlining Company, while the knitted polyester fabric was procured from Priontex Company with the assistance of Mr Rainer Absenger.

3.2.2 Plant sample preparation

The leaves of *Artemisia afra* and *Eucalyptus globulus* plants were harvested and thoroughly washed with distilled water to remove any dust particles. The leaves were then air-dried in the shade at room temperature for 30 days. Phytochemicals were extracted from the dried leaves using methanol and n-hexane solvents. The resulting extract was evaporated to determine the yield of the extractions.

3.2.3 Plant extraction

The solvent extracts were obtained using the pulsed ultrasound-assisted extraction (PUAE) method by applying a 1:10 (w/v) solid-solvent ratio (Green, 2004). Methanol and hexane were selected for their ability to extract polar and non-polar compounds, respectively, and their similarity with previous and future studies.

About 10 g of powdered leaves were extracted by sonicating in 100 ml of methanol and hexane. The PUAE was conducted at a solid-to-liquid ratio of 1:10 for 20 minutes at 40°C, at 50% amplitudes with a pulse of 0.5 s on and 5 s off at a 20 kHz frequency with a probe diameter of 25 mm placed at a depth of 10 mm into the medium. Post-extraction, the mixtures were filtered through a Whatman LSC601 70 mm filter paper disc. The solvents were removed using MiVac Sample Evaporation at 40°C.

The extraction yield of the plant extracts obtained through PUAE was determined, as depicted in Table 3.1. Subsequently, these extracts were reconstituted using their respective solvents and then stored at a temperature of -20°C.

Table 3.1: The average yield percentage of the plant extracts obtained by the PUAE method

Average Yield % = $\frac{\text{Weight of the extract after solvent removal}}{\text{Dry weight of plant sample}} * 100\%$			
Plants (Leaves)		*A. afra*	*E. globulus*
Solvents	Methanol	9.22	22.76
	Hexane	2.16	3.98

Table 3.2 shows the extraction conditions used in this study.

Table 3.2: Extraction conditions

No.	Parameter	PUAE extraction
1	Sample size (g)	10 g
2	Extraction solvent	n-hexane and methanol
3	Solvent volume (ml)	100 ml
4	Temperature (°C)	40 °C
5	Time (min)	20 minutes

Table 3.3 shows the physiochemical properties of the solvents used.

Table 3.3: Physiochemical properties of solvents used (n-Hexane and Methanol) [50]

No.	Solvent	Polarity index (pi)	Boiling point °C	Viscosity @ 20°C (cP)	Density @ 20°C (g/ml)
1	n-Hexane	0.1	67-69.5	0.31	0.672
2	Methanol	5.1	64.7	0.55	0.791

3.2.4 Preparation of antimicrobial cotton and polyester material

The fabric was cut into squares measuring 3 cm x 3 cm. Afterwards, it was washed with a mixture of ethanol and water using ultrasonication for 30 minutes to eliminate any wax and impurities on its surface. Next, the fabric was dried for an hour at 80 °C in a warm air oven. Plastic containers were then used to immerse the fabric cuttings in the chosen extracts. The cuttings were left to soak for 24 hours at 25 °C in an FHM incubator shaker and then dried in a warm air oven at 80 °C for one hour to eliminate the solvent. Finally, the fabric cuttings were utilized to determine the zone of inhibition against *S. aureus* ATCC 33591 and *E. coli* ATCC 25922.

3.2.5 Experimental method and procedure

The process flow diagram of the study is shown in Figure 3.1 below.

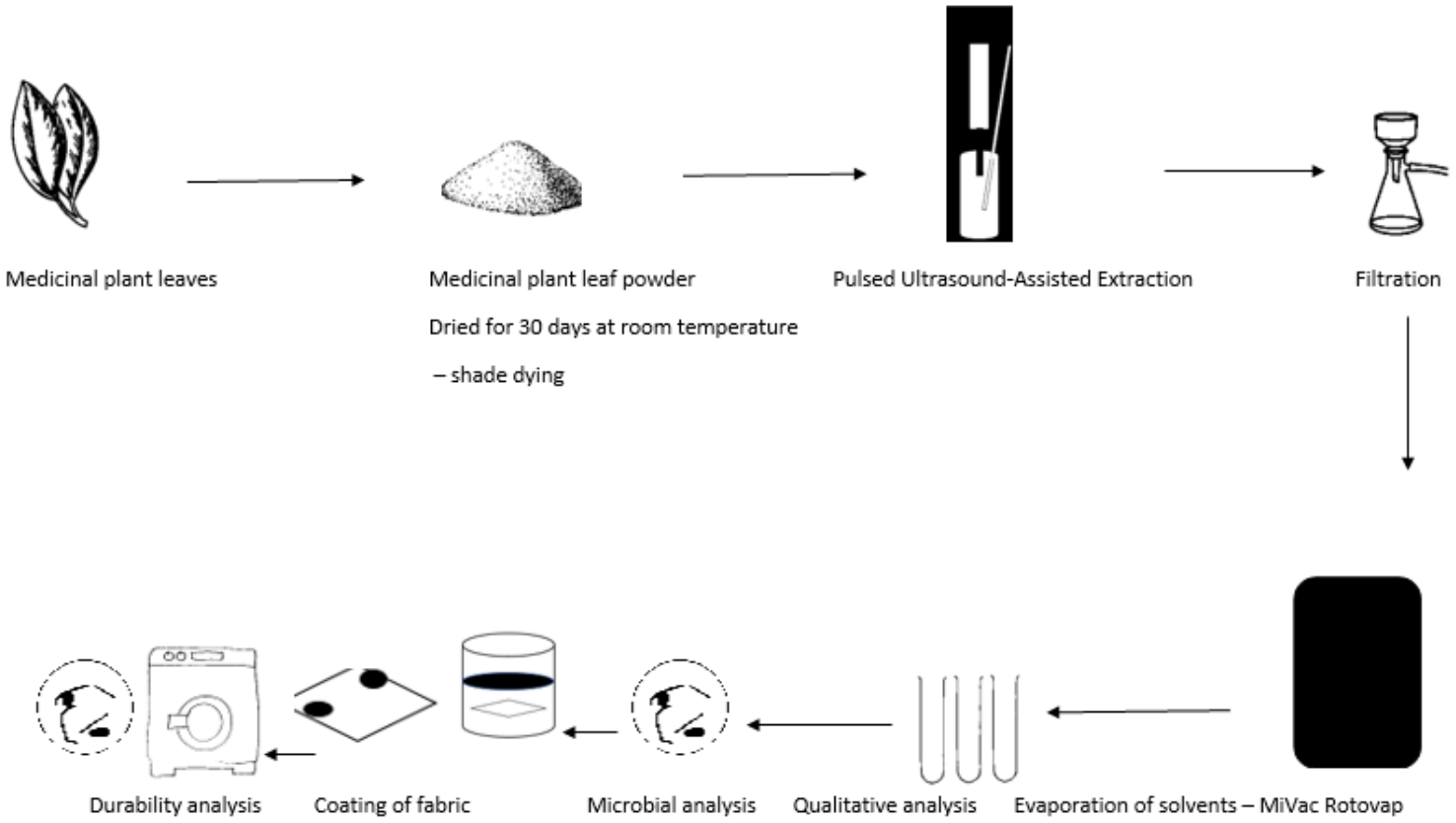


Figure 3.1: Process flow diagram of the experimental setup of the antimicrobial textiles

Experiments were carried out to determine various factors related to the effectiveness of coated fabrics in preventing microbial growth. Specifically, the Minimum Inhibitory Concentration (MIC), the Zone of Inhibition (ZOI), and the durability of the coated fabric after undergoing a laundering process according to ISO 6330:2012E were analyzed. The antimicrobial effectiveness of the coated fabric was then evaluated by measuring the ZOI against the selected strains *S. aureus* ATCC 33591 and *E. coli* ATCC 25922, as well as appropriate controls.

3.2.6 Characterizations

The functional groups present in the woven cotton and knitted polyester fabric were analyzed before and after the application of an antimicrobial finish. Fourier-transform infrared spectroscopy (FTIR) was used to perform the analysis, using a Cary 660 spectrometer from Agilent Technologies (USA). The wavenumber range of 400–4000 cm^{-1}

3.2.7 Determination of the inhibition zone

The nutrient and tryptic soy broth growth media for the selected test strains, *Staphylococcus aureus* ATCC 33591 and *Escherichia coli* ATCC 25922 respectively, were autoclaved for 25 minutes at 120 °C and under pressure. Once cooled, the liquid media were inoculated with agar plate cultures. The liquid cultures were incubated at 37 °C at 160 rpm for 24 hours. The next day, the cultures were analyzed by Gram staining to ensure no contamination. The optical density (OD) of the cultures was determined at a wavelength of 600 nm using a Perkin-Elmer Lambda-25 spectrophotometer and diluted to an OD600 = 0.2. Extracts were serially diluted in the range of 5 µg - 500µg, and 50µl of each sample was pipetted into a sterile 96-well plate in triplicate, and the plate was sealed with a Breath-Easy seal to allow for respiration. The plates were incubated at 37°C for 24 hours. Following that, 20 µl of 0.25 % (w/v) MTT solution (in phosphate-buffered saline) was added to each experimental well and incubated for 4 hours at 37 °C. The reaction was stopped by adding 100 µl of 100 % DMSO, and the OD was determined using a microtiter plate reader. To minimize errors, each sample was evaluated in its 96-well plate: for twelve samples, twenty-four plates of 96-well plates (two test strains). The minimum inhibitory concentration (MIC) is determined as the lowest concentration at which total inhibition of the test strain occurs. Controls included wells with media and test strains without any additions.

3.2.8 Determination of the zone of inhibition

Step 1: Preparation of liquid cultures
Specific liquid growth media (5 ml per 50 ml glass flask; five flasks per strain) were prepared for each test strain:

Staphylococcus aureus ATCC 33591	Nutrient broth
Escherichia coli ATCC 25922	Tryptic soy broth

The cultures were analysed by Gram staining to ensure that the cultures were not contaminated. The optical density of the cultures was determined at 600 nm using a Perkin-Elmer Lambda-25 spectrophotometer and diluted to an OD600 = 0.2. These diluted cultures were used in the agar diffusion test (Marković et al., 2018).

Step 2: Preparation of agar plates
A total of twelve samples, twelve agar plates per test strain were prepared. This required the preparation of the following (also including plates for controls):

25 tryptic soy agar plates = 500 ml of media
25 nutrient agar plates = 500 ml of media

Each sample was assessed in triplicate on each plate and was prepared as follows:
For each agar plate, 100 µl of the OD600 = 0.2 cultures was spread-plated onto the agar and allowed to diffuse into the agar. Each textile specimen was pre-treated with UV (30 minutes on each side) to kill off any contaminants and then placed onto the agar plate containing the test strain (to ensure that the UV treatment was effective, the material was also placed on agar containing no test strain). For each test strain, a commercial antibiotic was used as a positive control. Gentamicin and ampicillin were used for the bacterial test strains. Samples were plated on agar plates and incubated at 37 °C overnight. The zones of inhibition were measured to determine whether growth inhibition occurred as reported by (Marković et al., 2018).

3.2.9 Durability Tests

The durability of the extract coating applied to textiles under simulated home laundering conditions was assessed using the washing method ISO 6330:2012. The coated fabric samples were cut into 3 x 3 cm squares and weighed to determine the amount of washing powder and water required for laundering. The fabric samples were washed at 60 °C using the Pyrotec MB2 washing machine, rinsed in cold water, and dried in a hot air oven at 80 °C for 1

hour. Each textile sample was pre-treated with UV for 30 minutes on each side to kill off contaminants and then placed onto the agar plate containing the test strain. The material was also placed on agar containing no test strain to ensure that the UV treatment was effective. The agar plates were incubated at 37 °C overnight, and the zones of inhibition were measured.

3.2.10 Phytochemical screening of *Artemisia afra* and *Eucalyptus globulus*

The extracts were subjected to phytochemical screening of bioactive compounds. The methanolic and hexanoic extracts of the leaves were screened for the presence of phenols, flavonoids, quinones, tannins, saponins, terpenoids, and steroids. The quantitative results are expressed as (+) for positive and (−) for negative results.

3.2.10.1 Detection of Phenols

The extract of 1.0 ml was diluted in distilled water to 3.0 ml followed by filtration. Four drops of 5% iron (III) chloride solution were added to the solution. The dark green colour indicated the presence of phenol (Yimam & Desalew, 2022).

3.2.10.2 Detection of Flavonoids

The extract of 1.0 ml was transferred into a test tube and treated with four drops of sodium hydroxide solution. The yellow colour indicated the presence of flavonoids (Yimam & Desalew, 2022).

3.2.10.3 Detection of quinones

The extract of 1.0 ml was placed in a test tube and mixed with 1.0 ml of pure 98% sulphuric acid. The red colour showed the presence of quinones (Yimam & Desalew, 2022).

3.2.10.4 Detection of Tannins

About 1 ml aliquot of the extract was transferred into a 5 ml test tube followed by the addition of three drops of 5% Iron (III)chloride, and a greenish-black precipitate showed the presence of tannins (Yimam & Desalew, 2022).

3.2.10.5 Test for saponins

About 0.5 mg aliquot of the extract was mixed vigorously with 5 ml of distilled water. The formation of stable foam indicated the presence of saponins (Yimam & Desalew, 2022).

3.2.10.6 Test for steroids

About 1.0 ml of extract was shaken with 99% chloroform, and to the chloroform layer, 98% sulphuric acid was added slowly by the sides of the test tube. The red colour showed the presence of steroids (Yimam & Desalew, 2022).

3.2.10.7 Test for terpenoids

About 0.5 ml aliquot of the extract was mixed with 2.0 ml of 99% chloroform followed by 3 ml concentrated of 98% sulphuric acid added to form a layer. The formation of the reddish-brown colour showed the presence of terpenoids (Odutayo et al., 2017).

3.3 Results and Discussion

3.3.1 Extraction Yield

The average yields of methanolic and hexanoic extracts from *Artemisia afra* and *Eucalyptus globulus* are summarised in Table 3.1. All experiments were conducted in triplicates. The extraction yields of *A. afra* and *E. globulus* were investigated by studying the effects of various solvents, including methanol and hexane. The findings demonstrated that the extraction yields differed depending on the solvent used, attributed to variations in solvent polarity. The yield of extraction is a measure of solvent effectiveness in extracting phytochemicals from the material under selected conditions. The results in Table 3.1 show that the maximum yield was obtained with *E. globulus* methanol extraction at 22.76%, followed by hexane extraction at 3.98%. *A. afra* methanol extraction yielded 9.22%, followed by hexane extraction of 2.16%. Higher yields were observed in the methanolic extracts of *A. afra* and *E. globulus* compared to the hexanoic extracts. Notably, *A. afra* produced the lowest yield. Overall, the yields of methanolic and hexanoic extracts differed significantly. The improved extraction yields could be attributed to cavitation effects caused by high-intensity ultrasound and solvent polarity.

3.3.2 Phytochemical Screening Analysis

Plant parts contain bioactive compounds with various phytochemical compounds, including alkaloids, flavonoids, steroids, terpenoids, tannins, saponins, phenols, and quinones, all of which possess antimicrobial properties. In this study, the crude extracts were screened for the presence of phenols, flavonoids, quinones, tannins, saponins, terpenoids, and steroids. The phytochemical screening of *Artemisia afra* and *Eucalyptus globulus* crude leaf extracts is shown in Table 3.4, and it revealed the presence of different secondary metabolites, such as phenols, quinones, and steroids. Tannins, saponins, and terpenoids were not found in the hexanoic extracts of *Artemisia afra.* Flavonoids were absent in the methanolic extraction of *Eucalyptus globulus.* Tannins and saponins were also not found in the hexanoic extraction of *Eucalyptus globulus*.

Table 3.4: Phytochemical compounds of *A. afra* and *E. globulus* extract

Plant	*Artemisia afra*		*Eucalyptus globulus*	
Solvent	**Methanol**	**n-Hexane**	**Methanol**	**n-Hexane**
Phytochemicals				
Phenol	+	+	+	+
Flavonoids	+	+	-	+
Quinones	+	+	+	+
Tannins	+	-	+	-
Saponins	+	-	+	-
Terpenoids	+	-	+	+
Steroids	+	+	+	+

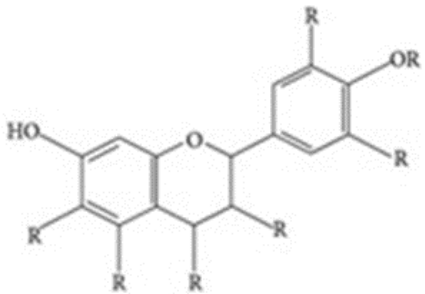
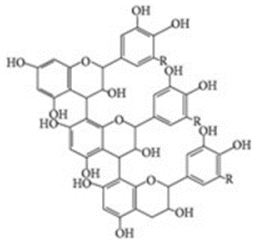
-: absence, +: presence

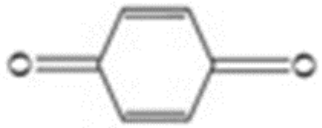
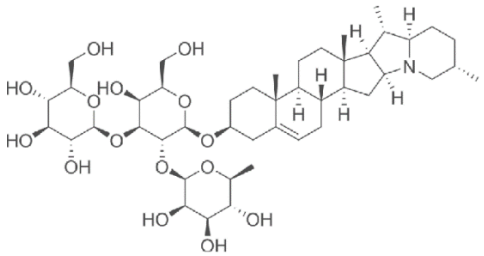
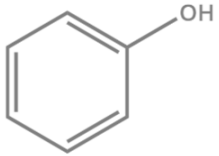
Phytochemical screening revealed the presence and absence of different secondary metabolic compounds such as phenols, quinones, terpenoids, flavonoids, saponins, steroids and tannins. This could be attributed to the different plants, plant parts, types of solvents used for extraction, and geographical areas exhibiting climatic conditions that determine their ability to support rain-fed cultivation (Kane et al., 2019).

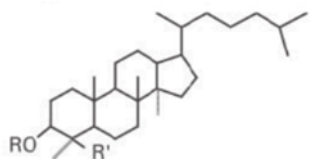
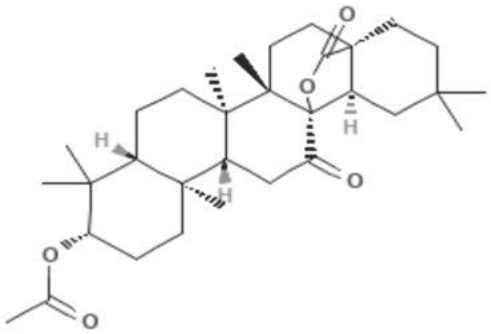
Phytochemical screening of crude leaf extracts of *A. afra* and *E. globulus* revealed that both plants had phenols, quinones, and steroids. The phytochemical screening indicates that the methanol extract produced positive results for the analysed phytochemicals. Many solvent extractions have been performed to obtain phytochemical compounds for use against pathogens. From the phytochemical assessment, phenols were found in all the extractions. The mechanism of action of polyphenols is to bind to adhesins, inhibit enzyme-substrate deprivation, complex with the cell wall, and disrupt the membrane and metal ion complexation (Cowan, 1999; Tiwari et al., 2011). Quinones were also found in all the extractions. The mechanisms of action of quinones include inactivating enzymes, complexing with the cell wall, and binding to adhesins (Tiwari et al., 2011; Cowan, 1999). Tannins were present in the methanolic extracts, as shown in Table 3.4. Tannins allow the binding of adhesins, inhibit enzyme-substrate deprivation, complex with the cell wall, and form metal ion complexes (Ameer et al., 2017). Flavonoids were present in most of the extracts except for the *Eucalyptus*

globulus methanolic extracts. Flavonoids form a complex with the cell wall and bind to adhesins (Cowan, 1999; Tiwari et al., 2011). The extracts were also screened for terpenoids which disrupt the membrane wall (Tiwari et al., 2011; Cowan, 1999) The extracts were screened for saponins, and they were only present in the methanolic extracts. The mechanism of action of saponins inhibits the growth of Gram-positive bacteria and Gram-negative bacteria by disrupting cell membranes (Tiwari et al., 2011; Cowan, 1999). The extracts were screened for steroids, and they were found in all the extracts. Steroids derived from medicinal plants are known to possess antibacterial and insecticidal properties (Ndezo Bisso et al., 2022). Table 3.5 displays the mechanisms of action of the phytochemical compounds found in the studied plant extracts.

Table 3.5 Phytochemical compounds and their mechanisms of action

Phytochemical compounds	Chemical profile	Mechanism of action
Flavonoids		The mechanism of action of flavonoids is complex with the cell wall, binds to proteins (adhesins), inhibits the secretion of autocoids and prostaglandins and inhibits contractions caused by spasms (Cowan, 1999; Tiwari et al., 2011).
Tannins		The mechanism of action of tannins allows the binding of proteins (adhesins), inhibits enzyme-substrate deprivation, complexes with the cell wall, makes intestinal mucosa more resistant and reduces secretion, increases the supply of digestible proteins by animals by forming protein

		complexes in the rumen, and causes a decrease in gastrointestinal-tract metabolism (Cowan, 1999; Tiwari et al., 2011).
Quinones		The mechanisms of action of quinones inactivate enzymes, maybe adhere to the cell wall, and bind to proteins (Cowan, 1999; Tiwari et al., 2011).
Saponins		The mechanism of action of saponins inhibits histamine release in-vitro (Cowan, 1999; Tiwari et al., 2011).
Phenols		The mechanism of action of polyphenols binds to proteins (adhesins), inhibit enzyme-substrate deprivation, complex with the cell wall, make intestinal mucosa more resistant and reduce secretion, increase the

		supply of digestible proteins by animals by forming protein complexes in the rumen, and causes a decrease in gastrointestinal-tract metabolism (Cowan, 1999; Tiwari et al., 2011).
Steroids		Steroids derived from medicinal plants are known to possess antibacterial and insecticidal properties (Ndezo Bisso et al., 2022).
Terpenoids		Disrupt the membrane wall (Tiwari et al., 2011; Cowan, 1999).

3.3.3 Antibacterial Activity of plant extracts

The antibacterial activity of plant extracts *A. afra* and *E. globulus* was investigated against Gram-positive bacteria, *Staphylococcus aureus* ATCC 33591, and Gram-negative bacteria, *Escherichia coli* ATCC 25922, and the results are shown in Figure 3.2.

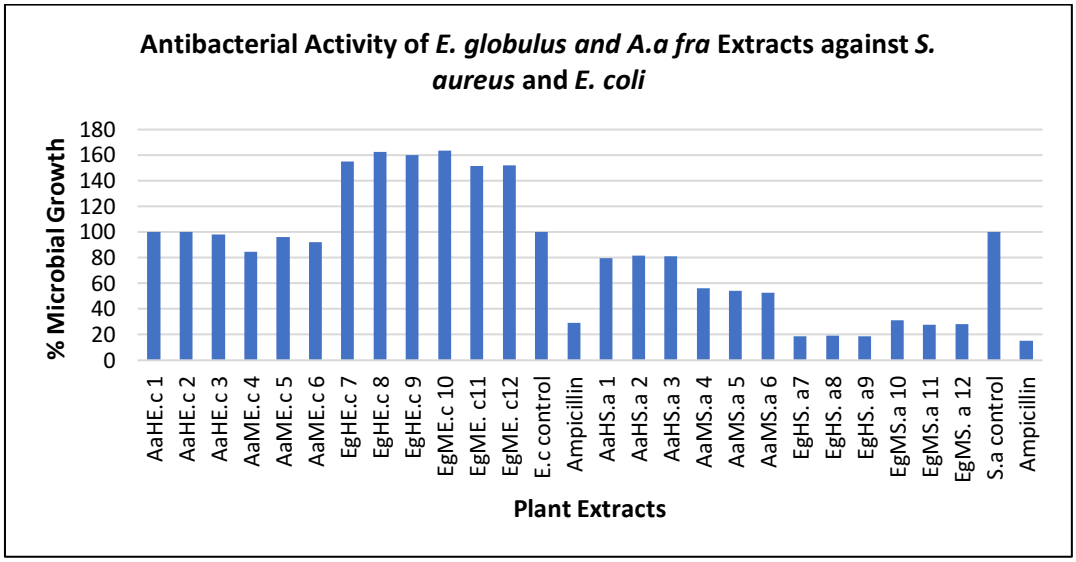


Figure 3.2: Antibacterial Activity of plant extracts *A. afra and E. globulus* against *S. aureus* and *E. coli*. A.a: *Artemisia afra*; E.g: *Eucalyptus globulus*; H: Hexane; M: Methanol.

The hexanoic *E. globulus* extracts showed good results in suppressing microbial growth of *S. aureus* ATCC 33591 at an average percentage of 18.70% growth due to a higher number of phytochemicals like polyphenolic compounds, present which are known to have antimicrobial activity, followed by the methanolic *E. globulus* extraction at an average of 29.03% growth. The *A. afra* methanolic extractions showed antibacterial activity against *S. aureus* at an average of 54.20%, and the hexanoic *A. afra* extracts at an average percentage growth of 80.90% exhibited the least activity against *S. aureus* ATCC 33591 strains at 250 µg/ml. The *S. aureus* microbial growth was observed to be more susceptible to the *E. globulus* hexanoic extracts, followed by the *E. globulus* methanolic extracts compared to the *A. afra* methanolic and hexanoic extracts. As expected, the control showed no antimicrobial activity (Figure 3). Ampicillin shows antimicrobial activity against *S. aureus* ATCC 33591 at an average growth of 15.27% as expected.

The evaluation of the *A. afra* and *E. globulus* extracts against *E. coli* ATCC 25922 was the least susceptible compared to *S. aureus*. The methanolic *A. afra* shows slight activity against *E. coli* ATCC 25922 at an average percentage growth of 91%, followed by the hexanoic *A. afra* extracts at 99.52% (no effect). The methanolic and hexane *E. globulus* extracts show no effect

against Gram-negative *E. coli* ATCC 25922. Instead, they showed enhanced growth of an average of 159.25% and 155.70% at 250 µg/ml, respectively. This could be due to compounds from plants that enhance bacterial growth. As expected, the control sample did not show any antimicrobial activity. Ampicillin shows antimicrobial activity against *E. coli* ATCC 25922 at an average growth of 29.34%. The results show that *E. coli* ATCC 25922 was the most resistant strain against the plant extracts. *S. aureus* ATCC 33591 was the most susceptible strain to the plant extracts.

3.3.4 Minimum Inhibition Concentration Analysis

The MIC, which stands for Minimum Inhibitory Concentration, is defined as the lowest concentration of the extract that can prevent the growth of microorganisms. To test the extract's effectiveness against different strains of bacteria, specific liquid growth media were prepared for each test strain in 50 ml glass flasks at a volume of 5 ml per flask, with three flasks per strain. The results showed that only some of the samples displayed activity in the liquid media against the tested ATCC bacterial strains, as detailed in Table 3.6.

Table 3.6: Summary of the antibacterial activity detected against two ATCC test strains when a concentration range of 5-500 μg/ml was evaluated in a 96-well bioactivity assay. *S. aureus*: *Staphylococcus aureus*; *E. coli*: *Escherichia coli;* A.a: *Artemisia afra*; E.g: *Eucalyptus globulus*; H: Hexane; M: Methanol

	MIC Values (μg/ml)	
	Microorganisms	
Sample No. **Plant extract**	**Gram-negative bacteria *E. coli* ATCC 25922 (Tryptic soy broth)**	**Gram-positive bacteria *S. aureus* subsp. *aureus* ATCC 33591 (Nutrient broth)**
1 A. aH	50-500 μg/ml	5-25 μg/ml; 250-500 μg/ml
2 A. aH	10-500 μg/ml	125-500 μg/ml
3 A. aH	None	50-500 μg/ml
4 A. aM	5-500 μg/ml	5-500 μg/ml
5 A. aM	5-500 μg/ml	5 μg/ml; 125-500 μg/ml
6 A. aM	10-500 μg/ml	100-500 μg/ml
7 E. gH	5-50 μg/ml*	None
8 E. gH	250-500 μg/ml	None
9 E. gH	100-500 μg/ml	None
10 E. gM	None	None
11 E. gM	None	250-500 μg/ml
12 E. gM	None	None

***If activity is only observed at lower concentrations, it usually means that a compound inhibitor is being diluted out, resulting in activity only being observed at the lower (more dilute) sample range. Samples 1-12 were performed in triplicates.**

The study evaluated the MIC values of methanolic and n-hexane extracts of *A. afra* and *E. globulus* leaf extracts against *S. aureus* and *E. coli*. Although some bioactivity was observed, complete inhibition of the cultures did not occur, and all MIC values were above 500 µg/ml. The antimicrobial tests yielded MIC values ranging from 5 µg/ml to above 500 µg/ml, as shown in Table 4. The methanol extract of *A. afra* exhibited sensitivity against the *S. aureus* ATCC33591 strain, with a MIC value ranging from 5 µg/ml to 25 µg/ml. However, some extracts, such as *E. globulus* hexanoic extract against *S. aureus* and *E. globulus* methanolic extract against *E. coli* ATCC 25922, did not show any MIC value. Bacterial culture growth exceeded 100% during sample analysis, possibly due to plant compounds that promote bacterial growth. Samples that exhibited antimicrobial activity in liquid-based assays did not show bioactivity in agar-based assays.

3.3.5 Zone of Inhibition Analysis

The zone of inhibition analysis (ZOI) was conducted against both bacterial types *S. aureus* and *E. coli*. The antimicrobial fabric was produced by using the *A. afra* and *E. globulus* methanolic and hexanoic extracts by immersion process for 24 hours. The green polyester and light blue cotton fabric per sample were used for the zone of inhibition analysis. The fabrics were cut into 2 cm diameter circles and subjected to the protocol described above. Only some samples exhibited bioactivity as shown in Table 3.7.

Table 3.7: Summary of the bioactivity detected for material samples infused with plant extracts evaluated against *E. coli* ATCC 25922 and *S. aureus* subsp. *aureus* ATCC 33591.

Sample No. Plant extract	Material type	Disk diameter	Zone diameter	Zone of inhibition
10 E. gMC	Light blue/grey cotton	20 mm	23 mm	101.32 mm^2
10 E. gMP	Turquoise polyester	20 mm	22 mm	65.97 mm^2
11 E. gMC	Light blue/grey cotton	20 mm	27 mm	258.40 mm^2
11 E. gMP	Turquoise polyester	19 mm	22 mm	96.60 mm^2
12 E. gMC	Light blue/grey cotton	20 mm	25 mm	176.71 mm^2

* **Sample 10 E.gMC: Light blue grey *E. globulus* methanolic coated cotton fabric; 10 E.gMP: Turquoise *E. globulus* methanolic coated polyester fabric; 11E.gMC: Light blue grey methanolic cotton coated fabric; 11E.gMP: Turquoise *E. globulus* methanolic coated polyester fabric; 12 E.gMC: Light grey/ blue methanolic coated cotton fabric. Samples 1-12 were performed in triplicates on the light blue/ grey cotton and turquoise polyester fabrics**.

Moist environments promote bacterial growth, and textiles often serve as a breeding ground for bacteria. To assess the antibacterial properties of fabrics, we examined coated and uncoated polyester and cotton samples against Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria. As indicated in Table 6, only samples 10 to 12 exhibited zones of inhibition. Cotton fabrics, which contain cellulose polymer, facilitate bacterial adherence, whereas polyester fabrics, with their polymer backbone, are less susceptible to bacterial attachment. However, the application of *Eucalyptus globulus* methanolic extracts onto fabrics altered the fabric's surface properties. Our study revealed that samples 10 to 12, which were coated with the extract, displayed antibacterial activity, as illustrated in Figure 3.3 by the presence of zones of inhibition around the material.

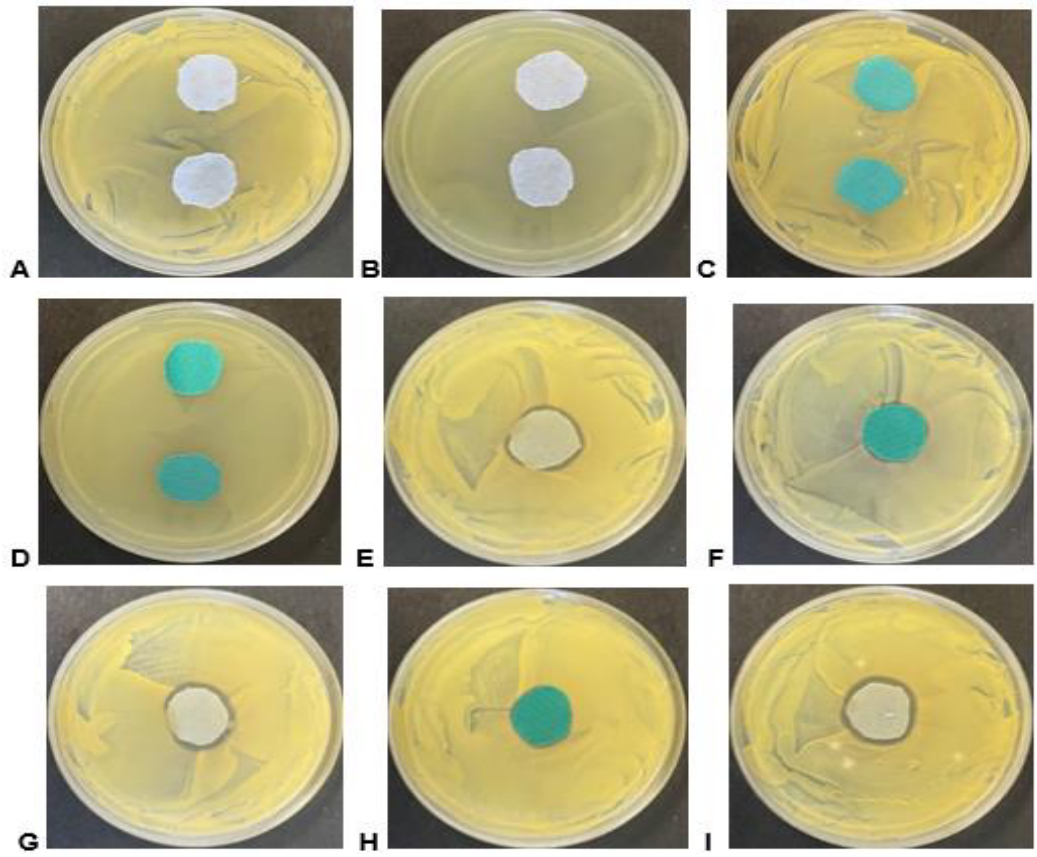


Figure 3.3 Bioactivity of material samples on agar plates containing the ATCC test strains. A: Light blue/grey cotton material control sample on *S. aureus*; B: Light blue/grey cotton material control sample on *E. coli*; C: Turquoise polyester material control sample on *S. aureus;* D: Turquoise polyester material control sample on *E. coli*; E: Sample 10 light blue/grey cotton material on *S. aureus;* F: Sample 10 turquoise polyester material on *S. aureus;* G: Sample 11 light blue/grey cotton material on *S. aureus;* H: Sample 11 turquoise polyester material on *S. aureus*; I: Sample 12 light blue/grey cotton material on *S. aureus.*

Figure 3.3 shows that the coated fabric demonstrated a larger zone of inhibition compared to the untreated samples. Among the coated fabrics, the highest zone of inhibition was observed

in cotton coated with *E. globulus* methanolic extract (258.4 mm^2) against *S. aureus*, while the smallest zone of inhibition was observed in polyester coated with *E. globulus* methanolic extract (65.97 mm^2) as depicted in Figure F, sample 10 (turquoise material) on *S. aureus*. The bioactivity was only evident against the *S. aureus* strain. Although some samples displayed activity in liquid media assays, they failed to exhibit bioactivity in the agar-based assay. The inactivation of active compounds during the treatment or preparation of the material samples or the enhanced absorption of other active compounds, when applied to the material, could be the potential reasons for this. The bioactivity seen in such assays also relies on the diffusibility of compounds from the material into the agar medium, leading to the inhibition of growth. It is essential to note that bacterial growth is rampant in humid conditions (Marković et al., 2018). Natural fibers like cotton are more susceptible to microbial attack because they easily retain water. In contrast, the microbial attack is slower in synthetic fibers such as polyester fabric due to their polymer backbone (Alaribe et al., 2018).

3.3.6 Durability to Washing

Frequent washing of coated fabrics may affect the durability of the coating on the fabric as it plays a key role in coated textiles. This study also focuses on the effect of laundering on the coated textiles against the selected bacterial strains. The ZOI of the microorganisms were measured to determine whether growth inhibition occurred.

After laundering, no activity was observed against *S. aureus* and *E. coli*. This could be due to the inactivation of active compounds during sample treatment or preparation, or the improved bioavailability of other active compounds when applied to the material. On agar plates containing ATCC test strains, no bioactivity was observed on the material samples after laundering. The antimicrobial activity of the samples that had previously shown activity against the selected bacterial strains decreased significantly after washing. The inactivity of the coated fabrics may be due to factors such as reduced extract concentration, variation in phytochemicals per extract, solvent polarity, and the coating technique used to apply the extracts onto the fabrics.

To further validate that the fabric was modified by the extracts of *A. afra* and *E. globulus*, an FTIR study was conducted to understand the chemical attachment. The FTIR spectra of uncoated and coated polyester and cotton fabric are presented in Figures 5-12.

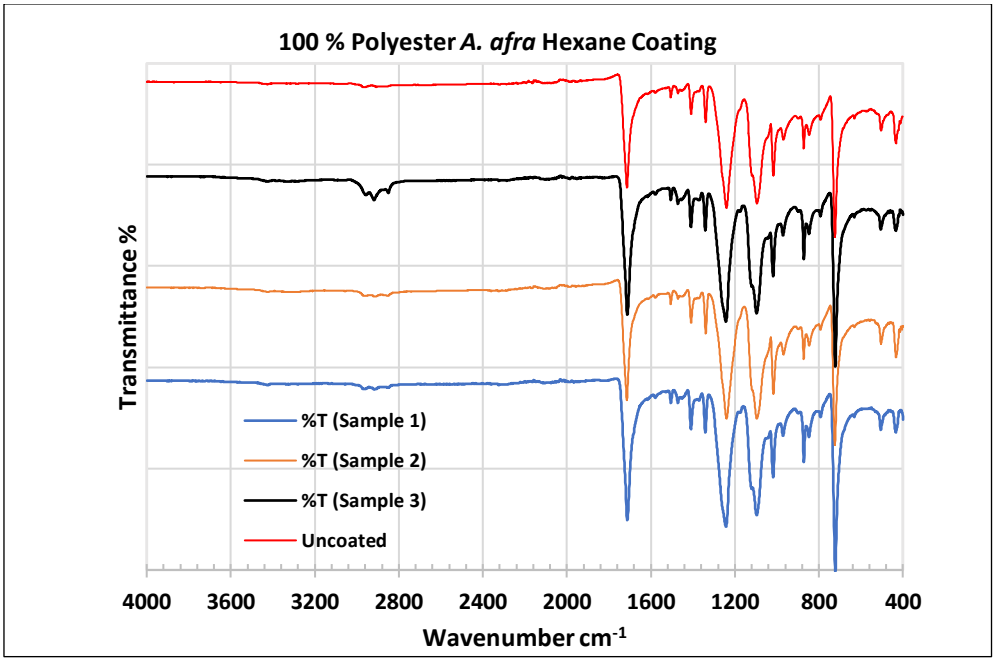


Figure 3.4: FTIR spectra of polyester fabrics uncoated (U) and coated (1-3) with *A. afra* hexane coating. Samples 1-12 were performed in triplicates.

FTIR spectra analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and Nandiyanto et al., (2019), for the uncoated polyester fabric, the peak range of 1715 cm $^{-1}$ indicates the C=O group. The peaks in the region 1502 cm^{-1} indicate vibrations of an aromatic ring and peaks at 1473 cm^{-1} indicate the CH_2 bending group, and at 1411-1409 cm $^{-1}$ indicate vibrations of an aromatic ring. The peaks at 1342-1340 cm $^{-1}$ represent a CH_2 wagging vibration of trans-co-formation, and the peak 1097 cm^{-1} indicates CH in-plane bending modes of an aromatic ring. Strong peaks at 1243-1241 cm^{-1} indicate C–O stretching bonds. Peak 967 cm^{-1} indicates trans-C-O stretching+ vibrations of the ester group and at peak 872 cm $^{-1}$ presents an aromatic ring. A peak range of 724 cm^{-1} indicates ring CH out-of-plane bending + C=O out-of-plane bending group.

For the coated *A. afra* hexane fabric, small peaks around 3427 cm^{-1} indicate the -OH group bond for samples 1-3. Peaks around 2958 cm^{-1}, 2918 cm^{-1} and 2852 cm^{-1} indicate C-H stretching bond from samples 1-3. Sample 3 shows strong peaks around this region whereas sample 1-2 peaks are weak but still indicate of C-H stretching bond for samples 1-3. Peaks from 1715 -1713 cm^{-1} indicate the C=O stretch group for samples 1-3. The peaks around 1502 cm^{-1} indicate vibrations of an aromatic ring for samples 1-3 and the peaks at 1473-1471 cm^{-1} indicate a CH_2 bending bond for samples 1-3. The peaks at 1410 cm^{-1} indicate an aromatic ring and the peak at 1340 cm^{-1} indicates a CH_2 bending functional group for samples 1-3. Peaks between 1242- 1232 cm^{-1} represent the C−O stretch (ester) for samples 1-3. The peaks in the range of 1096, cm$^{-1,}$ 1019-1017 cm^{-1} indicate vibrations of an aromatic ring for samples 1-3. Peak range from 973 cm^{-1} and 965 cm^{-1} indicates C=C stretching trans -C-H stretch out-

of-plane bend for samples 1-3. Peaks ranging between 725-720 cm^{-1} indicate ring -C-H out of plane bending + C=O out of plane bending for samples 1-3.

FTIR analysis reported by Arockia et al., (2015), for the coated polyester fabrics, from samples 1-3, the prominent peaks in the region of 2918 cm^{-1} indicate C-H symmetric stretching vibrations of CH_2 which is an indication of lipids and protein present and the vibrations of 1502 cm^{-1} indicate vibrations of an aromatic ring stretch indicating an aromatic compound and peaks at 1473 cm^{-1} indicate the C=C-C aromatic compound present, and at 1411-1409 cm^{-1} indicate vibrations of O-H bend indicating a phenol compound as well as the peaks at 1342-1340 cm^{-1}. The peak 1097 cm^{-1} indicates the C-O stretch indicating the presence of cyclic ethers. Strong peaks at 1243-1241 cm^{-1} indicate C–O stretching bonds. Peak 967 cm^{-1} and at peak 872 cm^{-1} presents a P-O-C stretch, indicating aromatic phosphates. A peak range of 724 cm^{-1} indicates the C-Cl stretch aliphatic chloro compound.

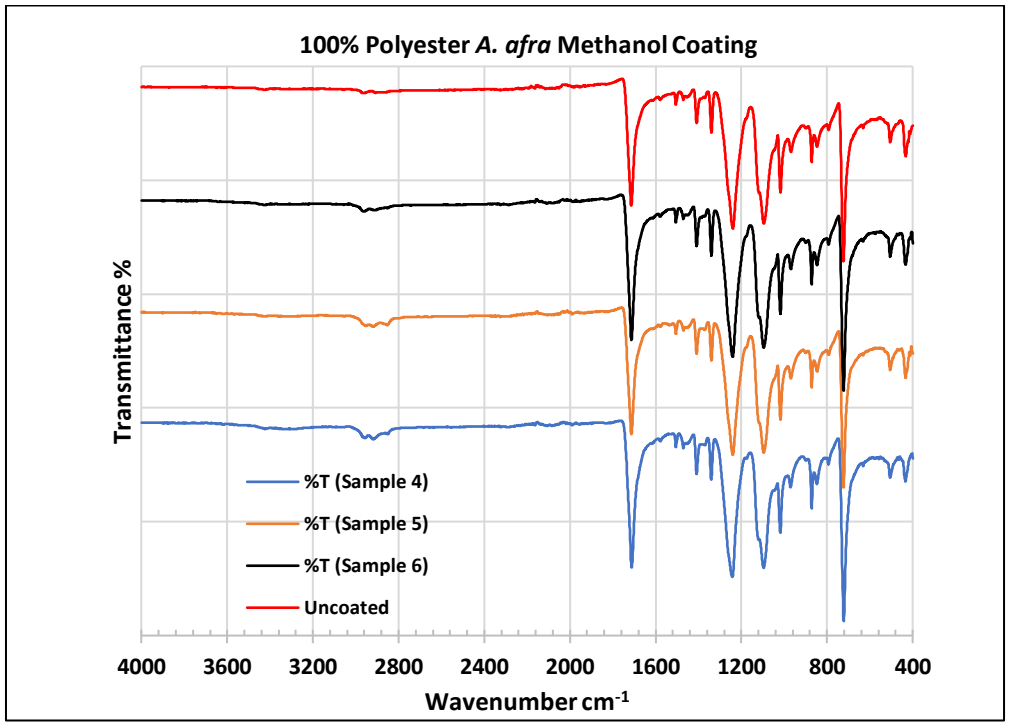


Figure 3.5: FTIR spectra of polyester fabrics uncoated (U) and coated (4-6) with *A. afra* methanol coating

In the FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020) and Nandiyanto et al., (2019), the coated *A. afra* methanol fabrics from samples 4-6, the peak ranging from 2965-2851 cm^{-1} indicates the presence of indicates C-H stretching bond (aliphatic compounds C-H stretching in methyl and methylene groups). They are also identified between this range followed by peaks between 1470-1414cm^{-1}. The peaks at 1714 cm^{-1} indicate the presence of indicates C=O stretching bond. The small peaks in the range of 1507 cm^{-1} indicate vibrations from an aromatic ring and the peaks at 1469 cm^{-1} CH_2 bending functional group. The peaks in

the range of 1414 cm^{-1} indicate vibrations of an aromatic ring. The peaks in the range of 1410 cm^{-1} indicate an aromatic ring. The peak at 1341 cm^{-1} indicates a CH_2 bending functional group. The wide peaks at the range of 1240 cm^{-1} indicate the C−O stretch group. The peaks at 1097 cm^{-1} and 1018 cm^{-1} indicate vibrations of an aromatic ring. The peaks in the range of 972 $cm^{=1}$ and 872 cm^{-1} also indicate an aromatic ring. The peaks in the range of 850 cm^{-1} indicate vibrations of an aromatic ring. The peak in the range of 725 cm^{-1} indicates -C-H out of plane bending + C=O out of plane bending.

FTIR analysis reported by Arockia et al., (2015), the coated *A. afra* methanol fabrics from samples 4-6, the prominent peaks ranging from 2965-2851 cm^{-1} indicate the presence of C-H stretching bond (aliphatic compounds). The peaks at 1714 cm^{-1} indicate the presence of C=O, indicating a carbonyl compound. The small peaks in the range of 1507 cm^{-1} and 1469 cm^{-1} indicate C=C-C vibrations, indicating an aromatic compound. The peaks in the range of 1414-1410 cm^{-1} indicate vibrations of the O-H bend, indicating phenol compounds. The peak at 1341 cm^{-1} indicates O-H vibrations, indicating the presence of phenol compounds. The wide peaks at the range of 1240 cm^{-1} indicate the C−O stretching bond, indicating. The peaks at 1097 cm^{-1} indicate the presence of the C-O stretch group, indicating the presence of cyclic ethers and 1018 cm^{-1} indicates the presence of phosphate ions. The peaks in the range of 972 cm^{-1} and 850 cm^{-1} also indicate the P-O-C stretch group, indicating aromatic phosphates. An aromatic ring. The peak in the range of 725 cm^{-1} indicates the C-Cl stretch group indicating an aliphatic chloro compound.

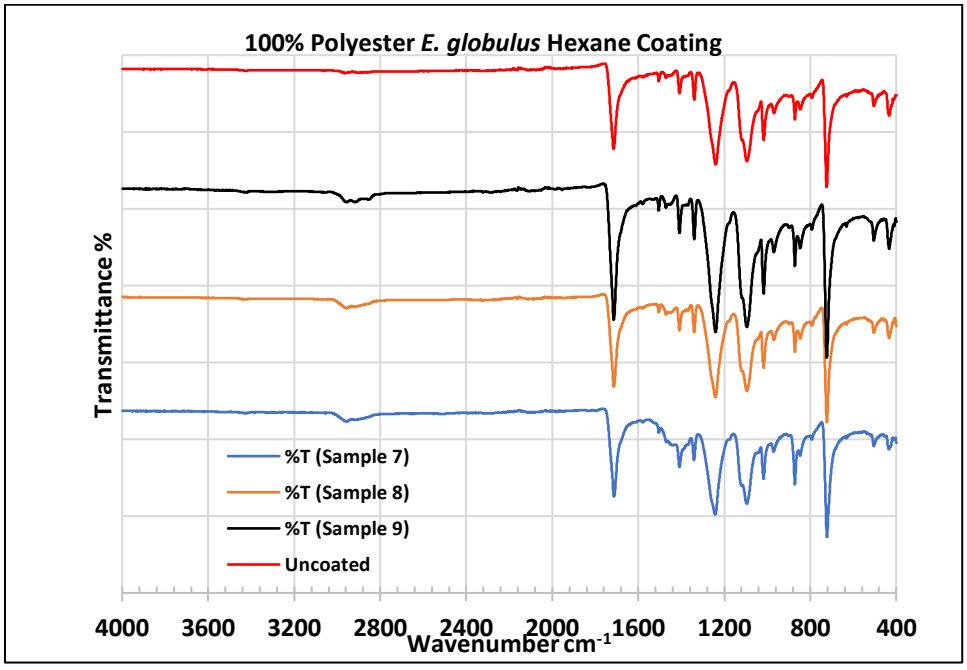


Figure 3.6: FTIR spectra of polyester fabrics uncoated (U) and coated (7-9) with E. *globulus* hexane coating

The FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and Nandiyanto et al., (2019), the coated *E. globulus* hexane-coated fabric from samples 7-9, peaks at 2952 cm^{-1} indicate C-H stretch in methyl and methylene groups (aliphatic compounds), followed by peaks at 1471 and 725 cm^{-1}. The peaks at 1714 cm^{-1} indicate C=O stretching bond. There are peaks at the band range 1506 cm^{1}, indicating that there are vibrations of an aromatic ring. The peaks around 1479 cm^{-1} indicate CH_2 bending. The peaks at 1412-1402 cm^{-1} indicate an aromatic ring and the peaks at 1342 cm^{-1} indicate a CH_2 bending functional group. Peaks between 1244-1237 cm^{-1} represent the C−O stretch group. The peaks at 1097 cm^{-1} indicate vibrations of an aromatic ring along with peaks in the range of 1017- 972 cm^{-1} and peaks at 871 cm^{-1}. Peaks ranging between 725-720 cm^{-1} indicate ring -C-H out of plane bending + C=O out of plane bending.

FTIR analysis reported by Arockia et al., (2015), the coated *E. globulus* hexane-coated fabric from samples 7-9, peaks at 2952 cm^{-1} indicating a C-H stretch group, indicating an alkane compound, followed by a peak at 1471 cm^{-1} indicating aromatic ring stretch indicating an aromatic compound. The peak at 725 cm^{-1} indicates the C-Cl stretch group indicating an aliphatic chloro compound. The peaks at 1714 cm^{-1} indicate the C=O group, indicating the carbonyl group. There are peaks at the band range 1506 cm^{1} and indicating the C=C-C group, indicating that there are vibrations of an aromatic ring. The peaks in the range of 1479 cm^{-1} indicate an aromatic ring stretch C=C-C group, indicating an aromatic compound. The peaks range 1412-1342 cm^{-1} indicates the O-H bend group, indicating phenol compounds. Peaks between 1244-1237 cm^{-1} represent the C−O stretch group. The peaks at 1097 cm^{-1} indicate the presence of the C-O stretch group, indicating the presence of cyclic ethers and 1018 cm^{-1} indicates the presence of phosphate ions. The peaks in the range of 972 cm^{-1} and 850 cm^{-1} also indicate the P-O-C stretch group, indicating aromatic phosphates. an aromatic ring. The peak in the range of 725 cm^{-1} indicates the C-Cl stretch group indicating an aliphatic chloro compound.

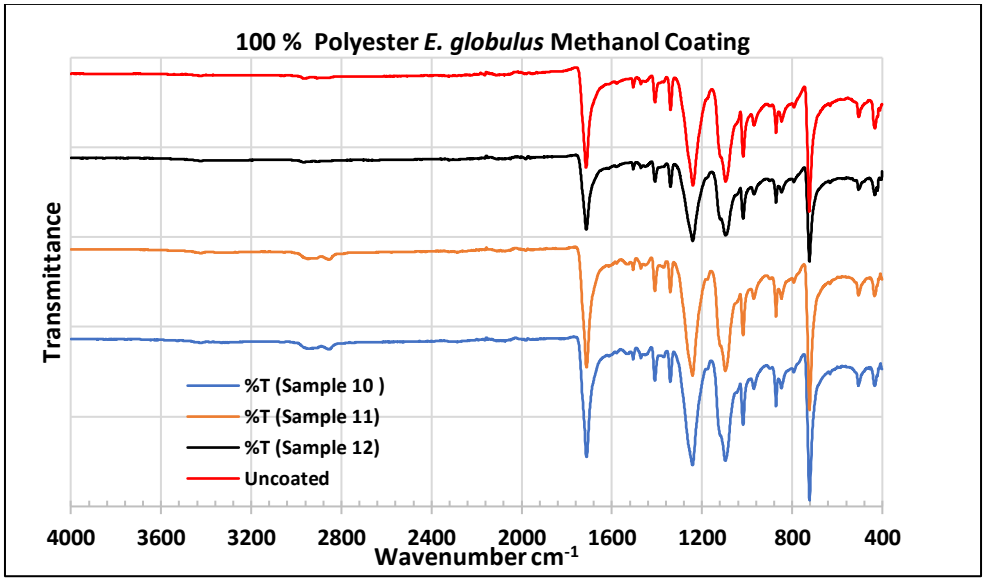


Figure 3.7: FTIR spectra of polyester fabrics uncoated (U) and coated (10-12) with *E. globulus* methanol coating.

In the FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and Nandiyanto et al., (2019) the *E. globulus* methanol-coated fabric from samples 10-12, the peaks around 2935 cm^{-1} In indicate in C-H stretching in methyl and methylene groups. The peaks at a range of 1714 -1710 cm^{-1} C=O stretching bond. The peak 1411 cm^{-1} indicates an aromatic ring and the peak 1342cm^{-1} indicates a CH_2 bending functional group. The peak range 1247-1237 cm^{-1} indicates the C-O stretch group. Peaks at 1092 cm^{-1} indicate vibrations of an aromatic ring. The peaks at 1018 cm^{-1} and 873 cm^{-1} indicate vibrations of an aromatic ring. A peak range of 847-735 cm^{-1} indicates vibrations of aromatic rings. Peaks at 725-722 cm^{-1} indicating CH out of plane bending + C=O out of plane bending.

FTIR analysis reported by Arockia et al., (2015), the *E. globulus* methanol-coated fabric from samples 10-12, the peaks around 2935 cm^{-1} indicate asymmetric stretching of -$CH(CH_2)$ vibration indicating the presence of lipids and protein compounds. The peaks at a range of 1714 -1710 cm^{-1} C=O stretching bond, indicating carbonyl compounds. The peaks range 1412-1342 cm^{-1} indicates the O-H bend group, indicating phenol compounds. Peaks between 1247-1237 cm^{-1} represent the C−O stretch group. The peaks in the range 1097 cm^{-1} indicate the presence of the C-O stretch group, indicating the presence of cyclic ethers and 1018 cm^{-1} indicate the presence of phosphate ions. The peaks in the range of 972 cm^{-1} and 850 cm^{-1} also indicate the P-O-C stretch group, indicating aromatic phosphates. An aromatic ring. The peak in the range of 725-722 cm^{-1} indicates the C-Cl stretch group indicating an aliphatic chloro compound.

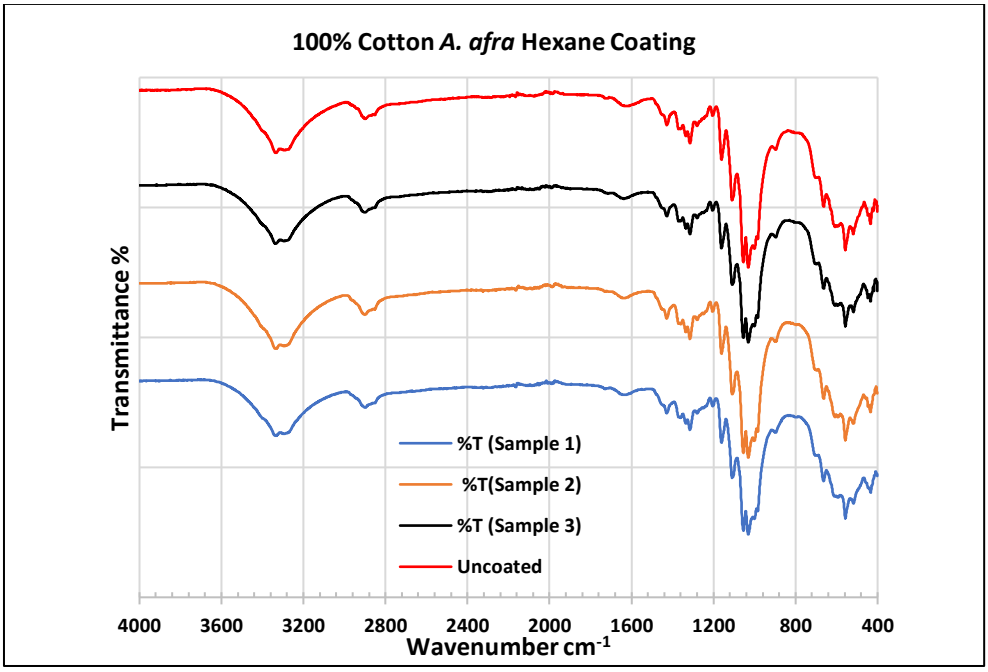


Figure 3.8: FTIR spectra of cotton fabrics uncoated (U) and coated (1-3) with *A. afra* Hexane coating

The FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020) and Nandiyanto et al., (2019), the uncoated cotton, peaks for cotton fabrics are found at around 3326 cm^{-1} corresponding to O–H stretching, 2897 cm^{-1} region for C-H stretching in methyl and methylene groups. The peak at 1611 cm^{-1} indicates the C=O carbonyl group. Peaks at 1417 cm^{-1} indicate vibrations of an aromatic ring. A peak at 1311 cm^{-1} is associated with the O-H group and peaks at 1120 cm^{-1}, 1066 cm^{-1} and 1120-1029 cm^{-1} indicate a C-O stretch. Peaks at 895 cm^{-1} indicate the C=C group and 680-629 cm^{-1} indicate the alkyne C-H bend.

For the cotton, *A. afra* hexane coated fabric from samples 1-3, with peaks of 3323 cm^{-1} indicates O-H stretch. A small wide absorption peak in 2889 cm^{-1} indicates C-H stretching in methyl and methylene groups. A peak in the range of 1429 cm^{-1} indicates the bending of the CH group and the peak range around 1316 cm^{-1} is associated with CH_2 groups. Peaks between the range of 1129 -1119 cm^{-1} indicate C-O stretching of trans conformer. The peak range at 1109 cm^{-1} indicates a C-O stretch is associated with CH_2 groups -cyclohexane vibrations at 1057 cm^{-1} and 1000 cm^{-1}. Peaks from 667-620 cm^{-1} indicate an alkyne C-H bend. For the cotton, *A. afra* hexane coated fabric from samples 1-3, with peaks of 3323 cm^{-1} indicates O-H stretch. A small wide absorption peak in 2889 cm^{-1} indicates C-H stretching in methyl and methylene groups. A peak in the range of 1429 cm^{-1} indicates the bending of the CH group and the peak range around 1316 cm^{-1} is associated with CH_2 groups. Peaks between the range of 1129 -1119 cm^{-1} indicate C-O stretching of trans conformer. The peak range at 1109 cm^{-1} indicates a C-O stretch is associated with CH_2 groups -cyclohexane vibrations at 1057 cm^{-1} and 1000 cm^{-1}. Peaks from 667-620 cm^{-1} indicate an alkyne C-H bend.

FTIR analysis reported by Arockia et al., (2015), the peaks for cotton fabrics are found at around 3326 cm^{-1} corresponding to O–H stretching group indicating polyhydroxy compounds. The peak at 1611 cm^{-1} indicates the C=O stretching vibrations, indicating a ketone group. A peak at 1311 cm^{-1} is associated with the O-H group indicating a phenol compound and peaks in the range of 1120 cm^{-1} indicate a C-O stretch indicating a cyclic ether compound. Peaks at 895 cm^{-1} indicate aromatic phosphates (P-O-C group) and 680-629 cm^{-1} indicate the C-Br group, indicating aliphatic bromo compounds.

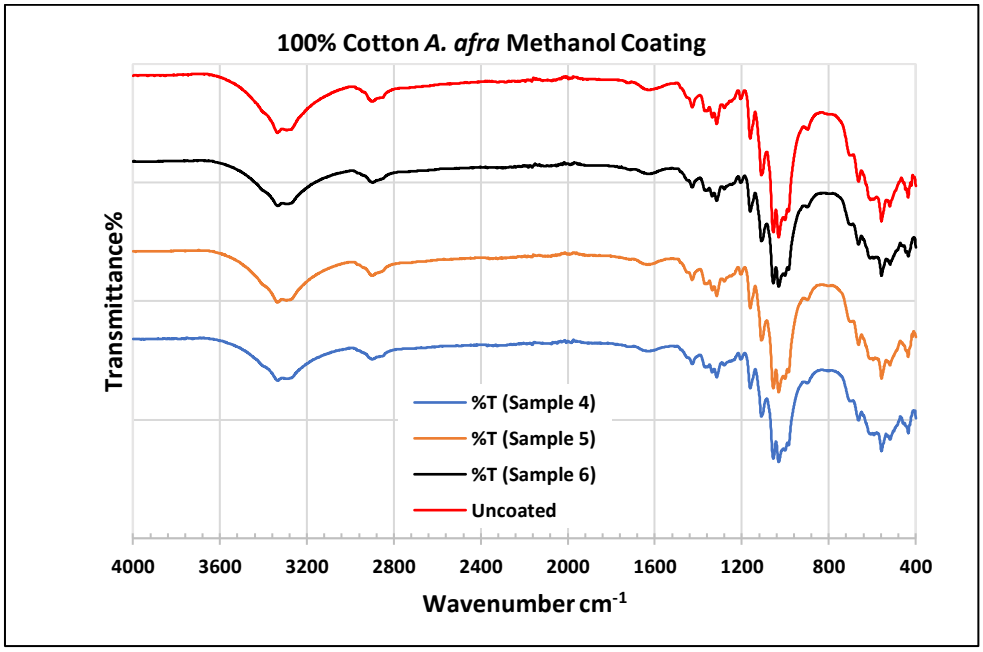


Figure 3.9: FTIR spectra of cotton fabrics uncoated (U) and coated (4-6) with *A. afra* methanol coating

The FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and (Nandiyanto et al., (2019), the cotton *A. afra* methanol coated fabric from samples 4-6, peaks between 3340-3268 cm^{-1} indicate the -OH group. Peaks at 2897-2823 cm^{-1} C-H stretching in methyl and methylene groups. Peaks at 1425 cm^{-1} indicate vibrations of an aromatic ring. Peaks at 1315 cm^{-1} are associated with the $–CH_2$ group. Peaks at 1163 cm^{-1}-1123 cm^{-1} are associated with C-O stretch as well as peaks at 1105 cm^{-1}-1068 cm^{-1}. Peaks at 1000 cm^{-1} indicate vibrations of an aromatic ring. Peaks at 681- 631 cm^{-1} indicate an alkyne C-H bend group.

FTIR analysis reported by Arockia et al., (2015), the cotton, *A. afra* methanol coated fabric from samples 4-6, peaks between 3340-3268 cm^{-1} indicating the O-H stretch group indicating a polyhydroxy compound. Peaks at 2897-2823 cm^{-1} C-H stretching in methyl and methylene groups. Peaks at 1425 cm^{-1} indicate vibrations of an aromatic ring. Peaks at 1315 cm^{-1} are associated with the O-H bend group indicating the presence of a phenol group. Peaks in the range 1105 cm^{-1}-1000 cm^{-1} indicate phosphate ion group indicating phosphate compounds. of an aromatic ring. Peaks at 681- 631 cm^{-1} indicate a C-Br stretch (aliphatic bromo compounds).

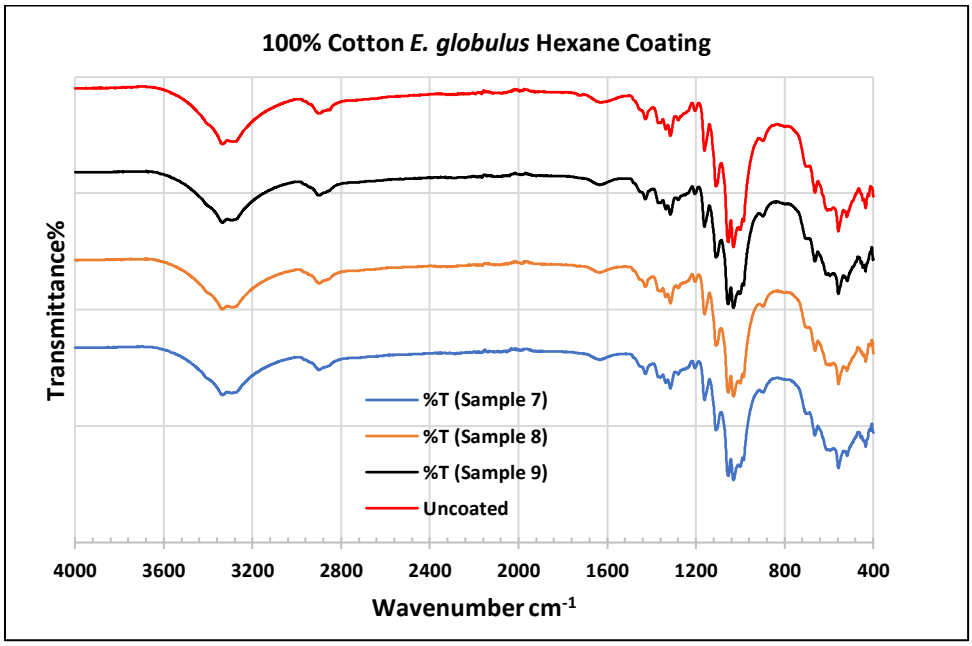


Figure 3.10: FTIR spectra of cotton fabrics uncoated (U) and coated (7-9) with *E. globulus* hexane coating

The FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and Nandiyanto et al., (2019), the cotton *E. globulus* hexane-coated fabric from samples 7-9, peaks ranging between 3333-3254 cm^{-1} indicate O-H stretching. Peaks at 2879-2823 cm^{-1} C-H stretching in methyl and methylene groups. Small peaks ranging from 1636-1626 cm^{-1} indicate asymmetrical stretching vibration of the COO- bond. Peaks at 1428 cm^{-1} indicate bending of the CH group and at 1372 cm^{-1} indicate CH_2 wagging vibration of gauche conformation. Peaks at 1317 -1307 cm^{-1} asymmetric stretching of C–O–C group. Peaks 1124-1068 cm^{-1} C-O stretching of trans conformer. Peaks at 1000 cm^{-1} indicate vibrations of an aromatic ring. Peaks at 622- 612 cm^{-1} indicate an alkyne C-H bend.

For FTIR analysis reported by Arockia et al., (2015), the cotton *E. globulus* hexane-coated fabric from samples 7-9, peaks ranging between 3333-3254 cm^{-1} indicates the O-H stretch group indicating polyhydroxy compound. Small peaks ranging from 1636-1626 cm^{-1} indicate the C-O stretch, indicating the ketone compound. Peaks at 1372- 1307cm^{-1} indicate O-H bending group indicating a phenol compound. Peaks at 1000 cm^{-1} indicate vibrations of the phosphate ion group indicating a phosphate compound. Peaks in the range of 622- 612 cm^{-1} indicate an alkyne C-Br vibration indicating an aliphatic bromo compound.

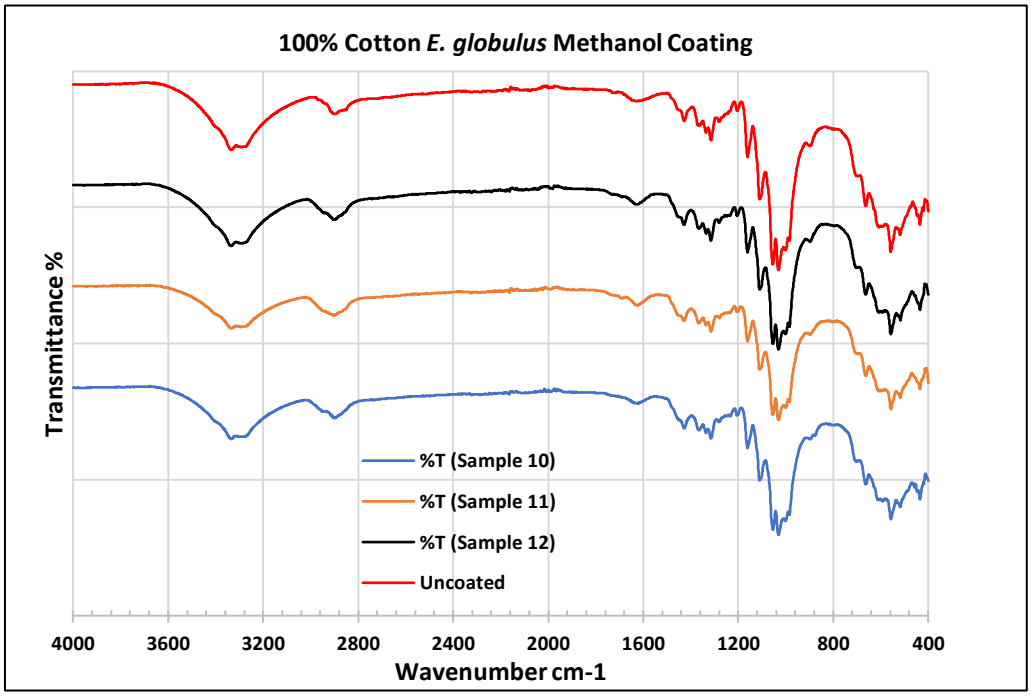


Figure 3.11: FTIR spectra of cotton fabrics uncoated (U) and coated (10-12) with *E. globulus* methanol coating.

The FTIR analysis reported by Sumantri et al., (2020), Hayat et al., (2020), and Nandiyanto et al., (2019), the cotton *E. globulus* methanol-coated fabric from samples 10-12, peaks around 3332-3270 cm^{-1} indicate -OH cm^{-1} group and peaks around 2935 cm^{-1} were observed and indicate C-H stretching in methyl and methylene group. The peaks at a range of 1714-1710 cm^{-1} indicate the C=O stretching bond. The peaks at 1411 cm^{-1} indicate an aromatic ring. The peak at 1342cm^{-1} indicates a CH_2 bending functional group. The peak range 1247-1237 cm^{-1} indicates the C-O stretch group. Peaks at 1092 cm^{-1} indicate vibrations of an aromatic ring. Peaks at 1018 cm^{-1} and 873 cm^{-1} indicate vibrations of the aromatic ring. A peak range of 847-735 cm^{-1} indicates vibrations of aromatic rings. Peaks at 725-722 cm^{-1} indicating CH out of plane bending + C=O out of plane bending.

The FTIR spectrum results demonstrated absorption For FTIR analysis reported by Arockia et al., (2015), the cotton *E. globulus* methanol coated fabric from samples 10-12, peaks around 3332-3270 cm^{-1} indicates O-H stretch group indicating polyhydroxy compound and peaks around 2935 cm^{-1} were observed and indicate asymmetric stretching of -$CH(CH_2)$ vibration indicating saturated aliphatic compound. The peaks at a range of 1714-1710 cm^{-1} indicate the C=O stretch indicating the carbonyl group. The peaks range 1412-1342 cm^{-1} indicates the O-H bend group, indicating phenol compounds. Peaks between 1247-1237 cm^{-1} represent the C−O stretch group. The peaks in the range 1097 cm^{-1} indicate the presence of the C-O stretch group, indicating the presence of cyclic ethers and 1018 cm^{-1} indicate the presence of

phosphate ions. The peaks in the range of 972 cm^{-1} and 850 cm^{-1} also indicate the P-O-C stretch group, indicating aromatic phosphates. an aromatic ring. The peak in the range of 725-722 cm^{-1} indicates the C-Cl stretch group indicating an aliphatic chloro compound.

The FTIR spectrum results reveal that the methanol and n-hexane extract functional groups exhibit absorption signals across multiple wavenumber ranges. These functional groups contain chemical bonds that may contribute to their antimicrobial properties. For instance, the presence of alcohols and phenols (O–H), polyhydroxy compounds (O-H stretch in the extracts), carboxylic acids (C-O stretching), and methyl and aldehyde groups (stretching of C-H bonds) may disrupt cell membranes or inhibit bacterial enzymes, thus potentially enhancing the extracts' antimicrobial activity. Moreover, alkenes (C=C stretching) and aromatics (C-C stretching group) may induce oxidative stress in bacterial cells, contributing to the extracts' anti-inflammatory and antimicrobial properties. Additionally, CH_2 methylene groups and aliphatic compounds (C-H stretching), aliphatic bromo compounds (C-Br), and P-O-C stretch (aromatic phosphates) may also be involved in the biological activity of the extracts. Polyhydroxy compounds (O-H stretch) may contribute to the extracts' anti-inflammatory and immunomodulatory properties, in addition to their antimicrobial activity. It's worth noting that the chemical composition of the extracts may vary depending on factors such as geographical location, climate conditions, drying methods, population variability, and extraction solvents.

The FTIR spectra were found to be similar to those reported by Sumantri et al., (2020), Hayat et al., (2020), and (Nandiyanto et al., (2019) but different from the FTIR spectra reported by Arockia et al., (2015). Overall, based on the FTIR spectrum results, it appears that the methanol and n-hexane extracts of *Artemisia afra* and *Eucalyptus globulus* contain a complex mixture of bioactive compounds with potential antimicrobial properties. (Liu et al., 2009).

Chapter 4

Conclusions and Recommendations

CHAPTER 4: CONCLUSIONS AND RECOMMENDATIONS

4. Conclusion

Eucalyptus globulus and *Artemisia afra* displayed antimicrobial activity against the Gram-positive *S. aureus* strain but poor activity against the Gram-negative bacteria *E. coli* strain. The methanolic extractions showed the highest yield compared to hexanoic extractions. The obtained results revealed that the different solvents resulted in different extraction yields due to the differences in solvent polarity. The *Eucalyptus globulus* hexanoic and methanolic extracts showed good antibacterial activity against the *S. aureus* strain and poor activity against the *E. coli* strain. The *Artemisia afra* hexanoic and methanolic extracts revealed poor antimicrobial activity against the *E. coli* strain. Microbial growths were rather observed when *Eucalyptus globulus* hexane and methanol extracts were tested against *E. coli*. The MIC results revealed that only some samples showed activity in the liquid media against the selected ATCC bacterial strains. The FTIR analysis confirmed the presence of a composition of alcohol and phenols, carboxylic acids, methyl and aldehyde group, alkenes, and aromatics functional groups. The results of this study show that hexane and methanol extract of *E. globulus* extractions could be a good candidate as an antibacterial agent to fight against Gram-positive bacteria. Only a few coated samples showed a zone of inhibition. No zone of inhibition was detected after one cycle of washing. This could be due coating technique used to apply the extracts onto the fabrics.

Plants play an essential role in the pharmaceutical and healthcare sectors due to their ability to fight pathogens. Unexplored medicinal plants with potent bioactive compounds should be studied, and various plant parts should be investigated to determine the plant part containing the highest bioactive concentration. More research on plant-based antimicrobial agents and finishing should be conducted to extend the antimicrobial activity and durability to laundering on textile substrates. The study's outcome suggests that freeze-drying should be used as a standard drying method at the pre-treatment stage, as it preserves bioactive compounds in the plant material, given that most phytochemicals and bioactive compounds are thermolabile. Freeze-drying is a very energy-consuming process but avoid the thermal decomposition of the valuable bioactive compounds during the drying process.

Finding a single method for a quick, efficient, and eco-friendly extraction has been a struggle due to the limitations of traditional extraction methods. The exploration of green extraction techniques has gained traction due to reduced time, energy, solvent consumption, and low operating costs and its positive impact on global warming and climate change, reducing toxic solvent usage and carbon emissions. Multiple extractions and green extraction methods should be used to increase the extraction yield. Only solvents regarded as safe with strong

polarity should be used for extraction to achieve maximum yield. Additionally, extracts should be further purified by eliminating compounds that might enhance bacterial growth.

Chapter 5

References

5. References

Afaneh, I., Yateem, H. & Al-Rimawi, F. 2015. Effect of Olive Leaves Drying on the Content of Oleuropein. *American Journal of Analytical Chemistry*, 06(03).

Ahmad-Qasem, M.H., Barrajón-Catalán, E., Micol, V., Mulet, A. & García-Pérez, J.V. 2013. Influence of freezing and dehydration of olive leaves (var. Serrana) on extract composition and antioxidant potential. *Food Research International*, 50(1).

Aisida, S.O., Batool, A., Khan, F.M., Rahman, L., Mahmood, A., Ahmad, I., Zhao, T., Maaza, M. & Ezema, F.I. 2020. Calcination induced PEG-Ni-ZnO nanorod composite and its biomedical applications. *Materials Chemistry and Physics*, 255.

Aisida, S.O., Ugwu, K., Akpa, P.A., Nwanya, A.C., Ejikeme, P.M., Botha, S., Ahmad, I., Maaza, M. & Ezema, F.I. 2019. Biogenic synthesis and antibacterial activity of controlled silver nanoparticles using an extract of *Gongronema latifolium*. *Materials Chemistry and Physics*, 237.

Alaribe, F.N., Maepa, M.J., Mkhumbeni, N. & Motaung, S.C. 2018. Possible roles of *Eucomis autumnalis* in bone and cartilage regeneration: A review. *Tropical Journal of Pharmaceutical Research*, 17(4): 741.

Ameer, K., Shahbaz, H.M. & Kwon, J.-H. 2017. Green Extraction Methods for Polyphenols from Plant Matrices and Their Byproducts: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 16(2): 295–315.

Araújo, F.O.L. de, Rietzler, A.C., Duarte, L.P., Silva, G.D. de F., Carazza, F. & Vieira Filho, S.A. 2010. Constituintes químicos e efeito ecotoxicológico do óleo volátil de folhas de *Eucalyptus urograndis* (Mirtaceae). *Química Nova*, 33(7).

A.Reshma, V.Brindha Priyadarisini & K. Amutha. 2022. Sustainable Antimicrobial Finishing of Fabrics Using Anitmicrobial Finishing of Fabrics Using Natural Bioactive Agents – A Review. *International Journal of Life Science and Pharma Research*, 8(4): 10–20. http://ijlpr.com/index.php/journal/article/view/483 6 August 2021.

Babu, A.K., Kumaresan, G., Raj, V.A.A. & Velraj, R. 2018. Review of leaf drying: Mechanism and influencing parameters, drying methods, nutrient preservation, and mathematical models. *Renewable and Sustainable Energy Reviews*, 90.

Bachir, R.G. & Benali, M. 2012. Antibacterial activity of the essential oils from the leaves of *Eucalyptus globulus* against *Escherichia coli* and *Staphylococcus aureus*. *Asian Pacific Journal of Tropical Biomedicine*, 2(9): 739–742.

Bajaj, Y.P.S. 1995. Somatic Embryogenesis and Synthetic Seed I. Springer. Y. P. S. Bajaj, ed. Berlin, Heidelberg: Springer Berlin Heidelberg. https://link.springer.com/10.1007/978-3-662-03091-2.

Bakal, S.N., Bereswill, S. & Heimesaat, M.M. 2017. Finding novel antibiotic substances from medicinal plants - Antimicrobial properties of Nigella sativa directed against multidrug resistant bacteria. *European Journal of Microbiology and Immunology*, 7(1): 92–98. https://akjournals.com/doi/10.1556/1886.2017.00001.

Bakkali, F., Averbeck, S., Averbeck, D. & Idaomar, M. 2008. Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46(2).

Barker, S.C. & Altman, P.M. 2011. An ex vivo, assessor blind, randomised, parallel group, comparative efficacy trial of the ovicidal activity of three pediculicides after a single application - melaleuca oil and lavender oil, eucalyptus oil and lemon tea tree oil, and a 'suffocation' pediculicide. *BMC Dermatology*, 11(1).

Baydoun, S., Chalak, L., Dalleh, H. & Arnold, N. 2015. Ethnopharmacological survey of medicinal plants used in traditional medicine by the communities of Mount Hermon, Lebanon. *Journal of Ethnopharmacology*, 173.

Behary, N., Smet, D.D., Campagne, C. & Vanneste, M. 2020. Antibacterial and Multifunctional Polyester Textile Using Plant-Based Cinnamaldehyde. *Journal of Textile Science and Technology 4*, 4(5): 1–11.

Bhatia, P., Sharma, A., George, A.J., Anvitha, D., Kumar, P., Dwivedi, V.P. & Chandra, N.S. 2021. Antibacterial activity of medicinal plants against ESKAPE: An update. *Heliyon*, 7(2): 1–12.

Bibi, R., Tariq, A., Mussarat, S., Niaz Khan, S., Rahman, H., Fathi, E., Allah, A., Ullah, R. & Adnan, M. 2017. Ethnomedicinal, phytochemical and antibacterial activities of medicinal flora of Pakistan used against *Pseudomonas aeruginosa* - A Review. *Pak. J. Pharm. Sci*, 30(6): 2285–2300.

Bouchekrit, M., Laouer, H., Hajji, M., Nasri, M., Haroutounian, S.A. & Akkal, S. 2016. Essential oils from *Elaeoselinum asclepium*: Chemical composition, antimicrobial and antioxidant properties. *Asian Pacific Journal of Tropical Biomedicine*, 6(10): 851–857.

Brophy, J.J., Forster, P.I., Goldsack, R.J., Hibbert, D.B. & Punruckvong, A. 2009. Essential oil variation in *Eucalyptus crebra*, *E. melanophloia* (Myrtaceae) and their hybrids. *Australian Journal of Botany*, 57(5): 425. http://www.publish.csiro.au/?paper=BT08171.

Cares, M.G., Vargas, Y., Gaete, L., Sainz, J. & Alarcón, J. 2010. Ultrasonically assisted extraction of bioactive principles from Quillaja Saponaria Molina. *Physics Procedia*, 3(1): 169–178.
Chandra, H., Bishnoi, P., Yadav, A., Patni, B., Mishra, A.P. & Nautiyal, A.R. 2017. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials - A review. *Plants*, 6(2): 457–462.
Chauhan, P. & Kumar, A. 2020b. Development of a microbial coating for cellulosic surface using aloe vera and silane. *Carbohydrate Polymer Technologies and Applications*, 1: 100015. https://linkinghub.elsevier.com/retrieve/pii/S2666893920300153.
Coskun, O. 2016. Separation Techniques: Chromatography. *Northern Clinics of Istanbul*, 3(2): 1–5. https://www.journalagent.com/nci/pdfs/NCI_3_2_156_160.pdf.
Cowan, M. 1999. Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, 12(4): 564–582.
Cragg, G.M. & Newman, D.J. 2001. Natural Product Drug Discovery in the Next Millennium. *Pharmaceutical Biology*, 39(1).
Cragg, G.M. & Newman, D.J. 2013. Natural products: A continuing source of novel drug leads. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1830(6).
Das, K., Shrivastava, D. & Tiwari, R. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agents: Current methods and future trends. *Journal of Medicinal Plants Research*, 4(2): 104–111. https://academicjournals.org/journal/JMPR/article-abstract/7B1A27415011 8 August 2021.
DeLeo, F.R., Otto, M., Kreiswirth, B.N. & Chambers, H.F. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*. *The Lancet*, 375(9725).
Djenane, D., Yangüela, J., Amrouche, T., Boubrit, S., Boussad, N. & Roncalés, P. 2011. Chemical composition and antimicrobial effects of essential oils of *Eucalyptus globulus*, *Myrtus communis*, and *Satureja hortensis* against *Escherichia coli* and *Staphylococcus aureus* in minced beef. *Food Science and Technology International*, 17(6).
Dube, P., Meyer, S. & Marnewick, J.L. 2017. Antimicrobial and antioxidant activities of different solvent extracts from fermented and green honeybush (*Cyclopia intermedia*) plant material. *South African Journal of Botany*, 110: 184–193.
Elbagory, A.M., Meyer, M., Cupido, C.N. & Hussein, A.A. 2017. Inhibition of Bacteria Associated with Wound Infection by Biocompatible Green Synthesized Gold Nanoparticles from South African Plant Extracts. *Nanomaterials,* 417(7): 1–22. www.mdpi.com/journal/nanomaterials.
FAO/WHO. 2018. *Joint FAO/ WHO Expert Committee on Food Additives. Summary and Conclusions*. FAO: Rome, Italy; WHO: Geneva, http://www.wipo.int/amc/en/mediation/rules 8 August 2021.
Fiore, E., van Tyne, D. & Gilmore, M.S. 2019. Pathogenicity of Enterococci. *Microbiology Spectrum*, 7(4).
Fortune Business Insights. 2021. *Antimicrobial Textiles Market Size, Share and COVID19, Impact Analysis, By Active Agents (Synthetic Organic Compounds, Bio-Based Compounds, Metal and Metallic Salts and Others) By Fabric (Cotton, Polyester and Others) By Application (Home, Commercial, Medical, Apparel, Industrial and Others and Regional Forecasts, 2021-2028)*. Global. https://www.fortunebusinessinsights.com/antimicrobial-textiles-market-102307 14 March 2023.
Foye, W.O., Lemke, T.L. & Williams, D.A. 2008. *Foye's Principals of Medicinal Chemistry*. 6th ed. Philadelphia: Lippincott Williams and Wilkins.
Gajalakshmi, S., Jeyanthi, P., Vijayalakshmi, S. & Devi, V. 2011. Phytochemcial constituent of Aconitium species - A Review. *International Journal of Applied Biology and Pharmaceutical Technology*, 2(4): 121–127.
Gao, Y., Cranston, R., Yuan, G. & Cranston, R. 2008. Recent Advances in Antimicrobial Treatments of Textiles. , 78(1): 60–72. www.trj.sagepub.com 12 August 2021.
Ghaffar, A., Yameen, M., Kiran, S., Kamal, S., Jalal, F., Munir, B., Saleem, S., Rafiq, N., Ahmad, A., Saba, I. & Jabbar, A. 2015. Chemical Composition and in-Vitro Evaluation of the Antimicrobial and Antioxidant Activities of Essential Oils Extracted from Seven Eucalyptus Species. *Molecules*, 20(11).
Ghareeb, M.A., Refahy, L.A., Saad, A.M., Osman, N.S., Abdel-Aziz, M.S., El-Shazly, M.A. & Mohamed, A.S. 2015. In vitro Antimicrobial Activity of Five Egyptian Plant Species. *Journal of Applied Pharmaceutical Science*, 5: 045–049.
Ghelichkhani, G., Modaresi, M.H., Rashidi, L., Shariatifar, N., Homapour, M. & Arabameri, M. 2019. Effect of the spray and freeze dryers on the bioactive compounds of olive leaf aqueous extract by chemometrics of HCA and PCA. *Journal of Food Measurement and Characterization*, 13(4).
Gilles, M., Zhao, J., An, M. & Agboola, S. 2010. Chemical composition and antimicrobial properties of essential oils of three Australian Eucalyptus species. *Food Chemistry*, 119(2).

Ginting, B., Maulana, I. & Karnila, I. 2020. Biosynthesis Copper Nanoparticles using *Blumea balsamifera* Leaf Extracts: Characterization of its Antioxidant and Cytotoxicity Activities. *Surfaces and Interfaces*, 21.

González-Burgos, E., Liaudanskas, M., Viškelis, J., Žvikas, V., Janulis, V. & Gómez-Serranillos, M.P. 2018. Antioxidant activity, neuroprotective properties and bioactive constituents' analysis of varying polarity extracts from *Eucalyptus globulus* leaves. *Journal of Food and Drug Analysis*, 26(4).

Graven, E.H., Dean, S.G., Svoboda, K.P., Mavi, S. & Gundidza, M.G. 1992. Antimicrobial and antioxidative properties of the volatile (essential) oil of *Artemisia afra* Jacq. *Flavour and Fragrance Journal*, 7(3).

Green, R.J. 2004. *Antioxidant activity of peanut plant tissues*. North Carolina: North Carolina State University.

Gulati, R., Sharma, S. & Sharma, R.K. 2022. Antimicrobial textile: recent developments and functional perspective. *Polymer Bulletin*, 79(8): 5747–5771. https://link.springer.com/10.1007/s00289-021-03826-3.

Gupta, D. & Bhaumik, S. 2007. Antimicrobial treatments for textiles. *Indian Journal of Fibre & Textile Research*, 32: 254–263.

Handa, S., Khanuja, S., Longo, G. & Rakesh, D. 2008. Extraction Technologies for Medicinal and Aromatic Plants. *International centre for science and high technology*: 21–25.

Harborne, J.B. 1984. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis.* 2nd ed. Berlin/Heidelberg, Germany.

Harborne JB. 1998. *Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis.* 3rd ed. London: Chapman and Hall.

Harnkarnsujarit, N., Kawai, K., Watanabe, M. & Suzuki, T. 2016. Effects of freezing on microstructure and rehydration properties of freeze-dried soybean curd. *Journal of Food Engineering*.

Hayat, J., Akodad, M., Moumen, A., Baghour, M., Skalli, A., Ezrari, S. & Belmalha, S. 2020. Phytochemical screening, polyphenols, flavonoids and tannin content, antioxidant activities and FTIR characterization of *Marrubium vulgare* L. from two different localities of Northeast of Morocco. *Heliyon*, 6(11): e05609.

Hipler, U.-C. & Elsner, P. 2006. *Biofunctional Textiles - New Development Changes for Dermatology*. 01/02.

Ibrahim, W., Sarwar, Z., Abid, S., Munir, U. & Azeem, A. 2017. Aloe Vera Leaf Gel Extract for Antibacterial and Softness Properties of Cotton. *Journal of Textile Science & Engineering*, 07(03).

Jager, A.K. 2003. Evaluation of antibacterial activity of traditionally prepared South African remedies for infections. *South African Journal of Botany*, 69(4): 595–598.

José Abad, M., Miguel Bedoya, L., Apaza, L. & Bermejo, P. 2000. molecules The *Artemisia* L. Genus: A Review of Bioactive Essential Oils. *Molecules*, 17: 2542–2566. www.mdpi.com/journal/molecules.

Joubert, E., Joubert, M.E., Bester, C., de Beer, D. & de Lange, J.H. 2011. Honeybush (*Cyclopia* spp.): From local cottage industry to global markets — The catalytic and supporting role of research. *South African Journal of Botany*, 77(4): 887–907.

Kamel, M.Y. & Hassabo, A.G. 2021. Anti-Microbial Finishing for Natural Textile Fabrics. *Journal of Textiles, Coloration and Polymer Science*, 18(2): 83–95. https://jtcps.journals.ekb.eg/ 6 August 2021.

Kandemir, F.M., Yıldırım, S., Kucukler, S., Caglayan, C., Darendelioğlu, E. & Dortbudak, M.B. 2020. Protective effects of morin against acrylamide-induced hepatotoxicity and nephrotoxicity: A multi-biomarker approach. *Food and Chemical Toxicology*, 138.

Kane, N.F., Kyama, M.C., Nganga, J.K., Hassanali, A., Diallo, M. & Kimani, F.T. 2019. Comparison of phytochemical profiles and antimalarial activities of *Artemisia afra* plant collected from five countries in Africa. *South African Journal of Botany*, 125: 126–133.

Kraker, M.E.A. de, Stewardson, A.J., Harbarth, S., De Kraker, M., Stewardson, A.J. & Harbarth, S. 2016. Will 10 Million People Die a Year due to Antimicrobial Resistance by 2050? 13(11). https://research.monash.edu/en/publications/will-10-million-people-die-a-year-due-to-antimicrobial-resistance 26 August 2021.

Kumar, D. 2015. *Herbal Bio-actives and Food Fortification: Extraction and Formulation*. Abingdon, UK: Taylor and Francis.

Kutama, R. 2018. Phytochemical Compositions in Some Nigerian Medicinal Plants and Their Pharmacological Properties: A Review. *Journal of Anesthesiology*, 6(1).

Lebreton, F., van Schaik, W., Manson McGuire, A., Godfrey, P., Griggs, A., Mazumdar, V., Corander, J., Cheng, L., Saif, S., Young, S., Zeng, Q., Wortman, J., Birren, B., Willems, R.J.L., Earl, A.M. &

Gilmore, M.S. 2013. Emergence of Epidemic Multidrug-Resistant *Enterococcus faecium* from Animal and Commensal Strains. *mBio*, 4(4).
Lediga, M.E., Malatjie, T.S., Olivier, D.K., Ndinteh, D.T. & van Vuuren, S.F. 2018. Biosynthesis and characterisation of antimicrobial silver nanoparticles from a selection of fever-reducing medicinal plants of South Africa. *South African Journal of Botany*, 119: 172–180.
Li, P., Xu, G., Li, S.-P., Wang, Y.-T., Fan, T.-P., Zhao, Q.-S. & Zhang, Q.-W. 2008. Optimizing ultra-performance liquid chromatographic analysis of 10 diterpenoid compounds in *Salvia miltiorrhiza* using central composite design. *Journal of Agricultural and Food Chemistry*, 56(4): 1164–71.
Liu, N.Q., Van der Kooy, F. & Verpoorte, R. 2009. *Artemisia afra*: A potential flagship for African medicinal plants? *South African Journal of Botany*, 75(2).
Lu, M., Ho, C.T. & Huang, Q. 2017. Extraction, bioavailability, and bio-efficacy of capsaicinoids. *Journal of Food and Drug Analysis*, 25(1): 27–36.
Luís, Â., Duarte, A., Gominho, J., Domingues, F. & Duarte, A.P. 2016. Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils. *Industrial Crops and Products*, 79.
Madubuonu, N., Aisida, S.O., Ali, A., Ahmad, I., Zhao, T., Botha, S., Maaza, M. & Ezema, F.I. 2019. Biosynthesis of iron oxide nanoparticles via a composite of *Psidium guavaja*, *Moringa oleifera* and their antibacterial and photocatalytic study. *Journal of Photochemistry and Photobiology B: Biology*, 199.
Magill, S.S., Edwards, J.R., Bamberg, W., Beldavs, Z.G., Dumyati, G., Kainer, M.A., Lynfield, R., Maloney, M., McAllister-Hollod, L., Nadle, J., Ray, S.M., Thompson, D.L., Wilson, L.E. & Fridkin, S.K. 2014. Multistate Point-Prevalence Survey of Health Care–Associated Infections. *New England Journal of Medicine*, 370(13).
Malek, A., McGlynn, K., Taffner, S., Fine, L., Tesini, B., Wang, J., Mostafa, H., Petry, S., Perkins, A., Graman, P., Hardy, D. & Pecora, N. 2019. Next-Generation-Sequencing-Based Hospital Outbreak Investigation Yields Insight into *Klebsiella aerogenes* Population Structure and Determinants of Carbapenem Resistance and Pathogenicity. *Antimicrobial Agents and Chemotherapy*, 63(6).
Marković, D., Milovanović, S., De Clerck, K., Zizovic, I., Stojanović, D. & Radetić, M. 2018. Development of material with strong antimicrobial activity by high pressure CO_2 impregnation of polyamide nanofibers with thymol. *Journal of CO_2 Utilization*, 26: 19–27. https://linkinghub.elsevier.com/retrieve/pii/S2212982018300258.
Marques, C., Belas, A., Franco, A., Aboim, C., Gama, L.T. & Pomba, C. 2018. Increase in antimicrobial resistance and emergence of major international high-risk clonal lineages in dogs and cats with urinary tract infection: 16-year retrospective study. *Journal of Antimicrobial Chemotherapy*, 73(2).
Martini, M.C., Zhang, T., Williams, J.T., Abramovitch, R.B., Weathers, P.J. & Shell, S.S. 2020. *Artemisia annua* and *Artemisia afra* extracts exhibit strong bactericidal activity against Mycobacterium tuberculosis. *Journal of Ethnopharmacology*, 262: 113191.
Mason, P.A., Ibrahim, K., Ingleby, K., Munro, R.C. & Wilson, J. 2000. Mycorrhizal development and growth of inoculated *Eucalyptus globulus* (Labill.) seedlings in wet and dry conditions in the glasshouse. *Forest Ecology and Management*, 128(3).
Metherel, A.H., Taha, A.Y., Izadi, H. & Stark, K.D. 2009. The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed). *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 81(5–6): 417–423.
Morais, D., Guedes, R. & Lopes, M. 2016. Antimicrobial Approaches for Textiles: From Research to Market. *Materials*, 9(6).
Mordeniz, C. 2019a. Integration of Traditional and Complementary Medicine into Evidence-Based Clinical Practice. In *Traditional and Complementary Medicine. London, UK.* London, UK: IntechOpen.
Mordeniz, C. 2019b. Introductory Chapter: Traditional and Complementary Medicine. In *Traditional and Complementary Medicine*. IntechOpen.
More, G., Lall, N., Hussein, A. & Tshikalange, T.E. 2012. Antimicrobial Constituents of *Artemisia afra* Jacq. ex Willd. against Periodontal Pathogens. *Evidence-Based Complementary and Alternative Medicine*, 2012.
Mulyaningsih, S., Sporer, F., Reichling, J. & Wink, M. 2011. Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens. *Pharmaceutical Biology*, 49(9).
Mulyaningsih, S., Sporer, F., Zimmermann, S., Reichling, J. & Wink, M. 2010. Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine*, 17(13).

Munoz-Price, L.S. & Weinstein, R.A. 2008. Acinetobacter Infection. *New England Journal of Medicine*, 358(12).
Muyima, N.Y.O., Zulu, G., Bhengu, T. & Popplewell, D. 2002. The potential application of some novel essential oils as natural cosmetic preservatives in an aqueous cream formulation. *Flavour and Fragrance Journal*, 17(4).
Nadi, F. 2017. Bioactive compound retention in *Echium amoenum* Fisch. & C. A. Mey. petals: Effect of fluidized bed drying conditions. *International Journal of Food Properties*, 20(10).
Nandiyanto, A.B.D., Oktiani, R. & Ragadhita, R. 2019. How to Read and Interpret FTIR Spectroscope of Organic Material. *Indonesian Journal of Science and Technology*, 4(1): 97.
Navon-Venezia, S., Kondratyeva, K. & Carattoli, A. 2017. *Klebsiella pneumoniae*: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiology Reviews*, 41(3).
Ndezo Bisso, B., Njikang Epie Nkwelle, R., Tchuenguem Tchuenteu, R. & Dzoyem, J.P. 2022. Phytochemical Screening, Antioxidant, and Antimicrobial Activities of Seven Under investigated Medicinal Plants against Microbial Pathogens. *Advances in Pharmacological and Pharmaceutical Sciences*, 2022: 1–8.
Neely, A.N. & Maley, M.P. 2000. Survival of *Enterococci* and *Staphylococci* on Hospital Fabrics and Plastic Go to: Survival of *Enterococci* and *Staphylococci* on Hospital Fabrics and Plastic. *J Clin Microbiol*, 38(2): 724–726.
Nguyen, V.T., van Vuong, Q., Bowyer, M.C., van Altena, I.A. & Scarlett, C.J. 2015. Effects of Different Drying Methods on Bioactive Compound Yield and Antioxidant Capacity of *Phyllanthus amarus*. *Drying Technology*, 33(8).
Nóbrega, E.M., Oliveira, E.L., Genovese, M.I. & Correia, R.T.P. 2015. The Impact of Hot Air Drying on the Physical-Chemical Characteristics, Bioactive Compounds and Antioxidant Activity of Acerola (Alphigia emarginata) Residue. *Journal of Food Processing and Preservation*, 39(2).
O. Oyedeji, A., Ekundayo, O., Olawore, O.N., Adeniyi, B.A. & Koenig, W.A. 1999. Antimicrobial activity of the essential oils of five Eucalyptus species growing in Nigeria. *Fitoterapia*, 70(5).
Odutayo, F., Ezeamagu, C., Kabiawu, T., Aina, D. & Mensah-Agyei, G. 2017. Phytochemical Screening and Antimicrobial Activity of *Chromolaena odorata* Leaf Extract against Selected Microorganisms. *Journal of Advances in Medical and Pharmaceutical Sciences*, 13(4): 1–9.
Oguntibeju, O.O., Aboua, Y. & Goboza, M. 2019. Vindoline—a natural product from *Catharanthus roseus* reduces hyperlipidemia and renal pathophysiology in experimental type 2 diabetes. *Biomedicines*, 7(3): 1–22.
O'Neill, J. 2016. *Tackling drug-resistant infections globally: Final report and recommendations. The review on antimicrobial resistance chaired by Jim O'Neill*. London, UK.
Orphanides, A., Goulas, V. & Gekas, V. 2013. Effect of drying method on the phenolic content and antioxidant capacity of spearmint. *Czech Journal of Food Sciences*, 31(No. 5).
Owira, P.M.O. & Ojewole, J.A.O. 2009. 'African potato' (*Hypoxis hemerocallidea* corm): a plant-medicine for modern and 21st century diseases of mankind? - a review. *Phytotherapy Research*, 23(2): 147–152. https://onlinelibrary.wiley.com/doi/10.1002/ptr.2595.
Oyedeji, A., Afolayan, A. & Hutchings, A. 2009. Compositional Variation of the Essential Oils of *Artemisia afra* Jacq. from three Provinces in South Africa - A Case Study of its Safety. *Natural Product Communications*, 4(6): 849–852.
P. Arockia, J. Gowri, V. Dharmalingam, R. Shobanaa & A. Prema. 2015. Phytochemical screening by FTIR spectroscopic analysis of leaf and stem extracts of *Wedelia biflora*. *International Journal of Nano Corrosion Science and Engineering,* 2(5): 322–334.
Patel, K., Panchal, N. & Ingle, P. 2019. Review of Extraction Techniques Extraction Methods: Microwave, Ultrasonic, Pressurized Fluid, Soxhlet Extraction, Etc. *International Journal of Advanced Research in Chemical Science*, 6(3): 1–16.
Paterson, D.L. & Bonomo, R.A. 2005. Extended-Spectrum β-Lactamases: a Clinical Update. *Clinical Microbiology Reviews*, 18(4).
Patil, G., Dass, S. & Chandra, R. 2011. Artemisia afra and Modern Diseases. *Journal of Pharmacogenomics & Pharmacoproteomics*, 02(03).
Pendleton, J.N., Gorman, S.P. & Gilmore, B.F. 2013. Clinical relevance of the ESKAPE pathogens. *Expert Review of Anti-infective Therapy*, 11(3).
Pereira, V., Dias, C., Vasconcelos, M.C., Rosa, E. & Saavedra, M.J. 2014. Antibacterial activity and synergistic effects between *Eucalyptus globulus* leaf residues (essential oils and extracts) and antibiotics against several isolates of respiratory tract infections (*Pseudomonas aeruginosa*). *Industrial Crops and Products*, 52.
Pham, H., Nguyen, V., Vuong, Q., Bowyer, M. & Scarlett, C. 2015. Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of *Helicteres hirsuta* Lour. Leaves. *Technologies*, 3(4).

Pino, J.A., Marbot, R., Delgado, A., Zumárraga, C. & Sauri, E. 2006. Volatile Constituents of Propolis from Honeybees and Stingless Bees from Yucatán. *Journal of Essential Oil Research*, 18(1).
Prasad, P., Sun, J., Danner, R.L. & Natanson, C. 2012. Excess Deaths Associated with Tigecycline After Approval Based on Noninferiority Trials. *Clinical Infectious Diseases*, 54(12).
Ramachandra, C.T. & Rao, P.S. 2008. Processing of *Aloe vera* Leaf Gel: A Review. *American Journal of Agriculture and Biological Sciences*, 3(2): 502–510.
Restuati, M. & Diningrat, D.S. 2018. Antimicrobial Profile of *Premna pubescens*. *Blume* and *Centella asiatica* Extracts Against Bacteria and Fungi Pathogens. *International Journal of Pharmacology*, 14(2): 271–275. https://www.scialert.net/abstract/?doi=ijp.2018.271.275.
Reza, A., Sutton, J.M. & Rahman, K.M. 2019. Effectiveness of Efflux Pump Inhibitors as Biofilm Disruptors and Resistance Breakers in Gram-Negative (ESKAPEE) Bacteria. *Antibiotics*, 8(4).
Rout, A., Jenna, P.K., Patrida U.K. & Bindhani, B.K. 2013. Green synthesis of silver nanoparticles using leaves extract of *Centella asiatica* L. for studies against human pathogens. *J. Pharma Bio Sci.*, 4: 661–674.
Safarzadeh Markhali, F. 2021. Roles of Drying, Size Reduction, and Blanching in Sustainable Extraction of Phenolics from Olive Leaves. *Processes*, 9(9).
Saifullah, M., McCullum, R., McCluskey, A. & Vuong, Q. 2019. Effects of different drying methods on extractable phenolic compounds and antioxidant properties from lemon myrtle dried leaves. *Heliyon*, 5(12).
Santhi, K. & Sengottuvel, R. 2016. Qualitative and Quantitative Phytochemical analysis of *Moringa concanensis* Nimmo. *International Journal of Current Microbiology and Applied Sciences*, 5(1).
Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K.M. & Latha, L.Y. 2011. *Extraction, Isolation and Characterization of Bioactive Compounds from Plants Extracts*.
Savoia, D. 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiology*, 7(8).
Shrestha, A.K., Ua-arak, T., Adhikari, B.P., Howes, T. & Bhandari, B.R. 2007. Glass Transition Behavior of Spray Dried Orange Juice Powder Measured by Differential Scanning Calorimetry (DSC) and Thermal Mechanical Compression Test (TMCT). *International Journal of Food Properties*, 10(3).
Sidana, J., Rohilla, R.K., Roy, N., Barrow, R.A., Foley, W.J. & Singh, I.P. 2010. Antibacterial *sideroxylonals* and *Loxophlebal* A from *Eucalyptus loxophleba* foliage. *Fitoterapia*, 81(7).
Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L. & Matos, F.J.A. 2003. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. *Journal of Ethnopharmacology*, 89(2–3).
Singh, A., Nautiyal, M.C., Kunwar, R.M. & Bussmann, R.W. 2017. Ethnomedicinal plants used by local inhabitants of Jakholi block, Rudraprayag district, western Himalaya, India. *Journal of Ethnobiology and Ethnomedicine*, 13(1).
Smeriglio, A., Denaro, M., Barreca, D., Calderaro, A., Bisignano, C., Ginestra, G., Bellocco, E. & Trombetta, D. 2017. In Vitro Evaluation of the Antioxidant, Cytoprotective, and Antimicrobial Properties of Essential Oil from Pistacia vera L. Variety Bronte Hull. www.mdpi.com/journal/ijms.
Sumantri, I.B., Wahyuni, H.S. & Mustanti, L.F. 2020. Total Phenolic, Total Flavonoid and Phytochemical Screening by FTIR Spectroscopic of Standardized Extract of *Mikania micrantha* Leaf. *Pharmacognosy Journal*, 12(6): 1395–1401.
Takahashi, T., Kokubo, R. & Sakaino, M. 2004. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*. *Letters in Applied Microbiology*, 39(1).
Tille, P. 2014. Section 3: Principles of Antimicrobial Action and Resistance. In *Bailey's and Scotts Diagnostic Microbiology. Elsevier, Missouri, US.* 153–186.
Tiwari, P., Kumar, B., Kaur, M., Kaur, G. & Kaur, H. 2011. Phytochemical screening and Extraction: A Review. *Internationale Scientia Pharmaceutica*, 1(1): 99–106.
Trease, G.E. & Evans, W.C. 1989. Trease and Evans Pharmacognosy. In *Pharmacognosy*. London: Bailliere Tindall Limited: 376–480.
Ubeda, C., Taur, Y., Jenq, R.R., Equinda, M.J., Son, T., Samstein, M., Viale, A., Socci, N.D., van den Brink, M.R.M., Kamboj, M. & Pamer, E.G. 2010. Vancomycin-resistant Enterococcus domination of intestinal microbiota is enabled by antibiotic treatment in mice and precedes bloodstream invasion in humans. *Journal of Clinical Investigation*, 120(12).
Ugwoke, E., Aisida, S.O., Mirbahar, A.A., Arshad, M., Ahmad, I., Zhao, T. & Ezema, F.I. 2020. Concentration induced properties of silver nanoparticles and their antibacterial study. *Surfaces and Interfaces*, 18.
Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A. & Venkitanarayanan, K. 2014. Combating Pathogenic Microorganisms Using Plant-Derived Antimicrobials: A Minireview of the Mechanistic Basis. *BioMed Research International*, 2014.
Vastrad, J., Goudar, G. & Byadgi, S. 2016. Characterisation of Phenolic Compounds *Eucalyptus globulus* and *Cymbopogan citratus* leaf extracts.: 1–10.

Velavan, S. 2015. Phytochemicals techniques - A review. *World Journal of Science and Research*, 1(2): 80–91.
World Health Organisation. 2017. *Global Priority List of Antibiotic Resistant Bacteria to Guide Research*. Geneva, Switzerland.
Worth, H., Schacher, C. & Dethlefsen, U. 2009. Concomitant therapy with Cineole (Eucalyptole) reduces exacerbations in COPD: A placebo-controlled double-blind trial. *Respiratory Research*, 10(1).
van Wyk, B.-E. 2008. A broad review of commercially important Southern African medicinal plants. *Journal of Ethnopharmacology*, 119(3).
van Wyk, B.E., Van Oudtshoorn, B. & Gericke, N. 1997. *Medicinal plants of South Africa.* Pretoria: Briza.
Yayan, J., Ghebremedhin, B. & Rasche, K. 2015. Antibiotic Resistance of *Pseudomonas aeruginosa* in Pneumonia at a Single University Hospital Centre in Germany over a 10-Year Period. *PLOS one*, 10(10).
Yimam, B.B. & Desalew, A. 2022. Phytochemical Screening, Antibacterial Effect, and Essential Oil Extract from the Leaf of *Artemisia afra* against on Selected Pathogens. *Advances in Microbiology*, 12(07): 386–397.
Zhang, Q.-W., Lin, L.-G. & Ye, W.-C. 2018. Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, 13(1).

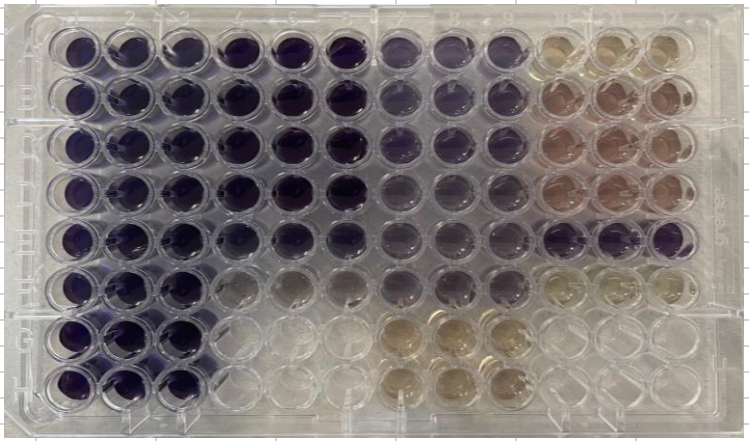
Chapter 6

Appendices

Addendum A: Antibacterial Activity analysis

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample12
A	1,331	1,393	1,329	2,144	2,203	2,128	0,998	0,91	1,064	0,222	0,224	0,246
B	1,344	1,349	1,354	2,168	2,281	2,157	0,893	0,85	1,294	0,365	0,386	0,406
C	1,309	1,305	1,354	1,917	2,028	2,19	1,054	0,861	1,099	0,317	0,363	0,348
D	1,075	1,119	1,224	2,014	2,152	1,973	0,659	0,695	0,73	0,35	0,345	0,359
E	1,22	1,305	1,357	1,338	1,381	1,323	0,667	0,663	0,684	1,188	1,168	1,363
F	1,309	1,211	1,215	0,374	0,409	0,403	0,612	0,675	0,665	0,198	0,185	0,185
G	1,934	2,208	2,132				0,226	0,238	0,227			
H	2,017	2,188	2,357				0,212	0,229	0,267			

Sample	Location	Average OD	%growth		NOTE:
E.c 1	A1-3	1,351	100,272143		No effect
E.c 2	B1-3	1,349	100,123701		No effect
E.c 3	C1-3	1,322666667	98,1692232		
E.c 4	D1-3	1,139333333	84,562098		
E.c 5	E1-3	1,294	96,0415636		
E.c 6	F1-3	1,245	92,4047501		
E.c 7	G1-3	2,091333333	155,220188		Enhanced growth
E.c 8	H1-3	2,187333333	162,345374		Enhanced growth
E.c 9	A4-6	2,158333333	160,192974		Enhanced growth
E.c 10	B4-6	2,202	163,433944		Enhanced growth
E. c11	C4-6	2,045	151,781296		Enhanced growth
E. c12	D4-6	2,046333333	151,880257		Enhanced growth
E.c control	E4-6	1,347333333	100		
Ampicillin	F4-6	0,395333333	29,3419099		
S.a 1	A7-9	0,990666667	79,9139554		
S.a 2	B7-9	1,012333333	81,661737		
S.a 3	C7-9	1,004666667	81,0432912		
S.a 4	D7-9	0,694666667	56,036569		
S.a 5	E7-9	0,671333333	54,1543426		
S.a 6	F7-9	0,650666667	52,4872277		
S. a7	G7-9	0,230333333	18,5802635		
S. a8	G7-9	0,236	19,0373756		
S. a9	A10-12	0,230666667	18,6071525		
S.a 10	B10-12	0,385666667	31,1105136		
S.a 11	C10-12	0,342666667	27,6418392		
S. a 12	D10-12	0,351333333	28,3409519		
S.a control	E10-12	1,239666667	100		
Ampicillin	F10-12	0,189333333	15,2729228		



Addendum B: Minimum Inhibitory Concentration Analysis

##BLOCKS= 16																																	
Plate:	S aureus P	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						S. aureus				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 1			Average	Stdev	Sample 2			Average	Stdev	Sample 3			Average	Stdev
	22,5	0,9413	0,964	0,8561	0,9416	0,7156	0,8457	1,0931	0,9848	1,0603				1,063966667	500ug/ml	88,47082	90,60434	80,46305	86,51274	5,346684	88,49901	67,25775	79,48557	78,41411	10,66109	102,7382	92,55929	99,65538	98,31762	5,219641
		0,8757	0,9215	0,9222	0,7028	0,8304	0,8692	0,933	0,9633	0,9451					250ug/ml	82,30521	86,60986	86,67565	85,1969	2,504497	66,0547	78,04756	81,69429	75,26552	8,182542	87,69072	90,53855	88,82797	89,01908	1,433503
		1,0986	1,1731	1,1889	0,8698	0,8244	0,8719	0,9607	0,9796	1,0297					125ug/ml	103,2551	110,2572	111,7422	108,4182	4,532579	81,75068	77,48363	81,94806	80,39412	2,522491	90,29418	92,07055	96,77935	93,04803	3,351259
		1,0967	1,1109	1,1931	1,0646	1,1027	1,1987	0,8943	0,9573	1,0511					100ug/ml	103,0765	104,4112	112,137	106,5416	4,891502	100,0595	103,6405	112,6633	105,4544	6,494741	84,05339	89,97462	98,79069	90,93957	7,415886
		1,1164	1,0296	1,0707	1,2032	1,1685	1,1716	0,954	1,0832	1,0963					50ug/ml	104,9281	96,76995	100,6329	100,777	4,080984	113,0862	109,8249	110,1162	111,0091	1,804739	89,66446	101,8077	103,0389	98,17037	7,39201
		0,9488	0,8022	1,0712	1,3148	1,1162	1,2392	1,2713	1,3199	1,3745					25ug/ml	89,17573	75,3971	100,6798	88,41756	12,65841	123,5753	104,9093	116,4698	114,9848	9,42119	119,4868	124,0546	129,1864	124,2426	4,852507
		0,6537	0,6522	0,627	1,0884	0,9193	0,9572	1,1048	1,2712	1,1918	0,9776	1,1053	1,109		10ug/ml	61,43989	61,29891	58,93042	60,55641	1,409913	102,2964	86,40308	89,96522	92,88825	8,340128	103,8378	119,4774	112,0148	111,7767	7,822512
		0,8574	0,8486	0,9322	1,176	1,12	1,2674	1,2164	1,2646	1,1669	0,1946	0,1701	0,1718		5ug/ml	80,58523	79,75814	87,61553	82,65297	4,317555	110,5298	105,2665	119,1203	111,6388	6,99318	114,3269	118,8571	109,6745	114,2862	4,591445
~End																																	
Plate:	S aureus p	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						S. aureus				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 4			Average	Stdev	Sample 5			Average	Stdev	Sample 6			Average	Stdev
	22,5	1,0412	1,1019	0,9416	0,8422	0,7778	0,8536	0,9315	1,0426	0,9607				1,0359	500ug/ml	100,5116	106,3713	90,8968	99,2599	7,812804	81,30128	75,08447	82,40178	79,59584	3,945523	89,92181	100,6468	92,74061	94,4364	5,559949
		1,0236	0,8887	0,9834	0,8691	0,9533	0,9544	0,9449	0,9521	1,0471					250ug/ml	98,81263	85,79013	94,93194	93,17823	6,686026	83,89806	92,02626	92,13245	89,35225	4,723769	91,21537	91,91042	101,0812	94,73566	5,506367
		0,8546	0,8785	0,8965	0,9844	0,8494	0,9149	0,9618	0,9981	0,9634					125ug/ml	82,49831	84,80548	86,5431	84,61563	2,029068	95,02848	81,99633	88,31934	88,44805	6,517026	92,8468	96,351	93,00125	94,06635	1,98007
		1,0231	1,0378	0,9835	1,2028	1,133	1,2093	0,838	0,9761	0,9422					100ug/ml	98,76436	100,1834	94,9416	97,96312	2,711208	116,1116	109,3735	116,7391	114,0747	4,083451	80,89584	94,22724	90,95473	88,6926	6,947624
		1,085	1,033	1,036	1,2775	1,141	1,1902	1,158	1,298	1,3282					50ug/ml	104,7398	99,72005	100,0097	101,4898	2,818298	123,3227	110,1458	114,8953	116,1212	6,673475	111,7869	125,3017	128,217	121,7685	8,766409
		1,0628	0,9916	0,9933	1,1482	1,0788	1,0559	1,2915	1,3604	1,4705					25ug/ml	102,5968	95,72353	95,88763	98,06931	3,921757	110,8408	104,1413	101,9307	105,6376	4,639691	124,6742	131,3254	141,9539	132,6512	8,715782
		0,6006	0,6122	0,6301	1,1631	1,1192	1,1948	1,2661	1,2153	1,2768	1,0257	0,9138	1,1682		10ug/ml	57,97857	59,09837	60,82633	59,30109	1,434665	112,2792	108,0413	115,3393	111,8866	3,664805	122,2222	117,3183	123,2551	120,9319	3,171803
		1,0324	0,9207	0,8785	0,9671	0,8833	0,8831	1,3393	1,429	1,4544	0,1976	0,1734	0,1792		5ug/ml	99,66213	88,87924	84,80548	91,11562	7,676655	93,35843	85,26885	85,24954	87,95894	4,676107	129,2885	137,9477	140,3997	135,8786	5,837374
~End																																	
Plate:	S aureus p	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						S. aureus				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 7			Average	Stdev	Sample 8			Average	Stdev	Sample 9			Average	Stdev
	22,5	2,093	2,2102	2,3138	1,3928	1,6866	1,4958	1,566	1,4258	1,507				1,075766667	500ug/ml	194,5589	205,4535	215,0838	205,0321	10,26894	129,4705	156,7812	139,045	141,7656	13,85714	145,5706	132,538	140,0861	139,3983	6,543457
		2,4298	2,461	2,6553	0,7276	0,686	0,6983	1,719	1,6428	1,6398					250ug/ml	225,8668	228,7671	246,8286	233,8208	11,35802	67,63548	63,76848	64,91185	65,4386	1,986591	159,793	152,7097	152,4308	154,9778	4,172391
		1,9301	1,8813	1,7992	2,2097	2,7517	2,7299	1,1095	1,1921	1,1557					125ug/ml	179,4162	174,8799	167,2482	173,8481	6,149305	251,8855	255,7897	253,7632	253,8128	1,952568	103,1357	110,814	107,4304	107,1267	3,848119
		1,5518	1,5922	1,693	2,1092	2,1579	2,285	0,8334	0,8446	0,89					100ug/ml	144,2506	148,0061	157,3761	149,8776	6,759942	196,0648	200,5918	212,4067	203,0211	8,437412	77,47033	78,51145	82,7317	79,57116	2,786167
		1,536	1,5924	1,6258	1,9267	1,7061	1,7475	2,8671	2,598	2,9727					50ug/ml	142,7819	148,0247	151,1294	147,312	4,219154	179,1002	158,5939	162,4423	166,7121	10,89957	266,5169	241,5022	276,3332	261,4507	17,95964
		1,6562	1,6863	1,5655	1,7249	1,8824	2,0252	2,4807	2,9148	2,7691					25ug/ml	153,9553	156,7533	145,5241	152,0776	5,845352	160,3415	174,9822	188,2564	174,5267	13,96306	230,5983	270,9509	257,4071	252,9855	20,53647
		1,6768	1,5842	1,4904	1,8927	1,9623	1,8232	2,2175	2,259	2,1562	1,0598	1,1553	1,0122		10ug/ml	155,8702	147,2624	138,5431	147,2252	8,663649	175,9396	182,4094	169,4791	175,9427	6,465157	206,1321	209,9898	200,4338	205,5185	4,807439
		1,4104	1,3063	1,5514	1,654	1,8091	1,7213	1,7709	1,7667	1,7933	0,1726	0,1666	0,1765		5ug/ml	131,1065	121,4297	144,2134	132,2499	11,43483	153,7508	168,1684	160,0068	160,642	7,229771	164,6175	164,2271	166,6997	165,1814	1,329295
~End																																	
Plate:	S aureus p	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						S. aureus				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 10			Average	Stdev	Sample 11			Average	Stdev	Sample 12			Average	Stdev
	22,5	2,0649	2,116	2,014	0,8432	0,9914	0,9317	1,4328	1,4396	1,4474				0,982066667	500ug/ml	210,2607	215,464	205,0777	210,2675	5,193133	85,85975	100,9504	94,87136	93,89383	7,592655	145,8964	146,5888	147,3831	146,6228	0,743911
		2,6217	2,2789	2,6413	0,7424	0,7739	0,5714	1,2535	1,194	1,223					250ug/ml	266,9574	232,0515	268,9532	255,9874	20,75312	75,59568	78,8032	58,18342	70,86077	11,09542	127,639	121,5803	124,5333	124,5842	3,029647
		1,8219	1,9175	2,1748	1,9749	1,8832	1,9982	1,0471	1,0834	1,1036					125ug/ml	185,5169	195,2515	221,4514	200,7399	18,58529	201,0963	191,7589	203,4689	198,7747	6,190599	106,6221	110,3184	112,3753	109,7719	2,915257
		2,029	1,9383	1,9133	2,415	2,2416	2,5309	0,9734	0,9676	1,0803					100ug/ml	206,6051	197,3695	194,8238	199,5995	6,199135	245,91	228,2533	257,7116	243,9583	14,8258	99,11751	98,52692	110,0027	102,549	6,461818
		1,6442	1,4856	1,5438	1,9466	1,8218	2,0757	2,5425	2,3131	2,0074					50ug/ml	167,4224	151,2728	157,1991	158,6315	8,169532	198,2146	185,5068	211,3604	198,3606	12,92744	258,8928	235,5339	204,4057	232,9441	27,33573
		1,4183	1,3877	1,4144	1,6892	1,8877	1,7246	2,2322	2,2383	2,3583					25ug/ml	144,4199	141,3041	144,0228	143,2489	1,695977	172,0046	192,2171	175,6093	179,9437	10,78083	227,2962	227,9173	240,1364	231,7833	7,240689
		1,5164	1,5625	1,4493	1,4765	1,54	1,5606	1,9196	1,9538	1,965	0,9377	1,0086	0,9999		10ug/ml	154,4091	159,1033	147,5765	153,6963	5,796319	150,3462	156,8122	158,9098	155,3561	4,463619	195,4653	198,9478	200,0882	198,1671	2,408296
		1,5374	1,4024	1,3666	1,5233	1,5591	1,4588	1,5554	1,5603	1,5392	0,2583	0,1476	0,1533		5ug/ml	156,5474	142,8009	139,1555	146,1679	9,171819	155,1117	158,757	148,5439	154,1375	5,175794	158,3803	158,8792	156,7307	157,9967	1,124445
~End																																	
Plate:	E coli plate	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						E. coli				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 1			Average	Stdev	Sample 2			Average	Stdev	Sample 3			Average	Stdev
	22,5	0,7472	0,7821	0,7634	0,8544	0,7454	0,8454	0,9177	0,8415	0,9854				0,927566667	500ug/ml	80,55486	84,31739	82,30136	82,3912	1,882875	92,11198	80,3608	91,1417	87,87149	6,522516	98,93628	90,72124	106,235	98,63083	7,761364
		0,7786	0,8685	0,6815	1,0491	0,9789	0,8554	0,976	0,8298	0,99					250ug/ml	83,94006	93,63208	73,47181	83,68132	10,08263	113,1024	105,5342	92,21979	103,6188	10,57224	105,2215	89,45988	106,7309	100,4708	9,565525
		0,8253	0,9045	0,8698	0,839	0,8906	0,9065	0,8472	0,8959	1,1463					125ug/ml	88,97474	97,51321	93,77224	93,42006	4,280115	90,45172	96,01466	97,72882	94,73174	3,804404	91,33575	96,58605	123,5814	103,8344	17,30172
		0,7987	0,9593	0,8949	0,868	0,7674	0,8539	0,9933	0,9788	1,0932					100ug/ml	86,10702	103,4211	96,47824	95,33547	8,713446	93,57818	82,7326	92,05807	89,45628	5,872277	107,0866	105,5234	117,8568	110,1556	6,715039
		0,8048	0,7644	0,8807	0,8769	0,7568	0,7467	0,8952	0,968	1,0455					50ug/ml	86,76465	82,40917	94,94735	88,04039	6,3657	94,53768	81,58982	80,50095	85,54282	7,808781	96,51058	104,3591	112,7143	104,528	8,103164
		1,0025	1,0121	1,0889	0,8792	0,8798	0,8686	0,9203	0,9564	1,0045					25ug/ml	108,0785	109,1135	117,3932	111,5284	5,105366	94,78564	94,85033	93,64286	94,42628	0,679225	99,21659	103,1085	108,2941	103,5397	4,554096
		0,7183	0,753	0,8943	0,9198	0,8199	0,8319	0,8636	1,0078	0,935	0,8798	0,8901	1,0128		10ug/ml	77,43918	81,18015	96,41356	85,01096	10,05053	99,16268	88,39257	89,68628	92,41384	5,880354	93,10382	108,6499	100,8014	100,8517	7,773148
		0,9178	1,0062	0,9707	0,9633	0,8744	0,9607	0,9748	0,9782	1,0386	0,1567	0,1617	0,1422		5ug/ml	98,94707	108,4774	104,6502	104,0249	4,795827	103,8524	94,26816	103,5721	100,5642	5,454334	105,0922	105,4587	111,9704	107,5071	3,869666
~End																																	
Plate:	E coli plate	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						E. coli				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 4			Average	Stdev	Sample 5			Average	Stdev	Sample 6			Average	Stdev
	22,5	0,9052	0,8914	0,9098	1,0584	1,0104	0,8409	0,9231	1,0224	1,1312				1,148333333	500ug/ml	78,82729	77,62554	79,22787	78,56023	0,833875	92,16836	87,98839	73,22787	84,46154	9,950607	80,38607	89,03338	98,50798	89,30914	9,064105
		0,9939	0,8629	0,816	1,0451	1,0531	0,9993	0,9906	1,0231	1,0659					250ug/ml	86,55152	75,14369	71,05951	77,58491	8,029341	91,01016	91,70682	87,02177	89,91292	2,52792	86,26415	89,09434	92,82148	89,39332	3,288873
		0,9035	0,8516	1,004	1,0359	0,8213	0,9261	0,9444	0,8792	0,9584					125ug/ml	78,67925	74,15965	87,43106	80,08999	6,747237	90,209	71,52104	80,64731	80,79245	9,344822	82,24093	76,56313	83,46009	80,75472	3,680843
		0,8248	0,8093	0,7976	0,8372	0,8008	0,9209	0,9791	1,0554	1,1102					100ug/ml	71,82583	70,47605	69,45718	70,58636	1,188171	72,90566	69,73585	80,19448	74,27866	5,3628	85,2627	91,90711	96,67925	91,28302	5,733803
		0,8302	1,0708	0,9028	1,1804	1,0338	0,9084	1,1659	1,0654	1,0378					50ug/ml	72,29608	93,24819	78,61829	81,38752	10,74705	102,7925	90,02612	79,10595	90,64151	11,85524	101,5298	92,77794	90,37446	94,89405	5,870997
		0,9617	0,9708	0,8262	0,843	0,8775	0,7978	0,7991	0,8937	0,8768					25ug/ml	83,74746	84,53991	71,94775	80,07837	7,052466	73,41074	76,41509	69,4746	73,10015	3,480656	69,58781	77,82583	76,35414	74,58926	4,393445
		0,8022	0,9056	0,824	1,0995	0,9142	0,9374	0,9389	1,0449	1,0365	1,1574	1,1565	1,1311		10ug/ml	69,85776	78,86212	71,75617	73,49202	4,746523	95,74746	79,61103	81,63135	85,66328	8,791384	81,76197	90,99274	90,26125	87,67199	5,131274
		1,0455	1,1134	1,0603	1,1662	1,0952	1,1531	1,1732	1,1994	1,186	0,1264	0,1018	0,1671		5ug/ml	91,04499	96,95791	92,33382	93,44557	3,109284	101,5559	95,373	100,4151	99,11466	3,290187	102,1655	104,447	103,2801	103,2975	1,140883
~End																																	
Plate:	E coli plate	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						E. coli				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 7			Average	Stdev	Sample 8			Average	Stdev	Sample 9			Average	Stdev
	22,5	1,3953	1,3112	1,2545	0,8148	0,977	0,9047	0,9116	0,8354	1,088				1,0208	500ug/ml	136,6869	128,4483	122,8938	129,343	6,939944	79,81975	95,70925	88,62657	88,05185	7,960324	89,30251	81,83777	106,5831	92,57445	12,69298
		1,4916	1,4033	1,2288	0,7755	0,7909	0,7786	0,8664	0,8486	0,8215					250ug/ml	146,1207	137,4706	120,3762	134,6558	13,10104	75,96983	77,47845	76,27351	76,57393	0,797917	84,87461	83,13088	80,4761	82,82719	2,214925
		1,6144	1,5691	1,4764	1,7931	1,8846	1,7497	0,8137	0,8065	0,8861					125ug/ml	158,1505	153,7128	144,6317	152,165	6,891032	175,6563	184,6199	171,4048	177,227	6,746119	79,71199	79,00666	86,80447	81,84104	4,312897
		1,2608	1,2291	1,1708	1,6357	1,66	1,5488	0,91	0,9815	0,9741					100ug/ml	123,511	120,4056	114,6944	119,537	4,472027	160,2371	162,6176	151,7241	158,1929	5,727176	89,14577	96,15008	95,42516	93,57367	3,851766
		1,0137	0,9381	0,9018	1,4837	1,4795	1,4411	1,741	1,8758	1,9992					50ug/ml	99,30447	91,89851	88,34248	93,18182	5,592537	145,3468	144,9353	141,1736	143,8186	2,299843	170,5525	183,5758	195,8464	183,3856	12,65105
		0,9366	0,9747	0,9204	1,224	1,1439	1,2762	1,7145	1,7476	1,8449					25ug/ml	91,75157	95,48393	90,16458	92,46669	2,730832	119,906	112,0592	125,0196	118,9949	6,528066	167,9565	171,1991	180,7308	173,2955	6,640166
		0,9834	1,0835	0,9804	1,0438	0,9236	0,9549	1,4982	1,6016	1,4943	0,9697	1,0077	1,085		10ug/ml	96,33621	106,1422	96,04232	99,50692	5,748233	102,2531	90,47806	93,54428	95,42516	6,108715	146,7672	156,8966	146,3852	150,0163	5,961511
		1,0387	0,9711	1,0226	1,0972	0,9527	1,12	1,4405	1,4059	1,491	0,1727	0,1742	0,1733		5ug/ml	101,7535	95,13127	100,1763	99,02038	3,459154	107,4843	93,32876	109,7179	103,5103	8,887927	141,1148	137,7253	146,0619	141,634	4,192481
~End																																	
Plate:	E coli plate	1,3	PlateForm	Endpoint	Absorbanc	Raw	FALSE	1						E. coli				% growth					% growth					% growth	
	Temperatu	1	2	3	4	5	6	7	8	9	10	11	12	100% (culture control)	Sample 10			Average	Stdev	Sample 11			Average	Stdev	Sample 12			Average	Stdev
	23	1,3385	1,3608	1,2624	0,8624	0,8941	0,8637	0,9802	1,1268	0,908				0,8434	500ug/ml	158,7029	161,3469	149,6799	156,5766	6,11727	102,2528	106,0114	102,4069	103,557	2,126927	116,2201	133,6021	107,6595	119,1605	13,21891
		1,2364	1,4282	1,4226	0,8984	0,8247	0,7605	0,8229	0,8532	0,906					250ug/ml	146,5971	169,3384	168,6744	161,5366	12,94227	106,5212	97,78278	90,17074	98,15825	8,181707	97,56936	101,162	107,4223	102,0512	4,986318
		1,2464	1,3765	1,222	1,3528	1,1957	1,2989	0,7759	0,7688	0,7708					125ug/ml	147,7828	163,2084	144,8897	151,9603	9,847976	160,3984	141,7714	154,0076	152,0591	9,465122	91,99668	91,15485	91,39198	91,5145	0,434083
		1,1525	1,087	1,0123	1,4283	1,3663	1,2474	0,8606	0,7996	0,8284					100ug/ml	136,6493	128,8831	120,0261	128,5195	8,317559	169,3502	161,9991	147,9014	159,7502	10,89985	102,0394	94,80673	98,22148	98,35586	3,618187
		1,0552	0,9476	1,0302	1,4492	1,4404	1,3882	1,2833	1,3322	1,2748					50ug/ml	125,1126	112,3548	122,1484	119,8719	6,676657	171,8283	170,7849	164,5957	169,0696	3,909523	152,1579	157,9559	151,1501	153,7546	3,673118
		0,8768	0,9156	0,7799	1,3209	1,118	1,2559	1,3975	1,2743	1,4539					25ug/ml	103,9602	108,5606	92,47095	101,6639	8,286961	156,6161	132,5587	148,9092	146,028	12,28476	165,6984	151,0908	172,3856	163,0583	10,8901
		0,8392	0,8458	0,7161	1,1485	1,1696	1,2008	1,1374	1,2887	1,2431	0,8248	0,786	0,9194		10ug/ml	99,50202	100,2846	84,90633	94,89764	8,661566	136,175	138,6768	142,3761	139,076	3,119758	134,8589	152,7982	147,3915	145,0162	9,202506
		0,9058	1	0,974	1,0097	1,0764	1,0571	1,1866	1,193	1,1802	0,1866	0,2006	0,1764		5ug/ml	107,3986	118,5677	115,4849	113,8171	5,768308	119,7178	127,6263	125,3379	124,2273	4,069522	140,6924	141,4513	139,9336	140,6924	0,758833

ADDENDUM C: Average Percentage Yield of Extractions

Mass of extract (g)	Sample	Mass of *A.afra* Hexane extract (	Sample	Mass of *A.afra* Methanol extract (g)	Sample	Mass of *E.globulus* Hexane extract (g)	Sample	Mass of *E.globulus* Methanol extract (g)
0,2587	1	0,2587	4	0,9787	7	0,3903	10	2,2328
0,1975	2	0,1975	5	0,8994	8	0,4032	11	2,2338
0,2015	3	0,2015	6	0,8881	9	0,4002	12	2,3621
0,9787		0,219233333		0,922066667		0,3979		2,276233333
0,8994								
0,8881								
0,3903								
0,4032								
0,4002								
2,2328								
2,2338								
2,3621