Green synthesis of copper nanoparticles using *Lannea discolor* (Sond.) Engl. and evaluation of their biological efficacy

By

Unarine Rambau U.Rambau 8/10/2024

Student no: 18018731

submitted in the Department of

Biological Sciences, Faculty of Sciences, Engineering and Agriculture

University of Venda in the fulfilment of the requirement of the degree of

Doctor of Philosophy in

Botany

Promoter: Dr N.A Masevhe 15/10/2024

Co-Promoter: Prof A Samie 16/10/2024

Abstract

Worldwide, herbal medicine has become a preferred source of therapy as it contains an array of bioactive phytochemicals, is biologically compatible, and has been used to synthesize nanoparticles with advanced therapeutic potential. *Lannea discolor*, commonly known as "dikbas," occurs in southern Africa and is extensively utilized as a traditional medicine. This study explores the phytochemical composition of the plant extracts, identifying potential active compounds. Qualitative and quantitative phytochemical screening and radical scavenging capabilities of the crude extracts of *Lannea discolor* revealed the presence and abundance of flavonoids, fatty acids, phenolics, and terpenoids and high antioxidant activity. Using LC-MS, several compounds were elucidated by comparing the molecular formulae and retention times with those already published in various chemical databases. The extracts displayed efficient antibacterial activity when evaluated against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Additionally, the methanolic and acetone extracts were highly toxic against the Caco-2 cancer cell line. The study also focuses on the green synthesis of copper, gold, and silver nanoparticles using *Lannea discolor* plant extracts as reducing and stabilizing agents.

Biosynthesis of copper, gold, and silver nanoparticles was executed using the different extracts at temperatures of 25 °C and 80 °C. The composition of the synthesized nanoparticles (NPs) was confirmed visually by the observation of color change and characterized by UV-Vis, FEGSEM, EDX, HRTEM, FTIR, and Zetasizer. Plasmon resonance peaks confirmed that the particle interfaces were coated with phytochemicals. Evaluation of the zeta potential affirmed the extent of the stability of the NPs, as substantiated by conclusive negative potential values. Elemental mapping revealed particles consisting of copper, gold, and silver among the main elements. The nanoflowers resulting from the reduction of leaf extracts and the nanoparticles acquired from the stem and root of *Lannea discolor* ranged from 30–97 nm and 9–37 nm, respectively, while the copper nanoparticles had sizes of 20–104 nm. Both nanoparticles showed potential for application in biomedicine and conductivity for manufacturing industries. The gold nanoparticles exhibited exceptional antibacterial activity, while the copper nanoparticles had no activities.

Furthermore, silver nanoparticles were synthesized to evaluate their antibacterial activity and cytotoxicity against Caco-2 cells. The spherical silver nanoparticles had notable activity against the bacteria *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, as well as *Klebsiella pneumoniae*, and were able to inhibit cell proliferation in Caco-2 cells, demonstrating their intrinsic potential as both antibacterial and anticancer agents. Additionally, the nanoparticles showed notable antioxidant activities. As the first study based on phytochemical profiling of *L. discolor*, this work highlights its potential for unexploited medicinal properties. This was the first study on nanoparticle synthesis of *L. discolor*, and it contributes positively to the eco-friendly and liberal

technique of fabricating nanoparticles for the manufacturing of novel drugs from plants used as alternative medicine.

DECLARATION

I, **Unarine Rambau**, hereby declare that this thesis is entirely my own work, with the exception of a few instances where duely indicated. All sources used and referenced in this thesis have been properly acknowledged. This work has not been submitted, in its entirety or partially, for any other academic purpose or qualification.

U. Rambau

Signed (Student): ... . Date: .08/10/2024.....

Signed (Promoter): .... Date: ....15/10/2024.....

Preface

This thesis is comprised of 8 chapters, which are compiled in the format of published and manuscripts under preparation. The outlines of each chapter are described below.

Disclaimer: Chapter 6 has been presented as a published paper, therefore, repetition was inevitable

Chapter 1. This is a general introduction that incorporates the background, aims and objectives of the study and highlights the problem statement and the hypothesis.

Chapter 2. This chapter encompasses the description of *Lannea discolor*, past phytochemical studies and an introduction to its potential in nanoparticle synthesis, by briefly revisiting nanosynthesis by few plants in family Anacardiaceae.

Chapter 3. This chapter reviews and briefly discusses on the dawn of nanotechnology on traditional medicine, illuminating the potential of medicinally used plants for nanosynthesis, the history of nanotechnology and recent nanotechnology developments in the South African landscape.

Chapter 4. This chapter details the phytochemical analysis and LC-MS metabolite profiling of *Lannea discolor* (Sond.) Engl. methanolic extracts.

Chapter 5. This chapter investigates the impact of acetone and water solvents on the yield and appearance of metabolites in the organs of *Lannea discolor* and reports their in vitro antioxidant, and antibacterial activities

Chapter 6. This chapter reports on an investigation on the green synthesis of gold and copper nanoparticles by the extracts of *Lannea discolor*. It also reports on the characterisation of the nanoparticles and assesses their ability to inhibit the growth of different bacteria (Published: Rambau, U., Masevhe, N. A., & Samie, A., 2024. Green Synthesis of Gold and Copper Nanoparticles by *Lannea discolor*: Characterization and Antibacterial Activity. Inorganics, 12(2), 36. https://doi.org/10.3390/inorganics12020036 (https://doi.org/10.3390/inorganics12020036).

Chapter 7. This chapter investigates the synthesis of nanoparticles by silver nitrate. It also reports the biological activity of the nanoparticles with focus on antioxidant, antimicrobial and cytotoxicity against Caco-2 cells. (*In preparation*)

Chapter 8. This chapter encompasses the general conclusion and future perspectives from the whole study.

Acknowledgements

First, I give praise and reverence to God Almighty for His favor and mercy, who sustained me until the completion of this project

This commemorates the finale of PhD program, during which I have learned a great deal about science, research project management, and myself.

With all of my heart, I want to thank my PhD promoter, Professor Nndivhaleni Anox Masevhe. I saw in him the spirit and character of a self-starter and pathfinder. The finalistaion of this work would not have been possible without his tenacious assistance, direction, and insightful counsel. With pleasure, I offer my sincere gratitude to my Co-promoter Professor Amidou Samie, for assisting me in thinking out of the box at the start and throughout my PhD project. The National Research Foundation (NRF) is highly appreciated for their financial resources.

I also want to express my gratitude to all of my laboratory buddies especially, Takalani M. Mulaudzi and Mueletshedzi Manyage, for their company and helpful tips in the laboratory. I would like to express my appreciation to everyone else in the departmental members i.e., of Department of Biological Sciences (Botany), for their occasional affirmations and guidance. Ms Mankone Legodi, thank you for your helpful spirit in the procuring of all laboratory resources needed in this study. A huge thanks to Dr Lutendo Mathomu for his valuable input and guidance for some aspects of my laboratory work. My greatest appreciation goes out to Dr Nneka Akwu, who fervently stood by me emotionally, and mentally, until the completion of this degree.

My family and close friends, thank you for your love, prayers, and support. A special thank you to my dad “Woodrow”, who took time out of his schedule for the location, and collection of the plant material utilised in this study. To my mom, brother, and knowledgeable grandmother, I appreciate you being my sources of support.

Table of Contents

Abstract……ii
Declaration……iv
Preface……v
Acknowledgments……vi
List of Figures……xii
List of Tables……xiv
List of Abbreviations……xv
CHAPTER 1
1.1 Background……1
1.2 Problem statement……3
1.3 Rationale of the study……3
1.4 Aim……4
1.5 Objectives of the Study……4
1.6 Hypothesis……4
1.7 References……5
CHAPTER 2
2.1 Literature Review……7
2.1.1 Plant description of *Lannea discolor* (Sond.) Engl……7
2.1.2 Phytochemistry and pharmacological history……8
2.1.3 The emergence of nanotechnology……9
2.1.4 Nanoparticles……10
2.1.5 Types of nanoparticles……10
2.1.5.1 Metal Nanoparticles……10
2.1.5.2 Polymer based Nanoparticles……11
2.1.5.3 Liposomes……12
2.1.6 Intrinsic properties of nanoparticles……12
2.1.6.1 Mechanical Properties……12
2.1.6.2 Optical Properties……14
2.1.6.3 Electrical Properties and Magnetic Properties……15
2.2 Plants and nanoparticles……15
2.3 Usefulness of nanotechnology for herbal medicines……16
2.4 Recent nanotechnology applications in the Anacardiaceae Family……17
2.5 References……18

CHAPTER 3: A review on the dawn of nanotechnology on African traditional medicine: A South African perspective

Abstract……..30

3.1 Introduction……..31

3.2 History and Development……32

3.3 Nanotechnology landscape in South Africa……..34

3.4 Green nanotechnology……..36

3.4.1 Metal nanoparticles in green nanotechnology……37

3.5 Nanoparticles in medicine and biology……....37

3.5.1 Nanoparticle roles Nanomedicine……..……37

3.5.2 Nanoparticle roles in biology……39

3.5.2.1 Drug Delivery……39

3.5.2.2 Imaging and Diagnostics……....39

3.5.2.3 Theranostics and Biomolecule Detection……...39

3.6 A South African perspective on nanomedicine……40

3.7 Green synthesis of nanoparticles from plant extracts……41

3.8 Significance of nanoparticles as an herbal medicine delivery system……43

3.8.1 Sufficient bioavailability……...44

3.8.2 Less Toxicity……..45

3.8.3 Pharmacological activity enhancement……45

3.9 Recent and present developments in nanotechnology……...…..46

3.10 Conclusions and future perspectives……..…48

3.10.1 Conclusions……..……...…48

3.10.2 Future prospectives ……..……..49

3.11 References……..50

CHAPTER 4: Phytochemical analysis and LC-MS metabolite profiling of *Lannea discolor* (Sond.) Engl. methanolic extracts

Abstract……..61

4.1 Introduction……..62

4.2 Materials and Methods……65

4.2.1. Preparation of plant extracts ……..……..…65

4.2.2 Qualitative Phytochemical screening ……...65

4.2.3 Quantitative Phytochemical screening ……66

4.2.3.1 Estimation of total phenolic contents……66

4.2.3.2 Estimation of total flavonoid contents……66

4.2.4 Secondary metabolite profiling by Liquid Chromatography Mass Spectrophotometry…..……..67

4.2.5 Antioxidant Assay……..67

4.2.5.1 *In vitro* antioxidant DPPH scavenging activity…….……67

4.2.6 Antimicrobial Assays…….……67

4.2.6.1 Sensitivity test ……..……..68

4.2.6.2 Determination of Minimal Inhibitory Concentration (MIC)……..……....68

4.3 Results and Discussion…….68

4.3.1 Qualitative phytochemical screening …….…...68

4.3.2 Quantitative phytochemical screening…….…….70

4.3.2.1 Effect of Extraction Parameters on Total Phenolic and Flavonoid Content ……....70

4.3.2.2 LC-MS Metabolite Profile of *Lannea discolor* Extracts…….……71

4.3.2.3 Chemo-taxonomical relevance of the LC-MS results ……...….85

4.3.3 Biological activities……...86

4.3.3.1 Antioxidant activity of *Lannea discolor* extracts……86

4.3.3.2 Antibacterial activities……87

4.4 Conclusions…….90

4.5 References……...……...……..91

CHAPTER 5: The effect of acetone and water solvents on the appearance of metabolites in the organs of *Lannea discolor* and the evaluation of their *in vitro* antioxidant, and antibacterial activities

Abstract……105

5.1 Introduction……106

5.2. Materials and Methods……..108

5.2.1 Plant Material ……..108

5.2.2 Extraction Process.……..108

5.2.3 Qualitative phytochemical screening ……..108

5.2.4 LC-MS analysis of water and acetone crude extracts of *Lannea discolor* ……..108

5.2.5 Evaluation of total phenolic contents …….……...…..108

5.2.6 Evaluation of total flavonoid contents ……109

5.2.7 Minimum inhibitory concentration assay …….……..109

5.3 Results and Discussion……..……..…….....109

5.3.1 Qualitative preliminary phytochemical screening ……109

5.3.2 LC-MS profiling of the metabolites in acetone and water extracts of *Lannea discolor*……..110

5.3.3 Quantitative phytochemical screening……116

5.3.3.1 Impact of Extraction Solvents on extraction yield, Total phenolic, flavonoid content, and antioxidant activity……116

5.3.4 Antibacterial activities of water and acetone extracts of *Lannea discolor*……118

5.3.5 Conclusion……119

5.4 References……120

CHAPTER 6: Green Synthesis of Gold and Copper Nanoparticles by *Lannea discolor*: Characterization and Antibacterial Activity

Abstract……126

6.1 Introduction……127

6.2 Materials and Methods……128

6.2.1 Collection of plant material ……128

6.2.2 Extraction of plant material ……128

6.2.3 Synthesis of Nanoparticles ……128

6.2.4 Characterisation……129

6.2.4.1 Physicochemical Characterisation of AuNPs and CuNPs……129

6.2.4.2 Scanning Electron Microscopy Analysis and Energy-Dispersive X-ray Microanalysis……129

6.2.4.3 Transmission Electron Microscopy Analysis and Measurements of Zeta Potential……129

6.2.4.4. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis……129

6.2.5 Antimicrobial Assays……130

6.3 Results and Discussion……130

6.3.1 Visual and UV-Vis Spectroscopic Analysis……130

6.3.2 The Morphology of the Synthesized Nanoparticles Using Scanning Electron Microscopy and Energy-Dispersive X-ray Analysis……132

6.3.3 Transmission Electron Microscopy, Particle Size, and Zeta Potential Analysis……133

6.3.4 FTIR Analysis……136

6.3.5. The Antibacterial Activity of AuNPs and CuNPs……137

6.4 Conclusion……139

6.5 References……139

CHAPTER 7: Plant mediated synthesis of silver nanoparticles by *Lannea discolor*: Antibacterial activities and cytotoxic effects on Caco-2 cell line

Abstract……146

7.1 Introduction……147

7.2 Materials and Methods……149

7.2.1 Plant collection and extraction ……149

7.2.2 Plant mediated Synthesis and collection of Silver Nanoparticles (AgNPs)……149

7.2.3 UV-Visible Spectral Analysis…….....149

7.2.4 Zeta Potential of Nanoparticles…….……...149

7.2.5 ATR-Fourier Transform Infrared Spectroscopy (FTIR) ……150

7.2.6 Scanning electron microscopy (SEM) analysis and energy dispersive x-ray (EDX) microanalysis……...…150

7.2.7 Transmission Electron Microscopy (TEM) Analysis ……150

7.2.8 Biological Evaluation of the plant synthesised nanoparticles……..…150

7.2.8.1 *In vitro* antioxidant DPPH scavenging activity……150

7.2.8.2 Antibacterial evaluation of AgNPs…….....151

7.2.8.2.1 Bacterial Strains and Antibacterial Activity Assay……151

7.2.8.3 Cytotoxicity evaluation of AgNPs against human colorectal adenocarcinoma cells (Caco-2)……..151

7.2.8.3.1 Sample preparation……151

7.2.8.3.2 Cell line maintenance……151

7.3 Results and Discussion…….…152

7.3.1 Synthesis, UV-Vis, and Zeta Potential analysis of AgNPs……..152

7.3.2 Surface Morphological analysis by SEM, and EDX Analysis……154

7.3.3 Microstructural studies by HRTEM and Size distribution……...154

7.3.4 FTIR analysis……...158

7.3.5 Antioxidant and Antibacterial assay……159

7.3.6 Comparative Cytotoxicity Activity……..163

7.3.7 Conclusion……...165

7.4 References…….....165

CHAPTER 8: General conclusion and recommendations for future research……161

8.1 General summary and conclusion…….…..172

8.2 Recommendations for future research……....174

9 APPENDICES……...176

9.1 APPENDIX A……176

9.2 APPENDIX B……182

9.3 APPENDIX C……183

List of Figures

Figure 2.1. The figure shows the whole tree (A), young leaves (B) and unripe fruits (C). The images were captured at Vuwani Village, Limpopo, South Africa……..8

Figure 2.2. A comparison of the size range of different nanoparticles in comparison to common materials. Image adapted from https://www.biorender.com/.....13

Figure 2.3. Different shapes of nanoparticles ……14

Figure 4.1. Peaks and the corresponding compounds detected in the leaves of *L. discolor* in the negative mode ionisation……..77

Figure 4.2. Peaks and the corresponding compounds detected in the stem bark of *L. discolor* in the negative mode of ionisation……...79

Figure 4.3. Peaks and the corresponding compounds detected in the root bark of *L. discolor* in the negative mode of ionisation……81

Figure 4.4. The molecular structures of major peaks of isolated compounds from *L. discolor* leaves…84

Figure 4.5. The molecular structures of major peaks of isolated compounds from *L. discolor* stems and root bark……..85

Figure 6.1. Visual representation of *Lannea discolor* extracts before and after NP synthesis. (A) Leaf, (B) stem and root before synthesis (C) AuNPs from leaves, and (D) AuNPs from stem and root bark..130

Figure 6.2. Representative UV-Vis spectra of SPR bands representing *Lannea discolor* (A) AuNPs/NFs and (B) CuNPs from methanol extracts……131

Figure 6.3. Representative SEM images of AuNFs from methanol leaf extracts. (A, B) AuNPs from methanol stem extract of *L. discolor* and (C, D) EDX spectra. Inset of (B) is image of fluorescence emission from AuNPs. Insets of (C, D) contain graphs of the abundance of Au to validate the formation of AuNFs and NPs……133

Figure 6.4. Representative SEM images and EDX spectra of CuNPs from the leaf methanol extracts of *Lannea discolor*. (A) Nanoparticles synthesized at 25 °C; (B) arrows showing sintering of CuNPs from leaf methanol extract. (C, D) show EDX spectra and abundance of Cu……134

Figure 6.5. Representative TEM images of the AuNFs (A) from methanol leaf extracts and AuNPs (B) from *L. discolor* methanol stem extract. Insets in B show triangular and hexagonal NPs from stem and root acetone extracts. Histograms show the size distribution of AuNFs (C) and spherical AuNPs (D).135

Figure 6.6. Schematic diagram showing proposed growth mechanism of AuNFs from *Lannea discolor* leaves……....136

Figure 6.7. Representative micrograph of (A) CuNPs from stem methanol extract and (B) size distribution. The inset shows a TEM image of sintering in CuNPs exposed to high temperatures…..136

Figure 6.8. FTIR spectrum of AuNPs from methanol stem extract (A) and CuNPs from methanol root extract (B) from *Lannea discolor*……..137

Figure 7.1. Representative images showing visual changes after synthesis of AgNPs using extracts of *Lannea discolor* leaves (A) and bark (B). Changes after synthesis observed in C and D……..152

Figure 7.2. The ultraviolet-visible (UV-vis) spectroscopy results show the absorbance spectra recorded for various reactions indicating the formation of silver nanoparticles (AgNPs). In each reaction, a 1 mM

aqueous silver nitrate (AgNO3) solution was reacted with different *Lannea discolor* extracts and incubated at temperatures of 25 °C and 80 °C. Each graph (A-I) represents AgNPs formation from methanol, acetone, and water extracts at varying times of synthesis (A – C) 0 minutes (D – F) 60 minutes (G – I) 24 hrs…….153

Figure 7.3. SEM images of AgNPs with their respective EDX spectra validating the presence of silver nanoparticles. Circle in (C) shows some spherical NPs. Insets in (A) and (B) showing signal peaks for the presence of elements……..155

Figure 7.4. TEM silver nanoparticles with different shapes of differently shaped nanoparticles. (Circled in red) Short silver nanorods (A, C, D), spheres (A – D) and triangular shaped (B) particles…….156

Figure 7.5. Representative TEM micrographs of AgNPs synthesized at 25 °C (A and B) and 80 °C (D and F) Shape and size at different magnification power. Notes: Ag nanoparticles are majorly spherical. AgNPs are dispersed homogeneously in solution and not agglomerated in some samples. The histograms (C and F) show silver nanoparticle size distribution of 2.85 to 46.6 nm……157

Figure 7.6. Representative Fourier transform infrared (FTIR) spectra are shown for silver nanoparticles (AgNPs) biosynthesized from various extracts of Lannea discolor. (A) leaf extract (25 °); (B) synthesis with stem bark (25 °C) (C) root bark (25 °); (E) leaf methanol extract (80 °C); (F) stem bark (80 °C) (G) root bark (80 °C)…….159

Figure 7.7. The figure shows the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity percentages of inhibition for the silver nanoparticles synthesised from the methanol, acetone, and water extracts of the leaves, stem, and root of *Lannea discolor* at room temperature and 80 °C……160

Figure 7.8. Results of minimum inhibitory concentration (MIC) of AgNPs particles and controls against bacteria…….163

Figure 7.9. The graph depicts the results of an MTT assay demonstrating the cytotoxic effect of extracts from L. discolor and AgNPs on Caco-2 colorectal cancer cell viability……..164

List of Tables

Table 3.1. Provides a number of studies of some bioactive medicinal plants that have been used for the green synthesis of various nanoparticles……42

Table 4.1. Compounds historically isolated from some plants in the *Lannea* genus……63

Table 4.2. Showing the presence of different phytochemicals after reagent based preliminary phytochemical screening of methanol extracts of the leaves, stem bark and root of *L. discolor*……..69

Table 4.3 Quantification of Total Polyphenol and Flavonoid Contents of *L. discolor*…..……71

Table 4.4. Compounds tentatively detected in the leaf extract of *L. discolor*……....78

Table 4.5. Compounds tentatively detected in the stem bark extract of *L. discolor*……..79

Table 4.6. Compounds tentatively detected in the root bark extract of *L. discolor*……...82

Table 4.7: Antioxidant activity of methanolic extracts of *L. discolor* using 2, 2-diphenyl1-pycrilhydrazil hydrate……87

Table 4.8. Sensitivity of methanolic extracts on sensitive microorganism's inhibition zones (mm) ± of three replicates……...…90

Table 4.9. MIC values in mg/ml of *L. discolor* after 24 incubation extract against different bacterial microorganisms……..90

Table 5.1. Qualitative phytochemical screening of acetone and water extracts of the leaves, stem bark and root of *Lannea discolor*……110

Table 5.2. Tentative identification of secondary metabolites in water extracts from leaves of *L. discolor* by LC-MS……....…113

Table 5.3. Tentative identification of secondary metabolites in acetone extracts from leaves of *L. discolor* by LC-MS……...…113

Table 5.4. Tentative identification of secondary metabolites in water extracts from the stem of *L. discolor* by LC-MS……..114

Table 5.5. Tentative identification of secondary metabolites in acetone extracts from the stem of *L. discolor* by LC-MS……..115

Table 5.6. Tentative identification of secondary metabolites in water extracts from the root of *L. discolor* by LC-MS……..115

Table 5.7. Tentative identification of secondary metabolites in acetone extracts from the root of *L. discolor* by LC-MS……...…115

Table 5.8. Extraction yield of *L. discolor* extracts……...118

Table 5.9. Quantification of Total Polyphenol and Flavonoid Contents from acetone and water extracts of *L. discolor*……118

Table 5.10. Antioxidant activity of methanolic, acetone and water extracts of *Lannea discolor* using 2, 2-diphenyl1-pycrilhydrazil hydrate……..118

Table 5.11. MIC values in mg/ml of *Lannea discolor* after incubation against different bacterial microorganisms……....119

Table 6.1. Antibacterial activity of AuNPs and NFs containing *Lannea discolor* extracts. Values of minimum inhibitory concentrations (MIC)…….....138

Table 7.1. Antibacterial activity of the synthesised nanoparticles from the methanol extracts of *Lannea discolor*……....161

Table 7.2. Antibacterial activity of the synthesised nanoparticles from the acetone extracts of *Lannea discolor*…….....161

Table 7.3. Antibacterial activity of the synthesised nanoparticle from the water extracts of *Lannea discolor*……....161

List of abbreviations

2,2'-diphenyl-1-picrylhydrazyl (DPPH)

adverse drug reactions (ADR)

Angiotensin converting enzyme (ACE)

base peak chromatograms (BPC)

Black Economic Empowerment (BEE)

colorectal adenocarcinoma cells (Caco-2)

computed tomography (CT)

Council for Scientific and Industrial Research (CSIR)

daunorubicin (DNR)

Department of Science and Innovation (DSI)

diethyl acetamidomalonate (DAA)

doxorubicin (DOX)

energy-dispersive X-ray (EDX)

Fourier-Transform Infrared Spectroscopy (FTIR)

gallic acid equivalents (GAE)

Human immunodeficiency virus (HIV)

hydrophilic anticancer drugs (ACD)

International Business Machines (IBM)

Liquid chromatography mass spectrometry (LC-MS)

minimum inhibitory concentration (MIC)

Mueller Hinton agar (MHA)

Muller Hinton broth (BMH)

Nanoflowers (NF

nanometre (nm)

nanoparticles (NPs)

National Research Foundation (NRF)

Nelson Mandela University (NMU)

polyvinyl alcohol (PVA)

quercetin equivalents (QE)

scanning electron microscopy (SEM)

Small Medium and Micro Enterprises (SMMEs)

South African Health Products Regulatory Authority (SAHPRA)

South African Nanotechnology initiative (SANi)

total phenolic content (TPC)

transmission electron microscopy (TEM)

Tuberculosis (TB)

University of the Free State (UFS)

University of the Western Cape (UWC)

World Health Organization (WHO)

CHAPTER 1

1.1 Background

Plants constitute the main sources of medicine as well as food for as long as man inhabited the earth. Medicinal plants have proved trustworthy, efficacious, economically viable, and easily obtainable for the medical management of an array of sicknesses and conditions (Ramaswamy et al., 2014). The World Health Organization (WHO) has always advocated for the use of traditional medicine because it has been the most common form of healthcare for more than four-fifths of the world's population (Ramaswamy et al., 2014; Sharma et al., 2016). Since mostly poverty-stricken people are at the disposal of natural products, a prompt corroboration of traditional medicine and its uses in developing countries would ensure they are properly identified, and their original uses are adhered to. This would help maximise benefit to the general health of the public, while simultaneously preserving natural remedies for generations to come (Boadu and Asase, 2017). Panday and Tripathi (2014) underlined the need to research the safety of the drug prior to their use and argued that herbal products had dramatically lower adverse effects and fatality rates compared to synthetic medicine. Throughout history, certain plant species utilized for medicinal purposes have sometimes been incorrectly associated with or attributed to necromancy and beliefs that are not scientifically supported. This is because the scientific basis of the therapeutic action of plants is not documented as well as understood. While some plant-derived medicines were incorporated into cultural traditions or practices considered superstitious, many demonstrated objective pharmacological effects and continue to do so, with documented clinical applications worth further rigorous study (Williams and Le Roux, 2012). People who prefer herbal medicine usually do not understand the scientific significance behind the medicines, but they know from personal experience some herbal remedies are curative when used as therapeutic treatments. Moving forward, evidence-based identification and investigation of biologically active compounds offers potential to advance human healthcare in a rational manner unburdened by unfounded supernatural or mystical claims (Sobiecki, 2014).

Medicinal plants also constitute combinations of different secondary compounds that could take individual action to treat illnesses, as additives or in a synergistic manner, to improve health (Mahomoodally, 2013; Yuan, et al., 2017). Observations have indicated that a significant portion of plant metabolic activity is devoted to the biosynthesis of secondary compounds (Kennedy and Wightman, 2011). Although they may have no apparent function in a plant's primary metabolism, secondary compounds often play an ecological role, as they attract pollinators by exuding favourable scents. They also serve as chemical defenses against an array of microorganisms, pests, and higher predators such as browsers and grazers. Secondary compounds also have potential as allelo-chemicals, thereby, inhibiting the growth of competing vegetation through direct or indirect means (Li et al., 2010; Kennedy and Wightman, 2011). Plants have developed and modified over millennia to be able to fight against insects, unfavourable weather, and pathogenic microorganisms, resulting in the production of

distinct, structurally diversified secondary metabolites. Historically, well known examples of plants used for medicine include *Salix alba* L. (willow tree) from which the flavonoid salicin was isolated, leading to the synthesis of acetylsalicylic acid (aspirin). One other ground-breaking discovery of drugs synthesised from plants is seen in the isolation of alkaloids such as morphine from the plant *Papaver somniferum* L. (opium poppy), which was also readily converted to codeine, which is also used as a painkiller (Dias et al., 2012). Medicinal compounds derived from plant sources through chemical synthesis are often developed and indicated for therapeutic applications consistent with those traditionally employed by indigenous medical practices. This provides recommendations about compounds that are therapeutically beneficial to humans as information on their pharmacology is always accessible. As such, an ethnobotanical approach in drug discovery is beneficial, as it would be more feasible to produce drugs based on ethnobotanical use (Atanasov et al., 2015). Secondary compounds produced by plants have potential as the sources of drug discovery. This has resulted in researchers being highly interested in studying plants with the aim of isolating novel active compounds to replace the synthetic drugs that are present in the market. The availability of the plant secondary compounds provides a source of natural drugs for modern medicine (Talib and Mahasneh, 2010; Ramaswamy et al., 2014). While access to plant materials containing bioactive compounds may present difficulties due to scale of collection needs, medicinal plants and their constituents deserve ongoing reappraisal to uncover valuable leads for new therapeutics to address the growing threat of untreatable infectious diseases (Islam et al., 2015).

In the advent of nanotechnology, evaluation of medicinal herbs and plants needs to be improved and advanced for better disease treatment and management. With the implementation of nanotechnology in healthcare and drug delivery spreading rampantly, herbal drugs have now occupied lead positions in the pharmacopoeia, improvement through nanoformulations using nanotechnology would prove efficient (Pandey and Pandey, 2013). Globally, there is a demand for high-quality healthcare delivery, and thus the dawn of nanotechnology-based herbal medicines. Therefore, it would be essential to integrate nanotechnology into traditional medicine by developing nanoparticles/nanosized-herbs with appropriate sizes and properties that best fit the action they are created for (antibacterial, anti-inflammatory, antioxidant, anti-plasmodial, anti-cancer, etc.) This would assist in overcoming challenges that are faced with dealing with traditional medicine and drug delivery (Makeen and Barik, 2016). Nano-sized herbal drugs would allow for efficient access to the cells (especially for activity against cancer, and other infectious diseases), as well as various cellular compartments, including the nucleus (Sachan and Gupta, 2015). In recent years, the elucidation of nano-sized herbal medicine or even drug delivery within plants has received considerable interest. Understanding the activities and significance of traditional medicine allows for further investigations into their manipulation, for the optimization of exudate output, quality, and the delivery (Chowdhury et al., 2017).

The *Lannea* A. Rich genus belongs to the Anacardiaceae family and comprises about 40 species and is distributed across tropical Africa and Indo-male-sia. Several of these species have been medicinally or scientifically exploited by numerous types of cultures and civilizations. Recently, investigators have focused their pursuits on the phytochemical and pharmaceutical properties of some members of *Lannea*. However, few have considered nanotechnology in relation to traditional medicinal use, particularly in family Anacardiaceae (Arunkumar et al., 2013; Muralikrishna et al., 2014; Santhoshkumar et al., 2017).

1.2 Problem statement

Human beings in developing countries experience high impaired health and increased mortality, therefore, pharmaceutical companies have been stirred towards developing novel drugs in recent years, particularly due to the perpetual rise in infectious diseases that are resistant to conventional antimicrobial remedies. Other diseases become a challenge to treat due to the solubility of herbal drugs in the human body, as well as the permeability in the cells during uptake (Ramakrishna and Setty, 2009).

Some common strategies embraced by pharmaceutical companies to meet the drug delivery demands with new and upcoming drugs may include changing the molecular structure of the existing medicines, to make them more effective or improve the activity lost caused by solubility and permeability issues (Ambwani et al., 2018). In the light of the above-mentioned constraints, it would be beneficial to look at the potential of nanoparticle synthesis using *Lannea discolor* and assess the properties of the said nanoparticles in comparison to the conventional plant extracts, this would prove useful in future drug delivery research.

1.3 Rationale of the study

Lannea discolor was the focal plant species of this investigation. It was chosen due to its documented traditional medicinal uses throughout Southern Africa and other regions. There may be several reports on the medicinal uses of the plant; however, none of them include determining the potential of using *Lannea discolor* to synthesise nanoparticles, and ultimately putting it on the trajectory of the integration of traditional medicine with nanotechnology. Based on literature survey on biological activities of *Lannea* species and other plant species, nanoparticle-based analysis would assist in overcoming the drawbacks of herbal delivery systems. Many *Lannea* species are used in ethno-medicine in the form of extracts or decoctions made from the leaves, stems, or the roots. The problem faced with conventional extracts or decoctions is that they are maybe poorly soluble or poorly absorbed, leading to high doses, and lengthy treatment periods (Chowdhury et al., 2017). The elevated requirement for traditional medicine and novel drug development merits the use of nanoparticle-based formulations for treating and managing diseases. Furthermore, nanoparticles synthesised from *Lannea discolor* may further alleviate the pressures of increasing medicinal doses, and advance drug delivery (Egwu et al., 2024). Furthermore, it appears nanotechnology maybe a potential solution for the reduction of particle size of herbal drugs and traditional medicine.

1.4 Aim

This study aimed to synthesize nanoparticles utilising extracts of *Lannea discolor* and subsequently characterize said nanoparticles. Assessment of the antibacterial efficacy against multiple pathogenic bacteria was conducted. Additionally, the nanoparticles were assessed for cytotoxicity effects on human colorectal adenocarcinoma cells (Caco-2) in vitro. Additionally, this study aimed to generate a metabolic profile through conventional phytochemical analysis as well as Liquid Chromatography Mass Spectrometry, with focus on antibacterial, antioxidant, and anticancer potential and validation of the phytochemical compounds within the plant.

1.5 Objectives of the Study

- To collect plant specimens of the species *Lannea discolor* from their natural habitat in Vuwani, Limpopo Province, South Africa, and to prepare each plant part for extraction using different solvents.
- To briefly examine the integration of nanotechnology into African traditional medicine from a South African viewpoint, assessing recent nanotechnology strategies, research gaps, and the potential impact of nanotechnology on alternative medicine.
- To synthesise copper sulphate and gold nanoparticles using *Lannea discolor* extracts, characterise them using various characterisation techniques and assess and compare their capabilities in preventing the growth of several bacteria.
- To determine the ability of the *Lannea discolor* synthesised silver nanoparticles as well as crude extracts in preventing the growth of several bacteria pathogens and preventing the proliferation of Caco-2 cells respectively.
- To perform a phytochemical analysis and tentatively provide identification of the compounds found in the leaves, roots and stems using phytochemical testing, liquid chromatography mass spectrometry.
- To contrast and compare the efficacy of acetone and water as extractants, with focus on extract yield, phytochemical content, and appearance of metabolites.

1.6 Hypothesis

The phytochemicals present in *Lannea discolor* methanol, acetone, and aqueous extracts have the ability to reduce copper sulfate, gold (III) chloride, and silver nitrate precursors to form bioactive nanoparticles. These green-synthesized nanoparticles exhibit greater antibacterial and anticancer activity (particularly on Caco-2 cells) compared to crude plant extracts, suggesting their potential as more effective treatments for colorectal cancer.

1.7 References

Ambwani, S., Tandon, R., Ambwani, T.K. and Malik, Y.S., 2018. Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. *Journal of Experimental Biology and Agricultural Sciences*, 6(1), 87 – 107.

Arunkumar, C., Astalakshmi A., Nima, P., and Ganesan, V., 2013. Plant mediated Synthesis of Silver nanoparticles using leaves of *Odina wodier* Roxb. *International Journal of Advanced Research*, 1, 265-272.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, E-. M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster, D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirscha, V.M. and Stuppner, H., 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Advances*, 33, 1582–1614.

Boadu, A.A. and Asase, A., 2017. Documentation of Herbal Medicines Used for the Treatment and Management of Human Diseases by Some Communities in Southern Ghana. *Evidence-Based Complementary and Alternative Medicine*, 1-12.

Chowdhury, A., Kunjiappan, S., Panneerselvam, T., Somasundaram, B. and Bhattacharjee, C., 2017. Nanotechnology and nanocarrier-based approaches on treatment of degenerative diseases. *International Nano Letters*, 7, 91-122.

Dias, D.A., Urban, S. and Roessner, U., 2012. A Historical Overview of Natural Products in Drug Discovery. *Metabolites*, 2, 303-336.

Egwu, C.O., Aloke, C., Onwe, K.T., Umoke, C.I., Nwafor, J., Eyo, R.A., Chukwu, J.A., Ufebe, G.O., Ladokun, J., Audu, D.T. and Agwu, A.O., 2024. Nanomaterials in Drug Delivery: Strengths and Opportunities in Medicine. *Molecules*, 29(11), 2584.

Islam, N.U., Khan, I., Rauf, A., Muhammad, N., Shahid, M. and Shah, M.R., 2015. Antinociceptive, muscle relaxant and sedative activities of gold nanoparticles generated by methanolic extract of *Euphorbia milii*. *BMC Complementary and Alternative Medicine*, 15 (1), 1-11.

Kennedy, D.O. and Wightman, E.L., 2011. Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of Human Brain Function. *Advances in Nutrition*, 2, 32-50.

Li, Z-. H., Wang, Q., Ruan, X., Pan, C-. D. and De-An Jiang, D-. A., 2010. Phenolics and Plant Allelopathy. *Molecules*, 15(12), 8933-8952.

Mahomoodally, M.F., 2013. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. *Evidence-Based Complementary and Alternative Medicine* 2, 1-14.

Makeen, H.A. and Barik, B.B., 2016. Nanotechnology and traditional medicine: challenges and opportunities. *Therapeutic Delivery*, 7(3), 157 – 162.

Muralikrishna, T., Malothu, R., Pattanayak, M. and Nayak, P.L., 2014. Green Synthesis of Gold Nanoparticles Using *Mangifera Indica* (Mango Leaves) Aqueous Extract. *World Journal of Nano Science & Technology*, 3(2), 66-73.

Pandey, A. and Pandey, G., 2013. Usefulness of nanotechnology for herbal medicines. *Plant Archives*, 13(2), 617-621.

Pandey, A. and Tripathi, S., 2014. Concept of standardization, extraction and prephytochemical screening strategies for herbal drug. *Journal of Pharmacognosy and Phytochemistry*, 2(5), 115-119.

Ramakrishna, V. A. D. D. E and Setty, O.H., 2009. Safety, Efficacy and Preclinical Evaluation of Plant Products. *Compendium of Bioactive natural products. Book series*, *3*, 124-146.

Ramaswamy, N., Samantha, T., Srinivas, P. and Chary, R.S., 2014. Phytochemical screening and TLC studies of leaves and petioles of *Oroxylum indicum* (L.) Kurz and endangered ethno medicinal tree. *International Journal of Pharmacy and Life Sciences,* 4(1), 2306-2313.

Sachan, A.K. and Gupta, A., 2015. A review on nanotized herbal drugs. *International Journal of Pharmaceutical Sciences and Research*, 6(3), 961-970.

Santhoshkumar, J., Rajeshkumar, S. and Kumar, S.V., 2017. Phyto-assisted synthesis, characterization and applications of gold nanoparticles – A review. *Biochemistry and Biophysics Reports*, 11, 46–57.

Sharma, A.K., Gangwar, M., Kumar, D., Nathi, G., Sinha, A.S.K. and Tripathi, Y.B., 2016. Phytochemical characterization, antimicrobial activity and reducing potential of seed oil, latex, machine oil and presscake of *Japtropha curcas*. *Avicenna Journal of Phytomedicine,* 6(4), 366-375.

Sobiecki, J-. F., 2014. The Intersection of Culture and Science in South African Traditional Medicine. *Indo-Pacific Journal of Phenomenology,* 14(1), 1-11.

Talib, W.H. and Mahasneh, A.M., 2010. Antiproliferative Activity of Plant Extracts Used Against Cancer in Traditional Medicine. *Scientia Pharmaceutica*, 78(1), 33–45.

Williams, G.P. and Le Roux, M., 2012. King Saul's mysterious malady. *HTS: Theological Studies*, 68(1), 1-6.

Yuan, H., Ma, Q., Cui, H., Liu, G., Zhao, X., Li, W. and Piao, G., 2017. How Can Synergism of Traditional Medicines Benefit from Network Pharmacology? *Molecules*, 22(7), 1-19.

CHAPTER 2

2.1 LITERATURE REVIEW

2.1.1 Plant description of *Lannea discolor* (Sond.) Engl.

Lannea discolor is locally named as “muvhumbu” and offers a broad range of ethno-medical uses amongst the Venda-speaking people of the Limpopo Province. The plant is also referred to as “xinkanyana”, by the Tsonga speaking people of Limpopo (Liengme, 1981). The tree is upright and grows up to 15 m, with a greyish trunk and branches. It is characterised by oval, compound soft and velvety leaves when young, while they appear green on the adaxial surface with a layer of trichomes on the abaxial surface (Palgrave, 2002). The flowers occur in a spike-like inflorescence with a soft white color. The fruits are round and fleshy with two to three spikes and turn from reddish to dark purple when ripe (Van Wyk and Van Wyke, 1997). Figure 1.1 A shows average mature *Lannea discolor* tree, while Figures 1.1 B and C show its young flowers and unripe fruits. The plant is used as treatment for dysentery, boils, and gynaecological complaints ((Fowler, 2002; Sobiecki, 2002; Steenkamp, 2003; Bruschi et al., 2011). The plant is also used for gonorrhoea, swollen legs, neutralizing lightening effects, respiratory infections, and used as a treatment for eye irritations (Hutchings et al., 1996, Van Wyk and Van Wyk., 1997; Maroyi, 2013). The soft, light wood, bark and roots are frequently utilized especially in the common rural African households. The Zulu use root infusions as a wash for convulsions and epilepsy in South Africa. Traditional healers in Zimbabwe have continuously used the plant for the treatment of schistosomiasis as well as treating and managing different bleeding disorders. The fibre of the stem can be used to wrap around wounds as a bandage (Mølgaard et al., 2001; Chinsembu et al., 2015; Thomford et al., 2015). The fruit pulp has been used to relieve diarrhoea, while the root infusion is drunk to relieve gonorrhoea. The fresh fruit is eaten by humans and animals but has not yet attained any commercial value (Chinsembu, 2016).

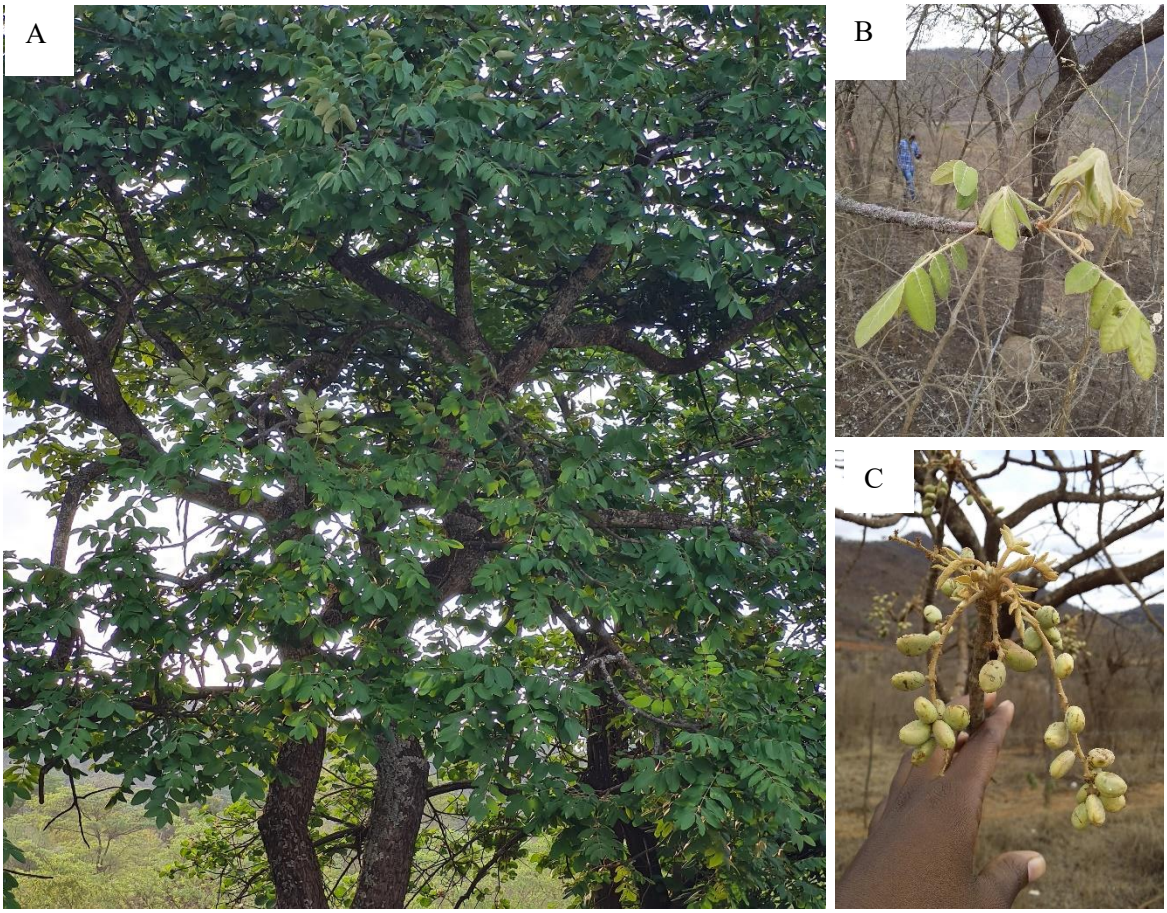


Figure 2.1. The figure shows the whole tree (A), young leaves (B) and unripe fruits (C). The images were captured at Vuwani Village, Limpopo, South Africa.

2.1.2 Phytochemistry and pharmacological history

Although no single bioactive compound has been documented to have been isolated from *Lannea discolor*, literature indicates that this species has potential therapeutic applications supported by various investigations (Clarkson et al., 2004; Chakuma et al., 2015; Nyoni et al., 2016). Recently, a study by Mwamatope et al. (2020) reported a high amount of phenolics in the barks of *L. discolor*, and significantly high antioxidant activity. Additionally, the study showed that a considerable positive relationship existed between the scavenging activities and abundance of phenolic compounds in *L. discolor*, suggesting that *L. discolor* may be a potential source of potent antioxidants with various biological activity. According to previous studies, the extracts of *L. discolor* have revealed a high amounts of phenolic flavones, which are responsible for antibacterial properties (Kabongo-Kayoka et al., 2016). The root bark as well as the leaves + stem extracts were effective against *Hymenolepsis diminuta* after 24 hours at lethal concentrations determined to be as little as 1.2 mg/ml (Mølgaard et al., 2001). A separate investigation analysed the *in vitro* antiplasmodial action of the medicinal preparation of fruit pulp against the malaria causing strain, *Plasmodium falciparum* D10, which exhibited high

antiplasmodial potency (Clarkson et al., 2004). In addition, some antimicrobial-related uses specific to skin diseases have been reported. Mabona et al. (2013) noted significant activity of *L. discolor* extracts against an array of acne-causing bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, *Brevibacillus agri, Pseudomonas aeruginosa*, *Propionibacterium acnes*, and *Trichophyton mentagrophytes*, demonstrating the plant's potential as an anti-acne active ingredient. Furthermore, crude extracts from the plant have expressed minimal cytotoxic effects on non-diseased cell lines, indicating minimal negative effects from oral intake, and also potent antimycobacterial properties against *Mycobacterium aurum* and *Mycobacterium fortuitum* (Kabongo-Kayoka et al., 2016). Ultimately, the pharmacological history of medicinal plants have long been central to traditional healing practices and over time, research into these phytochemicals has provided insights into their mechanisms of action, reinforcing their relevance in modern medicine. As scientific understanding deepens, the need to enhance the efficacy of these natural compounds has driven the exploration of new technologies (Ansari, 2023). This is where the emergence of nanotechnology plays a crucial role, providing innovative methods to manipulate and enhance the bioavailability and therapeutic potential of these plant-derived compounds at the nanoscale level (Malik et al., 2023).

2.1.3 The emergence of nanotechnology

Nanotechnology, which involves manipulating matter at the molecular level has become a field of study and innovation in the last few eras. The origins of nanotechnology can be traced back to 1959 when physicist, Richard Feynman, delivered his seminal lecture "There's Plenty of Room at the Bottom," in which he put forth the concept of manipulating individual molecules (Hulla et al., 2015; Sahu et al., 2023). The field of study began emerging as an area of research focus during the 1980s and 1990s when advanced microscopy techniques, like scanning tunnelling microscopy and atomic force microscopy were introduced. These powerful techniques enabled scientists to observe and manipulate different materials at a molecular scale, establishing the foundation for nanotechnology research (Bayda et al., 2019). Since that time nanotechnology has advanced due to contributions of research from physicists, chemists, biologists, engineers, and materials scientists. As such, scientists have been able to develop methods for producing nanomaterials studying their characteristics and integrating them into real-world applications (Mohammad et al., 2022; Taha et al., 2022). One of the most significant developments in nanotechnology has been the ability to manoeuvre the intelligible features of materials still in their original forms at nanoscales to produce novel nanomaterials with distinct characteristics and functions. These nano-based materials have found relevance in industries such as healthcare, energy production, and environmental science (Khan et al., 2019; Pokrajac et al., 2021). The significant relevance of nanotechnology has facilitated the miniaturization of electronic devices into smaller counterparts, resulting in quicker and more efficient computers, smartphones, and other electronic gadgets (Wu et al., 2013). Additionally, nanotechnology has been used to improve targeted drug delivery systems, diagnostic instruments, and therapeutic agents that are used in medicine (Singh et al., 2019).

Additionally, there is an opportunity to tackle worldwide issues, like the production of energy, environmental restoration and water purification using nanotechnology. For example, scientists are currently exploring the use of nanomaterials to enhance solar cell efficiency, catalyse chemical reactions for energy storage and to eliminate pollutants from both the atmosphere, springs and aquifers (Guerra et al., 2018; Anjum et al., 2019). Despite its enormous potential, nanotechnology presents concern about safety, ethics, and environmental effects. As nanomaterials grow more common in consumer products and industrial uses, researchers and policymakers are attempting to understand them better to reduce the unanticipated environmental risks associated with their use (Gupta et al., 2015; Hansson et al., 2020; Kumari et al., 2023). The emergence of nanotechnology represents a significant change in scientific capabilities to exploit and engineer materials at a miniscule level. This newfound ability holds wide-ranging implications for advancing scientific solutions as well as for industrial applications and improvements to societal well-being (Malik et al., 2023).

2.1.4 Nanoparticles

Nanoparticles are “minute particles with size dimensions varying from 1 to 100 nm” that have different properties, which render them admirable for uses in different fields (Khan et al., 2019). The word "nanoparticle" refers not just to metals, but also to other inorganic and organic substances such as silica, carbon and micelles aswell as liposomes. Because of their diversity in qualities based on size, shape, orientation, arrangement, and so on, nanoparticles are regarded as an effective subject of study with applications across all scientific branches, branch of science, including material sciences, chemistry, and biological systems (Joudeh and Linke, 2022). Manipulating morphology at the nanometre scale allows for the design and fabrication of materials with unique applications. Nanoparticles exhibit enhanced reactivity relative to larger orientations due to an increased specific outer surface, a property renders nanoparticles useful for various applications that require high reactivity. At the nanoscale, nanoparticles of various elements display extraordinary intrinsic properties (Yadav et al., 2020; Salem et al., 2023). Innumerable nanoparticles also tend to have lower melting points, can absorb light easily, have high diffusion coefficients, and tensile strength, as well as significant variations in their electromagnetic and catalytic capabilities. Nanoparticles also demonstrate reduced melting point, improved light absorption, electrical resistivity, specific heat, diffusivity, plasticity, and mechanical strength, as well as alterations in their electromagnetic and catalytic properties (Joudeh et al., 2022). As such, these properties are favourable in different fields where nanoparticles are required due to their significant properties.

2.1.5 Types of nanoparticles

2.1.5.1 Metal Nanoparticles

During the process of biosynthesis, various metals have been implemented that allow for the formation of nanoparticles through biological means (Liu et al., 2014; Bendicho et al., 2012). Certain metals have

always been concluded to exhibit differing levels of efficacy regarding diverse pharmacological effects and have a history of medical application; however, further research into their therapeutic efficacy is required due to their toxicity. Gold, silver, and copper/copper oxide nanoparticles are considered far superior to other metals for applications in internal medical treatment owing to their high renal clearance and low-level toxicity (Yaqoob et al., 2020). Copper nanoparticles have gained significant attention in numerous fields, including electronics, catalytic reactions, biomedical healthcare, and material science, owed to their beneficial properties, which include elevated electrical and some thermal conductivity and activity against different infectious microorganisms (Naika et al., 2015; Gawanda et al., 2016; Devipriya and Roopan, 2017; Al-Hakkani, 2020). The inclination towards the use copper nanoparticles over alternative metals in applications may also be attributed to copper's relative economic feasibility, the physical and chemical stability, longer shelf life after synthesis, and the ease of mixing with pectins in plant extracts (Din and Rehan, 2017; Ijaz et al., 2017). Gold (III) chloride nanoparticles, however, are renowned for demonstrating significance in optics and as such, find applications in medical imaging, sensing and precision drug delivery (Milan et al., 2022). Additionally, silver nanoparticles have been used widely in wound dressings and antimicrobials and have shown potential for use as water purification systems, by removal of approximately 98% of *Escherichia coli* colonies in the test water (Nqakala et al., 2021; Msoka et al., 2023). Nanoparticles synthesized from metal oxides are frequently incorporated in sunscreen formulations, antibacterial coatings, and gas sensors due to their UV-blocking properties (Lin et al., 2019). Iron and titanium oxides are widely employed in energy conversion, contrasting media in magnetic resonance imaging (MRI) machinery, storage devices, and gas sensors for environmental monitoring (Chavali and Nikolova, 2019; Dikshit et al. 2021).

2.1.5.2 Polymer based Nanoparticles

These are nanoparticles made up of biodegradable polymer and can encapsulate active compounds and essential oils for the management of neoplastic complaints, and pathogenic infections (Lammari et al., 2020; Szczęch et al., 2020). Their sizes range from 1 – 1000 nm in size and they have demonstrated high potential for targeted drug delivery. Most polymeric nanoparticles are spherical; however, a wide range of sizes can be produced during synthesis (Essa et al., 2020). These are comprised of synthetic or natural polymers such as chitosan, which makes them compatible to biological systems without exerting toxicity (Idrees et al., 2020). To date, numerous polymeric nanoparticles have been exploited in the precise administration of anti-cancer therapeutics such as camptothecin (Çırpanlı et al., 2011; Hu et al., 2017; Yuan et al., 2018). The use of these polymeric NPs can provide improvements in cancer therapy by exploring new routes of administration of some drugs, combining some active substances to potentiate their action, or combining with other therapies like gene therapy (Begines et al., 2020).

2.1.5.3 Liposomes

These are sphere-shaped vesicles made up of a single or multiple lipid bilayers around an aqueous core. In aqueous solutions, amphiphilic molecules, most often phospholipids, self-assemble into these structures. Liposomes have sparked broad interest in a variety of sectors due to their distinct features and diverse applications (Nsairat et al., 2022). One potential use of liposomes is drug delivery, as they can contain both water-soluble and water-insoluble drugs within their aqueous cores or lipid bilayers, respectively. This encapsulation protects the drug from degradation before use, increases its stability, and allows for controlled release kinetics (Xu et al., 2015). Liposomes can pass past various membrane barriers and disseminate in tissues before being eliminated by the elimination organs due to their similar structure to cell membranes. Furthermore, altering liposomes with various ligands and polymers optimizes the absorption of drugs and increases drug circulation within the bloodstream (Hossen et al., 2019).

2.1.6 Intrinsic properties of nanoparticles

Different nanomaterials are currently manufactured at an industrial scale, meanwhile, some are synthesised for research and development reasons. Nanoparticles can be industrially synthesized with variable specifications and compositions from different metals (Patra and Baek, 2015). They have revolutionised various fields due to their intrinsic properties, such as mechanical (size, shape and charge), optical, electrical and magnetic properties stemming from their nanoscale dimensions. These properties harness the full potentials of nanoparticles in diverse applications (Sim and Wong et al., 2021; Saleh and Hassan, 2023). Understanding these properties is crucial for harnessing the full potential of nanomaterials in diverse applications. Continued research efforts aimed at addressing challenges and exploring new avenues for nanomaterial synthesis, characterisation, and integration will drive further innovation and unlock exciting opportunities for technological advancement in the future (Sahu et al., 2023).

2.1.6.1 Mechanical Properties

The mechanical properties of nanoparticles exhibit significant size, shape, and charge dependence, with nanoscale materials often displaying enhanced strength, stiffness, and hardness in contrast to their original counterparts (Slavin et al., 2017).

(a) Size: The significance of size in nanoparticles cannot be overstated, as it fundamentally determines their properties and activity. Nanoparticles typically range from 1 to 100 nm (Figure 2.2), and this size exerts unique characteristics due to the large surface area. Nanoparticle size impacts various properties, making it a significant parameter when used in different disciplines such as material sciences, medicine, chemistry, and manufacturing (Hasan, 2015; Patra et al., 2018). Due to their miniature size, nanoparticles have been shown to effectively interact at the sub-cellular level and ability to easily go

through biological barriers. Nanoparticles with smaller sizes have more active moieties on their surface. It is also known that the biological activity of nanoparticles increases as their surface area increases, hence diminishing their size (Rizvi et al., 2018).

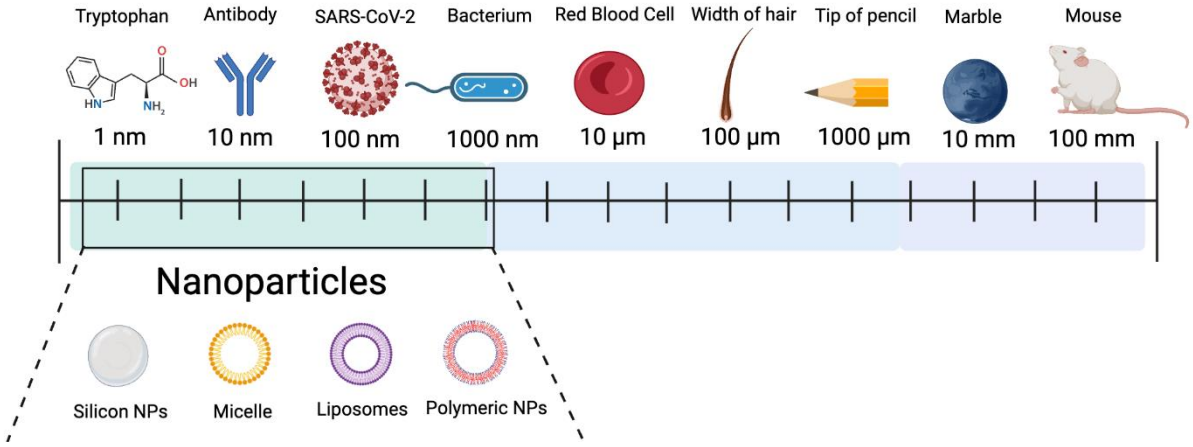


Figure 2.2. A comparison of the size range of different nanoparticles in comparison to common materials. Image adapted from https://www.biorender.com/.

(b) Shape: One of the most notable effects of nanoparticle shape is observed in plasmonic nanoparticles, such as gold and silver nanomaterials. Different shapes, such as spheres, rods, cubes, and triangles (Figure 2.3), exhibit distinct plasmonic resonances, resulting in adjustable properties (Loiseau et al., 2019; Hamida et al., 2020). For instance, rods, cubes, and triangles typically display longitudinal and transverse plasmon bands, enabling precise control over light transmission. This property is exploited in applications such biosensing, and photothermal therapy, where the shape-dependent plasmonic response enhances sensitivity (Khodashenas and Ghorbani, 2019; Khan et al., 2021). Conversely, though, in catalysis, stars, flowers and cluster shapes play a crucial part in ascertaining catalytic function, selectivity, and stability. The exposed crystal or petals planes and surface atom arrangements dictate the binding energies of reactant molecules and the kinetics of catalytic reactions. For example, high-index points on nanoflowers, stars and crystals may exhibit enhanced catalytic activity due to their unique surface reactivity compared to smoother surfaces (Ni and Wang, 2015). Biological interactions of nanoparticles are heavily influenced by their shape, affecting cellular uptake, bio-distribution, and toxicity. Nanoparticles with specific shapes, such as rods or disks, may exhibit enhanced cellular internalisation compared to spherical counterparts due to the way they are brought into the cell solely because of their shape (Augustine et al., 2020). Additionally, the shape-dependent surface curvature and exposed functional groups influence the adsorption of biomolecules, facilitating the fabrication of nanoparticles with tailored bio-conjugation and targeting capabilities for applications in targeted drug delivery, medical imaging, and drug treatment (Geißler et al., 2021).

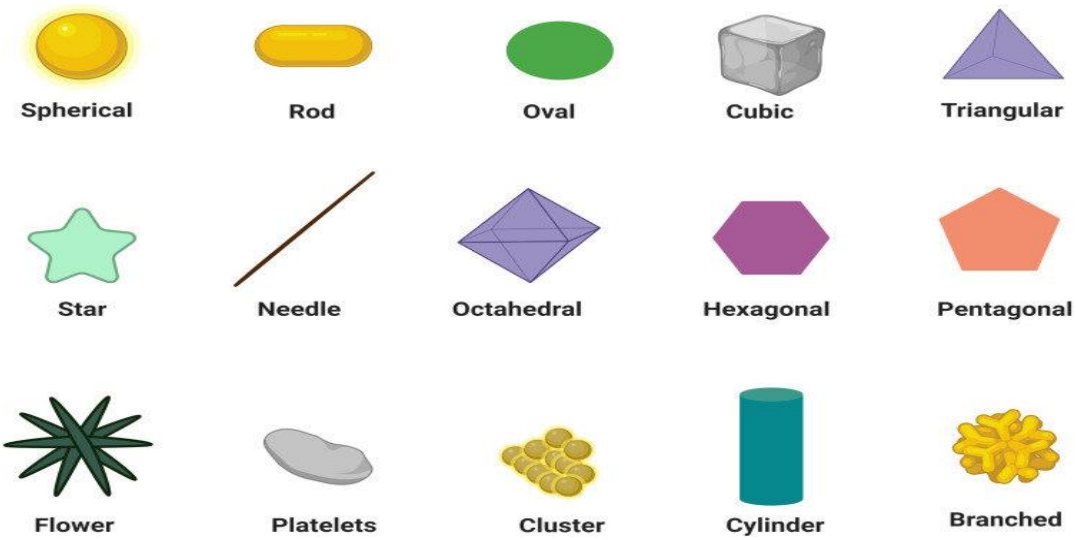


Figure 2.3. Different shapes of nanoparticles (Hamida et al., 2020).

(c) Charge: The electrical charge of nanoparticles is a factor with many implications, playing a key part in dictating particle behaviour, interactions, and applications across diverse disciplines. The charge of nanoparticles, which might be susceptible to incidents including surface functionalization, environmental conditions, and surrounding media, profoundly impacts their physical, chemical, biological, and conductive potentials (Behzadi et al., 2017). The electric charge that exists on the exterior of nanoparticles, which is typically characterised as positive, negative, or neutral, determines their stability. Electrostatic repulsion between like-charged nanoparticles prevents aggregation or agglomeration, maintaining colloidal stability (Bélteky et al., 2019). Controlling surface charge is essential for stabilising nanoparticles in various solvents or biological fluids, enabling their administration in clinical aspects including biomedical technology and catalysation of reactions in fuel cells. Positively charged nanoparticles may preferentially adsorb negatively charged biomolecules through electrostatic interactions, affecting cellular uptake, bioavailability, and toxicity profiles (Honary and Zahir, 2013a; Honary and Zahir, 2013b; Liao et al., 2019). Targeted drug delivery systems exploit these interactions to enhance precise cellular internalization (Kumar and Sharma, 2020). For example, a study by Huang et al. (2017) developed vancomycin-loaded composite nanoparticles which were surface charge switchable, as such, offering controlled drug delivery by switching from negative to positive charge for improved circulation and efficient cell internalisation. This suggests the crucial role that nanoparticle charge plays in the functionality of nanoparticles in various applications.

2.1.6.2 Optical Properties

Many metallic nanoparticles, such as those synthesised from gold or silver, exhibit plasmon resonance, where the extracted plasmon coupled conduction electrons respond collectively to an external light field. Interestingly, the resonance can lead to high light absorption and scattering or increased electric

field around the nanoparticle (Gentile et al., 2016). Nanoparticles with plasmonic characteristics can transform absorbed light into heat very efficiently, by methods such as plasmon-assisted photothermal heating. Such properties enable applications too numerous to mention, including photothermolysis or therapeutics in cancer treatment, and are a golden theme in photothermal nanomedicine. As such, the optical properties of nanoparticles can be tailored to the needs of various applications across nanotechnology, nanomedicine, and photonics (Kim et al.,2019; George et al., 2022).

2.1.6.3 Electrical Properties and Magnetic Properties

The properties of electrically charged nanoparticles is principally governed by the amount of surface on which the charges can interact with each other. Semiconductor nanoparticles have demonstrated size-tunable electrical conductivity and optical absorption, making them suitable for applications in electrical conduction (Coetzee et al., 2020). Generally, nanoparticles exhibit high electric double layer influence, mechanical and thermal stability and flexibility, making them ideal candidates for next-generation electronics, flexible displays, and energy storage devices (Qadir et al., 2021). They also demonstrate intriguing magnetic properties, including superparamagnetism, which makes them ideal for diagnostic applications, such as contrast medium in radiology and other imaging examinations. Magnetic nanoparticles and thin films also find applications in data storage and magnetic sensors, where precise control over magnetic properties is essential for device functionality (Avasthi et al., 2020; Montiel Schneider et al., 2022). According to Shukla et al. (2021), magnetic properties are also useful in other applications like environmental remediation, where plants may be the most suitable for green synthesis of nanoparticles with such properties. Ideally, the natural compounds in the plant are suggested to be major determining factors of the properties of nanoparticles, as they yield enhanced biocompatibility and reduced toxicity towards the environment and organisms (Stiufiuc and Stiufiuc, 2024).

2.2 Plants and nanoparticles

There has been an immense advancement in the application of nanoparticles and nanomaterials, owing to their morphology, size and how fast they are distributed (Li et al., 2007; Sharma et al., 2009). As an emerging technological revolution, nanotechnology has gained its fronts in different scientific disciplines (Ibrahim, 2015; Lateef et al., 2018). Conventionally, nanoparticles are synthesised by various methods such as laser ablation and gamma irradiation which have proven, to be costly and toxic to the surrounding environment. Both these synthesis methods involve the use of environmentally unfriendly chemicals that may pose an array of biological risks once they encounter most biotic factors. Additionally, most of these constituents used in synthesis are not soluble, furthermore, not biodegradable. Nanotechnology is partly built on the principles of solubility of chemicals which can be manipulated to yield a product from a variety of solvents or materials, containing the desirable

concentration or quality. In this regard, it is essential to come up with reliable, nontoxic, clean, eco-friendly protocols for nanosynthesis (Gopinath et al., 2012; Gopinath et al., 2013).

In the pursuit of more feasible and environmentally friendly ways of nanosynthesis, scientists have turned to "Green synthesis", which essentially uses the biomolecules in organisms to reduce the precursor employed in for the synthesis. Green synthesis is regarded as the reduction of any toxic chemical substances to zero level, in this context, the metals would be rid of all the potential toxicity they might bear (Thomas et al., 2018). The fabricating of nanoparticles utilizing extracts from plants is a strategy that has shown tremendous potential. In this approach, bioactive molecules in the extracts of plants serve as reductants, generating nanoparticles through this process (Das et al., 2017). Plant extract-based nanoparticle synthesis has since gained significance attention as it has a level of simplicity, and the reactions occur faster (Patil et al., 2016). In the preparation of nanoparticles, three main aspects to be considered for the process to be consider as "green synthesis" are the use of an unadulterated medium, a biodegradable reductant, and the use of an organic constituent for nanoparticle stabilization if external stabilization is required (Li et al., 2007). As such, plant extracts are suitable for this kind of synthesis, since they are readily accessible, not poisonous in most cases and constitute metabolites that are capable of completely reducing charged atoms. Additionally, nano-sizing of plant materials for different applications, may enable us to move from a crude, dried plant material to an applicable, "complete particle" based delivery and release system in just one or a few simple steps (Mittal et al., 2013; Ghorbani et al., 2015).

2.3 Usefulness of nanotechnology for herbal medicines

Plants are demonstrating significant potential as a derivation of novel medicinal agents and therapies for a wide range of medical conditions. Consequently, they have a track record of extended historical usage by different people as well as traditional healers and knowledgeable elders throughout the globe (Sabela et al., 2018). After the realisation of drawbacks in the efficiency of conventional/traditional herbal drug intake in the last couple of decades, many scientists have turned to nanotechnology research for advancements in drug administration for drugs such as curcurmin, caffeic acid and resveratrol (Guo et al., 2015; Park et al., 2016; Jaiswal et al., 2018). Ideally, when food or chemical items are taken orally, complicated processes of events take place, for example, absorption, the breakdown of large molecules through enzymatic reactions Consequently, medicinal plants contain numerous large chemical molecules which may not be absorbed directly in this way; hence, smaller absorbable molecules become imperative (Zhang et al., 2015).

To potentially address constraints encountered with the low emulsion and bio-distribution after consumption, reduction of extracts into particle form would be an effective solution (Medina et al., 2004; Angare et al., 2012; Agrawal et al., 2013). Herbal extracts have been converted into nano-formulations for many years, with pertinent findings documented in research, ultimately, it is vital to

completely break out of the limitations encountered in the use of extracts, for this reason. (Sundeep et al., 2017). In earlier research, synthesising nanoparticles was said to have several benefits for conventional herbal drugs, including reduced dosed, low apoptosis in normal cells, enhanced pharmacological action as well as enhanced stability as compared to crude drug preparation (Chen et al., 2004; Li et al., 2011; Wei et al., 2012; Khan et al., 2013; Alexandra et al., 2016). Nanoparticles can also be used to target herbal medicines at specific sites, due to their ability to cross endothelial cells in blood vessels, and reach targeted tissues through local administration, oral intake, inhalation or subcutaneous administration (Huckaby and Lai, 2018; Shen and Minko, 2020; Azagury et al.,2022; Liu et al., 2022). This is because they have an enhanced surface area, which allows for faster diffusion during blood circulation and decreases toxicity while preserving medicinal effects (Ambwani et al., 2018). For example, in tissues, nanoparticles would be able to easily pass cell membranes into the cells and enter the blood system. In a study by Singh et al. (2013), iron-oxide (Fe_2O_3) nanoparticles given to rats orally, were able to enter their organs, demonstrated by the bioavailability of iron in the liver, spleen, kidney, heart, blood and bone marrow, showing biocompatibility and the absence of genotoxicity. Most importantly, their ability to penetrate cells makes them a vital instrument for the administration of drug molecules (Bell et al., 2013).

Innovative fronts in the advancement of nanotechnology-driven drug administration systems are driven by the evaluation of the feasibly scalable processes that allow the transition of laboratory-based research into to practical use, which could be potentiated by the enhanced biological activity of the nanosized herbal medicines. However, further investigations are needed regarding mass production techniques for nanoparticles, to ensure safety and effectiveness even across large patient groups (Chakraborty et al., 2016). Al-Nadaf et al. (2024), successfully synthesised nanoparticles from silver and extracts of *Juglans regia*, which were potentiated to have the ability to treat wounds as tested using incision and excision wound healing models on rats. Evidently, *Juglans regia* nanoparticles showed significant effectiveness in various wound healing structures that were evident in the histopathology investigations. Similarly, Nagaraj et al. (2012) nanosized *Carthamus tinctorius* L, water extracts for the effective treatment against bacterial that causes gastrointestinal complains in human beings, ultimately showing some potential in nanoparticles from plants becoming reliable drug delivery vehicles (Herrero et al., 2010).

2.4 Recent nanotechnology applications in the Anacardiaceae Family

Within the family Anacardiaceae, extracts from *Mangifera indica* were utilized to synthesised nanoparticles from silver which demonstrated reasonable antibiofilm activity against microorganisms causing dental plaque. The silver nanoparticles exhibited potential utility for dental applications through inhibition of bacterial colony growth on tooth surfaces suggesting that silver nanoparticles may represent a novel approach for therapeutic applications in dentistry (Philip, 2010; Sundeep et al., 2017). Gannimani et al., (2014), utilized *Protorhus longifolia* seed extracts to synthesise nanoparticles from

silver and gold, that were highly effective against several bacteria, due to the inert chemical nature of the metal precursors. Earlier studies by Philip, (2010) and Muralikrishna et al., (2014), have documented green synthesis from *Mangifera indica* extracts, wherein several metals were used, including gold and silver. Other plants in family Anacardiaceae, whose extracts have been used to synthesise nanoparticles, include *Odina wadier* Roxb. (Kumar et al., 2013), *Buchanania lanzan* (Suresh et al., 2015), *Anacardium occidentale* (Edison et al., 2016; Araújo et al., 2020), *Spondias dulcis* (Pechyen et al., 2022).

Based on the information provided, there have been several reviews and studies examining the use of various plant species and constituents to biologically synthesise nanoparticles of significant biological importance. Prior studies have not documented the utilization of *L. discolor* for the fabrication of nanoparticles. Consequently, this investigation will potentially represent one of the first reports exploring the utilisation of *L. discolor* extracts and the phytochemical constituents contained therein as reduction and stabilisation agents during synthesis, furthermore, considering them potential for use in nanoparticle based herbal treatment.

2.5 References

Agrawal, S., Giri, T.K., Tripathi, D.K. and Ajazuddin, A.A., 2012. A Review on novel therapeutic strategies for the enhancement of solubility for hydrophobic drugs through lipid and surfactant based self-micro emulsifying drug delivery system: a novel approach. *American Journal of Drug Discovery and Development*, 2, 143–183.

Alexander, A., Ajazuddina, A., Patel, R.J., Saraf, S. and Saraf, S., 2016. Recent expansion of pharmaceutical nanotechnologies and targeting strategies in the field of phyto-pharmaceuticals for the delivery of herbal extracts and bioactives. *Journal of Controlled Release*. 241,110–124.

Al-Hakkani, M.F., 2020. Biogenic copper nanoparticles and their applications: A review. *SN Applied Sciences*, 2(3), 505.

Al-Nadaf, A.H., Awadallah, A. and Thiab, S., 2024. Superior rat wound-healing activity of green synthesized silver nanoparticles from acetonitrile extract of Juglans regia L: Pellicle and leaves. Heliyon, 10(2).

Ambwani, S., Tandon, R., Ambwani, T.K. and Malik, Y.S., 2018. Current knowledge on nanodelivery systems and their beneficial applications in enhancing the efficacy of herbal drugs. *Journal of Experimental Biology and Agricultural Sciences*, 6(1), 87 – 107.

Angare, D., Giri, T., Tripathi, D.K. and Ajazuddin, A.A., 2012. Unexplored areas and new findings in lipid emulsion serving as a potential drug carrier for lipophilic drugs: a review. *Trends in Medical Research*, 7, 1–24.

Anjum, M., Miandad, R., Waqas, M., Gehany, F. and Barakat, M.A., 2019. Remediation of wastewater using various nanomaterials. *Arabian Journal of chemistry*, 12(8), 4897-4919.

Ansari, M.A., 2023. Nanotechnology in food and plant science: challenges and future prospects. *Plants*, 12(13), 2565.

Araújo, J. T. C. D., Lima, L. A., Vale, E. P., Martin-Pastor, M., Lima, R. A., Silva, P. G. D. B. and Sousa, F. F. O. D., 2020. Toxicological and genotoxic evaluation of anacardic acid loaded-zein nanoparticles in mice. *Toxicology Reports*, 7, 1207-1215.

Augustine, R., Hasan, A., Primavera, R., Wilson, R.J., Thakor, A.S. and Kevadiya, B.D., 2020. Cellular uptake and retention of nanoparticles: Insights on particle properties and interaction with cellular components. *Materials Today Communications*, 25, 101692.

Avasthi, A., Caro, C., Pozo-Torres, E., Leal, M.P. and García-Martín, M.L., 2020. Magnetic nanoparticles as MRI contrast agents. *Surface-modified Nanobiomaterials for Electrochemical and Biomedicine Applications*, 49-91.

Azagury, A., Baptista, C., Milovanovic, K., Shin, H., Morello III, P., Perez-Rogers, J., Goldenshtein, V., Nguyen, T., Markel, A., Rege, S. and Hojsak, S., 2022. Biocoating—A Critical Step Governing the Oral Delivery of Polymeric Nanoparticles. Small, 18(26), 2107559.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M. and Rizzolio, F., 2019. The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. *Molecules*, 25(1), 112.

Begines, B., Ortiz, T., Pérez-Aranda, M., Martínez, G., Merinero, M., Argüelles-Arias, F. and Alcudia, A., 2020. Polymeric nanoparticles for drug delivery: Recent developments and future prospects. *Nanomaterials*, 10(7), 1403.

Behzadi, S., Serpooshan, V., Tao, W., Hamaly, M.A., Alkawareek, M.Y., Dreaden, E.C., Brown, D., Alkilany, A.M., Farokhzad, O.C. and Mahmoudi, M., 2017. Cellular uptake of nanoparticles: journey inside the cell. *Chemical society reviews*, 46(14), 4218-4244.

Bell, I.R., Schwartz, G.E., Boyer, N.N., Koithan, M. and Brooks, A.J., 2013. Advances in integrative nanomedicine for improving infectious disease treatment in public health. *European Journal of Integrative Medicine*, 5, 126–140.

Bélteky, P., Rónavári, A., Igaz, N., Szerencsés, B., Tóth, I.Y., Pfeiffer, I., Kiricsi, M. and Kónya, Z., 2019. Silver nanoparticles: Aggregation behavior in biorelevant conditions and its impact on biological activity. *International journal of nanomedicine*, 667-687.

Bendicho, C., De La Calle, I., Pena, F., Costas, M., Cabaleiro, N. and Lavilla, I., 2012. Ultrasound assisted pre-treatment of solid samples in the context of green analytical chemistry. *Trends in Analytical Chemistry*, 31, 50–60.

Bruschi, P., Morganti, M., Mancini, M.M. and Signorini, M.A., 2011. Traditional healers and laypeople: A qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). *Journal of Ethnopharmacology*, 138, 543– 563.

Chakraborty, K., Shivakumar, A. and Ramachandran, S., 2016. Nanotechnology in herbal medicines: A review. *International Journal of Herbal Medicine*, 4(3), 21-27.

Chakuma, N., Chipurura, B., Muchuweti, M., Chitindingu, K., Bhebhe, M. and Chagonda, L., 2015. Total phenolic content, free radical scavenging and antioxidant potential of *Lannea discolor* (Sond.) Engl bark and root extracts. *Journal of Biologically Active Products from Nature*, 5(1), 71-77.

Chavali, M.S. and Nikolova, M.P., 2019. Metal oxide nanoparticles and their applications in nanotechnology. *SN applied sciences*, 1(6), 607.

Chen, H., Chang, X. and Weng T., 2004. A study of microemulsion systems for transdermal delivery of triptolide. *Journal of Controlled Release*, 98, 427–36.

Chinsembu, K.C., 2016. Ethnobotanical Study of Plants Used in the Management of HIV/AIDS-Related Diseases in Livingstone, Southern Province, Zambia. *Evidence-Based Complementary and Alternative Medicine*, 4238625.

Chinsembu, K.C., Hijarunguru, A. and Mbangu, A., 2015. Ethnomedicinal plants used by traditional healers in the management of HIV/AIDS opportunistic diseases in Rundu, Kavango East Region, Namibia. *South African Journal of Botany*, 100, 33–42.

Çırpanlı, Y., Allard, E., Passirani, C., Bilensoy, E., Lemaire, L., Çalış, S. and Benoit, J.P., 2011. Antitumoral activity of camptothecin-loaded nanoparticles in 9L rat glioma model. *International journal of pharmaceutics*, 403(1-2), 201-206.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, O.M., Pillay, P., Matsabisa, M.G., Bhagwandhin, N., Smith, P.J. and Folb, P.I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *Journal of Ethnopharmacology*, 92, 177–191.

Coetzee, D., Venkataraman, M., Militky, J. and Petru, M., 2020. Influence of nanoparticles on thermal and electrical conductivity of composites. *Polymers*, 12(4), 742.

Das, R.K., Pachapur, V.L., Lonappan, L., Naghdi, M., Pulicharla, R., Maiti, S., Cledon, M., Dalila, L.M.A., Sarma, S.J. and Brar, S.K., 2017. Biological synthesis of metallic nanoparticles: plants, animals, and microbial aspects. *Nanotechnology for Environmental Engineering*, 2(18), 1 – 21.

Devipriya, D. and Roopan, S.M., 2017. *Cissus quadrangularis* mediated ecofriendly synthesis of copper oxide nanoparticles and its antifungal studies against *Aspergillus niger*, *Aspergillus flavus*. *Materials Science and Engineering*, 80, 38–44.

Dikshit, P.K., Kumar, J., Das, A.K., Sadhu, S., Sharma, S., Singh, S., Gupta, P.K. and Kim, B.S., 2021. Green synthesis of metallic nanoparticles: Applications and limitations. *Catalysts*, 11(8), 902.

Din, M.I. and Rehan, R., 2017. Synthesis, Characterization, and Applications of Copper Nanoparticles. *Analytical Letters*, 50 (1), 50-62.

Edison, T. N. J. I., Atchudan, R., Sethuraman, M. G. and Lee, Y. R., 2016. Reductive degradation of carcinogenic azo dyes using *Anacardium occidentale* testa derived silver nanoparticles. *Journal of Photochemistry and Photobiology B: Biology*, 162, 604-610.

Essa, D., Kondiah, P.P., Choonara, Y.E. and Pillay, V., 2020. The design of poly (lactide-co-glycolide) nanocarriers for medical applications. *Frontiers in bioengineering and biotechnology*, 8, 48.

Fowler, D.G., 2002. Traditional Ila plant remedies from Zambia. *Kirkia*, 18(1), 35-48.

Gannimani, R., Perumal, A., Krishna, S. B., Sershen, M., Mishra, A., & Govender, P. (2014). Synthesis and antibacterial activity of silver and gold nanoparticles produced using aqueous seed extract of *Protorhus longifolia* as a reducing agent. *Digest Journal of Nanomaterials and Biostructures,* 9(4),1669–1679.

Gawande, M.B., Goswami, A., Felpin, F.X., Asefa, T., Huang, X., Silva, R., Zou, X., Zboril, R. and Varma, R.S., 2016. Cu and Cu-based nanoparticles: synthesis and applications in catalysis. *Chemical reviews*, 116(6), 3722-3811.

Geißler, D., Nirmalananthan-Budau, N., Scholtz, L., Tavernaro, I. and Resch-Genger, U., 2021. Analyzing the surface of functional nanomaterials—how to quantify the total and derivatizable number of functional groups and ligands. *Microchimica Acta*, *188*,1-28.

Gentile, A., Ruffino, F. and Grimaldi, M.G., 2016. Complex-morphology metal-based nanostructures: Fabrication, characterization, and applications. *Nanomaterials*, 6(6), 110.

George, B.P., Chota, A., Sarbadhikary, P. and Abrahamse, H., 2022. Fundamentals and applications of metal nanoparticle-enhanced singlet oxygen generation for improved cancer photodynamic therapy. *Frontiers in Chemistry*, 10, 964674.

Ghorbani, P., Soltani, M., Homayouni-Tabrizi, M., Namvar, F., Azizi, S., Mohammad, R. and Moghaddam, A.B., 2015. *Sumac* Silver Novel Biodegradable Nano Composite for Bio-Medical Application: Antibacterial Activity. Molecules, 20, 12946-12958.

Gopinath, V., Mubarakali, D., Priyadarshini, S., Priyadharsshini, N. M., Thajuddin, N. and Velusamy, P., 2012. Biosynthesis of silver nanoparticles from *Tribulus terrestris* and its antimicrobial activity: a novel biological approach. *Colloids and Surfaces, B: Biointerfaces*, 96, 69-74.

Gopinath, V., Priyadarshini, S., Priyadharsshini, M.N., Pandian, K. and Velusamy, P., 2013. Biogenic synthesis of antibacterial silver chloride nanoparticles using leaf extracts of *Cissus quadrangularis* Linn. *Materials Letters*, 91, 224–227.

Guerra, F.D., Attia, M.F., Whitehead, D.C. and Alexis, F., 2018. Nanotechnology for environmental remediation: materials and applications. *Molecules*, 23(7), 1760.

Guo, D., Dou, D., Ge, L., Huang, Z., Wang, L., and Gu, N, 2015. A caffeic acid mediated facile synthesis of silver nanoparticles with powerful anti-cancer activity. Colloids and Surfaces B: Biointerfaces, 134, 229 -234.

Gupta, N., Fischer, A.R.H. and Frewer, L.J., 2015. Ethics, risk, and benefits associated with different applications of nanotechnology: a comparison of expert and consumer perceptions of drivers of societal acceptance. *NanoEthics*, 9, 93-108.

Hamida, R.S., Ali, M.A., Redhwan, A. and Bin-Meferij, M.M., 2020. Cyanobacteria–a promising platform in green nanotechnology: a review on nanoparticles fabrication and their prospective applications. *International Journal of Nanomedicine*, 6033-6066.

Hansson, S.O., 2020. How extreme is the precautionary principle?. *NanoEthics*, 14(3), 245-257.

Hasan, S., 2015. A review on nanoparticles: their synthesis and types. *Research Journal of Recent Sciences*, 2277, 2502.

Herrero, M., Plaza, M., Cifuentes, A. and Ibáñez, E., 2010. Green processes for the extraction of bioactives from rosemary: chemical and functional characterization via ultra-performance liquid chromatography-tandem mass spectrometry and in-vitro assays. *Journal of Chromatography A*, 1217, 2512–2520.

Honary, S. and Zahir, F., 2013. Effect of zeta potential on the properties of nano-drug delivery systems-a review (Part 1). *Tropical journal of pharmaceutical research*, 12(2), 255-264.

Hossen, S., Hossain, M.K., Basher, M.K., Mia, M.N.H., Rahman, M.T. and Uddin, M.J., 2019. Smart nanocarrier-based drug delivery systems for cancer therapy and toxicity studies: A review. *Journal of advanced research*, 15, 1-18.

Hu, J., Fu, S., Peng, Q., Han, Y., Xie, J., Zan, N., Chen, Y. and Fan, J., 2017. Paclitaxel-loaded polymeric nanoparticles combined with chronomodulated chemotherapy on lung cancer: In vitro and in vivo evaluation. *International journal of pharmaceutics*, 516(1-2), 313-322.

Huang, W.F., Tsui, C.P., Tang, C.Y., Yang, M., and Linxia, Gu, 2017. Surface charge switchable and Ph-responsive chitosan/polymer core-shell composite nanoparticles for drug delivery application. *Composites Part* B, 121,83 -91.

Huckaby, J.T. and Lai, S.K., 2018. PEGylation for enhancing nanoparticle diffusion in mucus. Advanced drug delivery reviews, 124, 125-139.

Hulla, J.E., Sahu, S.C. and Hayes, A.W., 2015. Nanotechnology: History and future. *Human & experimental toxicology*, 34(12), 1318-1321.

Hutchings, A., Scott, A.H., Lewis, G. and Cunningham A.F., 1996. Zulu Medicinal Plants: An Inventory. University of Natal Press, Pietermaritzburg.

Ibrahim, H.M.M., 2015. Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms. *Journal of Radiation Research and Applied Sciences*, 8, 265 – 275.

Idrees, H., Zaidi, S.Z.J., Sabir, A., Khan, R.U., Zhang, X. and Hassan, S.U., 2020. A review of biodegradable natural polymer-based nanoparticles for drug delivery applications. *Nanomaterials*, *10*(10), 1970.

Ijaz, F., Shahid, S., Khan, S.A., Ahmad, W. and Zaman, S., 2017. Green synthesis of copper oxide nanoparticles using Abutilon indicum leaf extract: Antimicrobial, antioxidant, and photocatalytic dye degradation activities. *Tropical Journal of Pharmaceutical Research*, 16 (4), 743-753.

Jaiswal, S., and Mishra, P, 2018. Antimicrobial and Antibiofilm activity of Curcumin-silver nanoparticles with improved stability and selective toxicity to bacteria over mammalian cells. Medical microbiology and immunology, 207,39-53.

Joudeh, N. and Linke, D., 2022. Nanoparticle classification, physicochemical properties, characterization, and applications: a comprehensive review for biologists. *Journal of Nanobiotechnology*, 20(1), 262.

Kabongo-Kayoka, P.N., Eloff, J.N., Obi, C.L. and McGaw, L.J., 2016. Antimycobacterial Activity and Low Cytotoxicity of Leaf Extracts of Some African Anacardiaceae Tree Species. *Phytotherapy Research*, 30, 2001–2011.

Khan, I., Saeed, K. and Khan, I., 2019. Nanoparticles: Properties, applications, and toxicities. *Arabian journal of chemistry*, 12(7), 908-931.

Khan, J., Alexander, A., Ajazuddin, A. and Saraf S., 2013. Recent advances and future prospects of phyto-phospholipid complexation technique for improving pharmacokinetic profile of plant actives. *Journal of Controlled Release*, 168, 50–60.

Khan, N.U., Lin, J., Younas, M.R., Liu, X. and Shen, L., 2021. Synthesis of gold nanorods and their performance in the field of cancer cell imaging and photothermal therapy. *Cancer Nanotechnology*, 12(1), 1-33.

Khodashenas, B. and Ghorbani, H.R., 2019. Synthesis of silver nanoparticles with different shapes. *Arabian Journal of Chemistry*, 12(8), 1823-1838.

Kim, M., Lee, J.H. and Nam, J.M., 2019. Plasmonic photothermal nanoparticles for biomedical applications. *Advanced Science*, 6(17), 1900471.

Kumar, S. and Sharma, B., 2020. Leveraging electrostatic interactions for drug delivery to the joint. *Bioelectricity*, *2*(2), 82-100.

Kumari, R., Suman, K., Karmakar, S., Lakra, S.G., Saurav, G.K. and Mahto, B.K., 2023. Regulation and Safety Measures for Nanotechnology-based Agri-Products. *Frontiers in Genome Editing*, 5, 1200987.

Lammari, N., Louaer, O., Meniai, A.H. and Elaissari, A., 2020. Encapsulation of essential oils via nanoprecipitation process: Overview, progress, challenges, and prospects. *Pharmaceutics*, *12*(5), 431.

Lateef, A., Folarin, B.I., Oladejo, S.M., Akinola, P.O., Beukes, L.S. and Gueguim-Kana, E.B., 2018. Characterization, antimicrobial, antioxidant, and anticoagulant activities of silver nanoparticles synthesized from *Petiveria alliacea* L. leaf extract. *Preparative Biochemistry and Biotechnology*, 29, 1-7.

Li, S., Ji, Z. and Zou, M., 2011. Preparation, characterization, pharmacokinetics and tissue distribution of solid lipid nanoparticles loaded with tetrandrine. *AAPS PharmSciTech*, 12, 1011–1018.

Li, S., Shen, Y., Xie, A., Yu, X., Qui, L., Zhang, L. and Zhang, Q., 2007. Green synthesis of silver nanoparticles using *Capsicum annuum* L. extract. *Green Chemistry*, 9, 852-858.

Liao, C., Li, Y. and Tjong, S.C., 2019. Bactericidal and cytotoxic properties of silver nanoparticles. *International journal of molecular sciences*, 20(2), 449.

Liengme, C.A., 1981. Plants used by the Tsonga people of Gazankulu. *Bothalia*, *13*(3/4), 501-518.

Lin, C.C., Lee, M.H., Chi, M.H., Chen, C.J. and Lin, H.Y., 2019. Preparation of Zinc oxide nanoparticles containing spray and barrier films for potential photoprotection on wound healing. *Acs Omega*, 4(1), 1801-1809.

Liu, S., Chang, C., Verma, M., Hileeto, D., Muntz, A., Stahl, U., Woods, J., Jones, L. and Gu, F., 2015. Phenylboronic acid modified mucoadhesive nanoparticle drug carriers facilitate weekly treatment of experimentally induced dry eye syndrome. *Nano Research*, *8*, 621-635.

Liu, Y., Xu, L., Zhang, Q., Kang, Y., Liu, L., Liu, Z., Wang, Y., Jiang, X., Shan, Y., Luo, R. and Cui, X., 2022. Localized myocardial anti-inflammatory effects of temperature-sensitive budesonide nanoparticles during radiofrequency catheter ablation. Research, 9816234.

Loiseau, A., Asila, V., Boitel-Aullen, G., Lam, M., Salmain, M. and Boujday, S., 2019. Silver-based plasmonic nanoparticles for and their use in biosensing. *Biosensors*, 9(2), 78.

Mabona, U., Viljoen, A., Shikanga, E., Marston, A. and Van Vuuren, S., 2013. Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethnopharmacological screening approach to combination studies and the isolation of a bioactive compound. *Journal of Ethnopharmacology*, 148, 45–55.

Malik, S., Muhammad, K. and Waheed, Y., 2023. Nanotechnology: A revolution in modern industry. *Molecules*, *28*(2),661.

Maroyi, A., 2013. Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. *Journal of Ethnobiology and Ethnomedicine*, 9, 1–18.

Medina, O.P., Zhu, Y. and Kairemo, K., 2004. Targeted liposomal drug delivery in cancer. *Current Pharmaceutical Design*, 10, 2981–2989.

Milan, J., Niemczyk, K. and Kus-Liśkiewicz, M., 2022. Treasure on the Earth—gold nanoparticles and their biomedical applications. *Materials*, 15(9), 3355.

Mittal, A. K., Chisti, Y. and Banerjee, U. C., 2013. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology advances*, *31*(2), 346-356.

Mohammad, Z.H., Ahmad, F., Ibrahim, S.A. and Zaidi, S., 2022. Application of nanotechnology in different aspects of the food industry. *Discover Food*, 2(1), 12.

Mølgaard, P., Nielsen, S.B., Rasmussen, D.E., Drummond, R.B., Makaza, N. and Andreassen, J., 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology*, 74, 257–264.

Montiel Schneider, M.G., Martín, M.J., Otarola, J., Vakarelska, E., Simeonov, V., Lassalle, V. and Nedyalkova, M., 2022. Biomedical applications of iron oxide nanoparticles: Current insights progress and perspectives. *Pharmaceutics*, 14(1), 204.

Msoka, M., Jacob, F. and Mahadhy, A., 2023. Development of a filter system using silver nanoparticles modified silica sand for drinking water disinfection. *African Journal of Environmental Science and Technology*, 17(7), 146-156.

Muralikrishna, T., Malothu, R., Pattanayak, M. and Nayak, P.L., 2014. Green Synthesis of Gold Nanoparticles Using *Mangifera Indica* (Mango Leaves) Aqueous Extract. *World Journal of Nano Science & Technology*, 3(2), 66-73.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E. and Nyirenda, C., 2020. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*, 9, e00481.

Nagaraj, B., Malakar, B., Divya, T.K., Krishnamurthy, N., Liny, P., Dinesh, R., Iconaru, S. and Ciobanu, C., 2012. Synthesis of plant mediated gold nanoparticles using flower extracts of Carthamus tinctorius L.(safflower) and evaluation of their biological activities. *Digest Journal of Nanomaterials and Biostructures*, 7, 1289-1296.

Naika, H.R., Lingaraju, K., Manjunath, K., Kumar, G.D., Nagaraju, G., Suresh, D. and Nagabhushana, H., 2015. Green synthesis of CuO nanoparticles using *Gloriosa superba* L. extract and their antibacterial activity. *Journal of Taibah University for Science*, 9, 7–12.

Ni, B. and Wang, X., 2015. Face the edges: catalytic active sites of nanomaterials. *Advanced Science*, 2(7), 1500085.

Nqakala, Z.B., Sibuyi, N.R., Fadaka, A.O., Meyer, M., Onani, M.O. and Madiehe, A.M., 2021. Advances in nanotechnology towards development of silver nanoparticle-based wound-healing agents. *International Journal of Molecular Sciences*, 22(20), 11272.

Nsairat, H., Khater, D., Sayed, U., Odeh, F., Al Bawab, A. and Alshaer, W., 2022. Liposomes: Structure, composition, types, and clinical applications. *Heliyon*. 8, e09394.

Nyoni, M., Muzemu, S., Chinheya, C., Mushayabasa, T. and Ncube, B., 2016. Screening indigenous nematicidal plants in Zimbabwe against *Meloidogyne javanica*: paper presented at a conference held on the 14th-16th July 2015, Elephant Hills Resort, Victoria Falls, Zimbabwe. Available from: http:// www.ir.msu.ac.zw:8080/jspui/bitstream/11408/1319/1/muzemu.pdf

Palgrave, C.M., 2002. Keith Coates Palgrave Trees of Southern Africa. Struik Publishers, Cape Town.

Park, S., Cha, S.H., Cho, I., Park, S., Park, Y., Cho, S., and Park, Y, 2016. Antibacterial nanocarriers of resveratrol with gold and silver nanoparticles. Materials Science and Engineering : C, 58, 1160-1169.

Patil, M.P., Rokade, A.A., Ngabire, D. and Kim, G-. D., 2016. Green Synthesis of Silver Nanoparticles Using Water Extract from Galls of *Rhus Chinensis* and Its Antibacterial Activity. *Journal of Cluster Science*, 27, 1737-1750.

Patra, J.K. and Baek, K.H., 2015. Green nanobiotechnology: factors affecting synthesis and characterization techniques. *Journal of Nanomaterials*, 219-219.

Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.D.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S. and Habtemariam, S., 2018. Nano based drug delivery systems: recent developments and future prospects. *Journal of nanobiotechnology*, 16(1), 1-33.

Pechyen, C., Ponsanti, K., Tangnorawich, B. and Ngernyuang, N., 2022. Biogenic synthesis of gold nanoparticles mediated by *Spondias dulcis* (Anacardiaceae) peel extract and its cytotoxic activity in human breast cancer cell. *Toxicology Reports*, 9, 1092-1098.

Philip, D., 2010. Rapid green synthesis of spherical gold nanoparticles using *Mangifera indica* leaf. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *77*(4), 807-810.

Pokrajac, L., Abbas, A., Chrzanowski, W., Dias, G.M., Eggleton, B.J., Maguire, S., Maine, E., Malloy, T., Nathwani, J., Nazar, L. and Sips, A., 2021. Nanotechnology for a sustainable future: Addressing global challenges with the international network4sustainable nanotechnology. *ACS Nano*, 15, 18608-18623.

Qadir, A., Le, T.K., Malik, M., Min-Dianey, K.A.A., Saeed, I., Yu, Y., Choi, J.R. and Pham, P.V., 2021. Representative 2D-material-based nanocomposites and their emerging applications: A review. *RSC advances*, 11(39), 23860-23880.

Raveendran, P., Fu, J. and Wallen, S.L., 2003. Completely "green" synthesis and stabilization of metal nanoparticles. *Journal of the American Chemical Society*, 125(46), 13940-13941.

Rizvi, S.A. and Saleh, A.M., 2018. Applications of nanoparticle systems in drug delivery technology. *Saudi pharmaceutical journal*, 26(1), 64-70.

Sabela, M.I., Makhanya, T., Kanchia, S., Shahbaaz, M., Idress, D. and Bisetty, K., 2018. One-pot biosynthesis of silver nanoparticles using *Iboza Riparia* and Ilex Mitis for cytotoxicity on human embryonic kidney cells. *Journal of Photochemistry and Photobiology, B: Biology,* 178, 560–567.

Sahu, M.K., Yadav, R. and Tiwari, S.P., 2023. Recent advances in nanotechnology. *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, 9(1), 015-023.

Saleh, H.M. and Hassan, A.I., 2023. Synthesis and characterization of nanomaterials for application in cost-effective electrochemical devices. *Sustainability*, 15(14), 10891.

Salem, S.S., Hammad, E.N., Mohamed, A.A. and El-Dougdoug, W., 2022. A comprehensive review of nanomaterials: Types, synthesis, characterization, and applications. *Biointerface Research in Applied Chemistry*, 13(1), 41.

Sharma, V.K., Yngard, R.A. and Lin, Y., 2009. Silver Nanoparticles: Green Synthesis and Their Antimicrobial Activities. *Advances in Colloid and Interface Science*, 145, 83–96.

Shen, A.M. and Minko, T., 2020. Pharmacokinetics of inhaled nanotherapeutics for pulmonary delivery. Journal of Controlled Release, 326, 222-244.

Shukla, S., Khan, R., and Davery, A, 2021. Synthesis and characterization of magnetic nanoparticles and their applications in wastewater treatment: A review. *Environmental Technology & Innovation*, 24, 101924.

Sim, S. and Wong, N.K., 2021. Nanotechnology and its use in imaging and drug delivery. *Biomedical reports*, 14(5),1-9.

Singh, A.P., Biswas, A., Shukla, A. and Maiti, P., 2019. Targeted therapy in chronic diseases using nanomaterial-based drug delivery vehicles. *Signal transduction and targeted therapy*, 4(1), 33.

Singh, S.P., Rahman, M.F., Murty, U.S.N., Mahboob, M. and Grover, P., 2013. Comparative study of genotoxicity and tissue distribution of nano and micron sized iron oxide in rats after acute oral treatment. Toxicology and applied pharmacology, 266(1), 56-66.

Slavin, Y.N., Asnis, J., Hńfeli, U.O. and Bach, H., 2017. Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *Journal of nanobiotechnology*, 15, 1-20.

Sobiecki, J. F. (2002) A preliminary inventory of plants used for psychoactive purposes in Southern African healing traditions. *Transactions of the Royal Society of South Africa*, *57*(1/ 2):1–24.

Sovová, H. and Stateva R.P., 2011. Supercritical fluid extraction from vegetable materials. *Reviews in Chemical Engineering*, 27 (3-4), 79-156.

Steenkamp, V., 2003. Traditional herbal remedies used by South African women for gynaecological complaints. *Journal of Ethnopharmacology*, 86, 97–108.

Stiufiuc, G.F., and Stiufiuc, R.I, 2024. Magnetic Nanoparticles: Synthesis, characterization, and their use in biomedical field. *Applied Sciences*, 14(4), 1623.

Sundeep, D., Kumar, T.V., Rao, P.S.S., Ravikumar, R.V.S.S.N., Krishna A.G., 2017. Green synthesis and characterization of Ag nanoparticles from *Mangifera indica* leaves for dental restoration and antibacterial applications. *Progress in biomaterials*, *6*, 57-66.

Suresh, D., Nethravathi, P. C., Kumar, M. P., Naika, H. R., Nagabhushana, H. and Sharma, S. C., 2015. *Chironji* mediated facile green synthesis of ZnO nanoparticles and their photoluminescence, photodegradative, antimicrobial and antioxidant activities. *Materials Science in Semiconductor Processing*, 40, 759-765.

Szczęch, M. and Szczepanowicz, K., 2020. Polymeric core-shell nanoparticles prepared by spontaneous emulsification solvent evaporation and functionalized by the layer-by-layer method. *Nanomaterials*, *10*(3), 496.

Taha, T.B., Barzinjy, A.A., Hussain, F.H.S. and Nurtayeva, T., 2022. Nanotechnology and computer science: Trends and advances. *Memories-Materials, Devices, Circuits and Systems*, 2, 100011.

Thomas, B., Vithiya, B.S.M., Prasad, T.A.A., Mohamed, S.B., Magdalane, C.M., Kaviyarasu, K. and Maaza, M., 2018. Antioxidant and Photocatalytic Activity of Aqueous Leaf Extract Mediated Green Synthesis of Silver Nanoparticles Using *Passiflora edulis* f. flavicarpa. *Journal of Nanoscience and Nanotechnology*, 18, 1–9.

Thomford, N.E., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D., Chirikure, S. and Dandara, C., 2015. Pharmacogenomics Implications of Using Herbal Medicinal Plants on African Populations in Health Transition. *Pharmaceuticals*, 8, 637-663.

Van Wyk B., and Van Wyk P., 1997. Field Guide to Trees of Southern Africa. Struik Publishers (Pty) Ltd.; Cape Town, South Africa.

Wei, L., Li, G., Yan, Y.D., 2012. Lipid emulsion as a drug delivery system for breviscapine: formulation development and optimization. *Archives of Pharmacal Research*, 35,1037–1043.

Wu, J., Shen, Y.L., Reinhardt, K., Szu, H. and Dong, B., 2013. A nanotechnology enhancement to Moore's law. *Applied Computational Intelligence and Soft Computing*, 2-2.

Xu, X., Ho, W., Zhang, X., Bertrand, N. and Farokhzad, O., 2015. Cancer nanomedicine: from targeted delivery to combination therapy. *Trends in molecular medicine*, 21(4), 223-232.

Yadav, S., Sharma, A.K. and Kumar, P., 2020. Nanoscale self-assembly for therapeutic delivery. *Frontiers in Bioengineering and Biotechnology*, 8, 127.

Yaqoob, A.A., Ahmad, H., Parveen, T., Ahmad, A., Oves, M., Ismail, I.M., Qari, H.A., Umar, K. and Mohamad Ibrahim, M.N., 2020. Recent advances in metal decorated nanomaterials and their various biological applications: A review. *Frontiers in chemistry*, 8, 341.

Yuan, J.D., ZhuGe, D.L., Tong, M.Q., Lin, M.T., Xu, X.F., Tang, X., Zhao, Y.Z. and Xu, H.L., 2018. pH-sensitive polymeric nanoparticles of mPEG-PLGA-PGlu with hybrid core for simultaneous encapsulation of curcumin and doxorubicin to kill the heterogeneous tumour cells in breast cancer. *Artificial cells, nanomedicine, and biotechnology*, 46(1), 302-313.

Zhang, L., Zhang, I., Zhang, M., Pang, Y., Li, Z., Zhao, A. and Feng, J., 2015. Self-emulsifying drug delivery system and the applications in herbal drugs. *Drug Delivery*, 22(4), 475-486.

CHAPTER 3

A review on the dawn of nanotechnology on African traditional medicine: A South African perspective

Abstract

Worldwide, herbal medicine has become a preferred source of therapy as it contains an array of bioactive phytochemicals, which have antioxidants, are biologically compatible and have been used to synthesise nanoparticles with efficient therapeutic potential. Thus, it can be stated that metal nanoparticles, synthesised medicinal herbs, may serve as potent therapeutics. Numerous scientific studies and reports were thoroughly examined and evaluated to identify areas that may need further investigation. An overview of fifteen plant species in Southern Africa, along with biological evaluations, were recorded as traditional remedies that were utilized to create nanoparticles. Technology, human civilisation, and vast ethnobotanical records have had a long presence in South Africa. However, nanoparticle-based properties of Southern African medicinal plants remain poorly investigated. There also remains a gap between nanotechnology and African traditional medicine, one which would take years to fill, despite the achievements according to the 10-year strategic plan by the Department of Science and Innovation (DSI). This study highlights the southern African perspective on the gap needed to be filled between African traditional medicine and nanotechnology. It provides a summary on the scientific studies that include the synthesis of nanoparticles from plants that have historically served as sources of medicinal benefits.

Keywords: Nanotechnology, South Africa, nanoscience, Nanomedicine, green synthesis, traditional medicine, plant extracts.

3.1 Introduction

The prefix "nano" in "nanotechnology" emanates from the Greek-derived word, "nanos", translating to "dwarf" that denotes to one billionth of a meter. Specifically, one nanometre (nm) is equivalent to one part in a billion of a meter, providing context for the diminutive scale associated with nanoscale materials and devices within the field of nanotechnology. With nanotechnology, the aim is to produce particles that interact on an atomic level and forms of dosages of sizes below 100 nm (Ansari et al., 2012). Nanotechnology is a scientific technique that has seen applications spanning from the 19th century into the present era. In the drug industry, it has the major property of producing self-targeting nanoparticles which are able to work efficiently due to their distinctively small size, on or around the affected areas. Their miniature sizes have turned them into a novel approach to overcome drawbacks in drug administration (Pandey and Pandey, 2013; Kaul et al., 2018). Nanotechnology is expected to spread rapidly in all areas of medicine and, more specifically, in drug delivery. Lately, pharmaceutical research uses nanoparticles to moderate drug toxicity and side effects. Furthermore, the nanosize, whether inherent or even when tuned for enhanced functionality, also provides access to the cell and different cell compartments, including the nucleus, as well as access to the blood-brain barrier (De Jong and Borm, 2008; babu Maddinedi et al., 2017). Scientists have since turned to a variety of organic substances for their potential in the tailored synthesis of nanoparticles for the administration of drugs. These range from biological substances such as proteins, collagen, gelatin and to more chemical substances such as different polymers and solid metals (De Jong and Borm, 2008; Ahmad and Hashim, 2012; Kunwar et al., 2019).

Traditionally-prepared herbal drugs and extracts are presently considered significant and are occupying lead positions in the global pharmacopoeia. For instance, traditional medicine is widely practiced in Latin America; 40% of people in Colombia and 71% of people in Chile, respectively, rely on it. Despite the availability of Western treatment, traditional medicine is still widely practiced in many Asian nations with 60–70% of physicians in Japan recommending traditional remedies. About 200 million patients are treated by traditional medicine each year in China, where it accounts for about 40% of total medical care (Karunamoorthi et al., 2013). Additionally, over 28 million people use medicinal plant items in South Africa, which are industrialized after collection straight from the wild (Khumalo, 2018; Khumalo et al., 2023). Since they have known effects as well as little to no side effects, this has made herbal drugs a considerable preference as therapy. Thus, this opens a gap for advanced research and development through green synthesis of nanoparticles. However, the delivery of herbal molecules as a treatment has proven challenging as some molecules are too polar to be solubilised and membranes are not permeable enough for them to pass through. Such molecules are at most times not readily absorbed, leading to an unstable biological environment. It is possible to resolve these drawbacks of herbal medicines by capping or encapsulating them with relevant nanomaterials (Kumari et al., 2012; Pandey and Pandey, 2013; Patil et al., 2018). In this way, the bioavailability and relative safety of medicinal

plant preparations can be greatly improved by nanomaterials. Furthermore, the therapeutic value of medication made from herbs can be significantly improved by targeting delivery of their nanoforms into specific cells or tissues (Chenthamara et al., 2019).

This review aims to give a concise background and synopsis of innovative investigations into nanotechnology in South Africa, as well as future trends in herbal medicine due to nanotechnology. It starts with the description of the history and development, with a brief discussion on what "nanotechnology is, and its applications. It goes on to summarise the landscape of nanotechnology in South Africa, revisiting the 10-year strategic plan for nanotechnology. South Africa's view on nanomedicine is briefly outlined, as well as the publications that have been part of the outputs within the 10 years of the plan. The review culminates with a short assessment of recent developments in nanotechnology in South Africa as well as a future prospective according to the plans that were initially placed. This review, as such, places focus on understanding current research trends, identifying gaps in knowledge, and evaluating the promising avenues of nanotechnology in African traditional medicine.

In this brief analysis, references were searched in databases include PubMed, Google Scholar, Scopus, and specialised databases focusing on African or south African nanotechnology. Data bases were searched using "nanotechnology in south Africa", "nanoparticles and herbal medicine", and "nanomedicine" as the keywords. Articles before and after the year 2010 were selected and sorted according to how pertinent the research is to the objectives. The findings were organised in summaries, and tables, to synthesize the information and facilitate analysis for a proper literature review.

3.2 History and Development

The principle of "nanoscience" has consistently been associated with Nobel Prize Recipient Richard Feynman (American physicist, 1918-1988), who gave an influential address in 1959, in which he stated "The principles of physics, as far as I can see, do not speak against the possibility of manoeuvring things atom by atom" (Feynman, 1960). Furthermore, he emphasized the significance of manipulating things at a scale imperceptible to the human eye and how physical processes change their expression depending on scale, such as the diminution of an object and alphabet letters such that a huge amount of information is stored in a minute storage device (Feynman, 1992). Additionally, the Japanese scientist, Norio Taniguchi (1912-1999), described nanotechnology as a science that primarily involves the synthesis or production of materials from a single atom or molecule (Taniguchi, 1974). When Iijima, another Japanese scientist, developed carbon nanotubes, the study of nanotechnology was further advanced, and to date, an increased fascination with the new areas of nanoscience and nanotechnology has been observed since the beginning of the 21st century (Taniguchi, 1974; Hulla et al., 2015; Schaming and Remita, 2015). Nanotechnology has been applied in metallurgy and art, being in use 100 of years before the science was formally introduced as a field of study. Earlier researchers in the field include Scottish physicist mathematician, James Clark Maxwell (1831-1879), and Austrian chemist,

Richard Adolf Zsigmondy (1865-1929), that investigated colloidal nanoparticles, gold, sol gel and other types of nanoparticles from different materials. Other significant nanotechnology earlier contributions include work from physicists Irvin Langmuir (1881-1957) and Katherine B. Blodgett who discovered unique properties of thin films which were a nanometer thick and used in the manufacture of electronic semiconductor devices (Jasmin and Ryan, 2016).

Although nanotechnology appears to be a modern field of scientific research that has only just been established on the face of technology and civilisation, it should be noted that the nanosynthesis of metallic nanoparticles predates records of its exploration and development. In the past, scientists have studied many ancient remains that have been associated with the existence of metal nanoparticles due to their unusual colors and attributes (Schaming and Remita, 2015). Nanoparticles synthesis and utilization seem to have been already occurring between years 1200 and 1400 BCE with the beginning of glassmaking in Egypt and Mesopotamia, where the world earliest civilisations can be found. Examples of such include the manufacturing of ancient red glasses, whose appearance and properties could only be explained by the presence of nanoparticles, as confirmed by analysis. It was suggested that the nanoparticles were a product of metallic copper or copper oxide nanoparticles as a range of ancient pieces from the 6th century were also discovered to contain nanoparticles (Brill and Cahill, 1988). The Lycurgus cup, produced in the 4th century in Rome, provides a compelling example of the use of nanoparticles for decorative purposes in artefacts. The cup's color is green when light is shone at it but turns red when the same light disperses through it because of the impregnation of the translucent material with 50 nm size nanoparticles synthesised from gold and silver. To date, the Romans have inspired exploitations of the interactions between nanoparticles and light (Schaming and Remita, 2015; Reeve, 2019).

A scanning tunnelling microscope, invented in 1981 by Swiss scientists and International Business Machines (IBM) corporation, enabled them to see particles at imperceptible levels. The widespread availability of higher-powered computer technologies during this period and after has brought about the possibility of large-scale simulations of nanomaterials using advanced computerised systems and machine learning. These studies have continuously provided a clear understanding into nanoscale material structures and their properties. Since then, research in nanotechnology has been driven by the activities of material modelling and simulation, atomic scale graphic imaging and characterisation, as well as studies based on experimental synthesis (Hulla et al., 2015). `In the past, humans have used nanotechnology without the actual awareness of its existence. In many instances, techniques of nanosynthesis have simply been passed from one generation to the next, without going into detail about why and how the materials and products developed from them acquired their unique properties (Bayda et al., 2020). However, recently, scientists are focused not only on techniques of nanosynthesis, but on their reproducibility as well (Lin et al., 2014; Roger and Amri, 2022). Ever since the inception of these findings, the innovations of nanotechnology and its applications in daily living, has greatly advanced,

not only in the application of microchip technology with the need of more minute components. Nanotechnology has also found applications in daily life items, such as cosmeceuticals, medication, smart appliances and weather resistance paints and surface sealants (Schaming and Remita, 2015).

Nanotechnology as the technical vehicle for the next industrial revolution has since gained global attention. South Africa has adopted nanotechnology as part of its scientific pursuits, aligning itself with numerous developing African nations exploring applications at the nanoscale to improve global productivity and sustainable economic development. As early as 2005, South Africa demonstrated interest in nanotechnology through the publication and subsequent implementation of the nanotechnology policy and a 10-year plan. The strategy laid out how nanotechnology could help improve specifically the niche areas of the country, specifically at application levels. The applications focused primarily on areas expected to benefit the country, since the overall goal was to promote growth. A little over a decade has lapsed since the implementation of the 10-year strategic plan mentioned above, however an inevitable question as to how nanotechnology has contributed to the development of South African continues to rise (Saidi and Douglas, 2017).

Since the early 2000s, South Africa has invested in nanotechnology and recognised the threats to human and environmental health that surround this technology as an important issue for governance. This serves as an example of a broader trend in which developing countries play an increasingly visible role in developing, producing, and using emerging technologies. This also validates the claim of the idea that countries around the world now face the risks of emerging technologies. Faced with the enormous potential that nanotechnology holds for the growth of Africa, it is evident that the continent, however, is reluctant to accept it (Beumer, 2018).

The first innovation centre for nanotechnology in South Africa was opened in November 2007, driven from the fact that the country has a lot to gain from pursuing nanotechnology. A minister from the government indicated that South Africa was ready to create an environment that would be able to channel the prospective benefits of nanotechnology in future years. In 2008, noteworthy nanotechnology research investment and research were provided, for instance, when the Sci-Bono Discovery Centre in Newtown, Johannesburg, hosted and launched the International La Villette nanotechnology exhibition. Towards the betterment of the research itself, in 2011, South Africa became the first country on the African continent to own and house a new US$15 million (R 218 million) electron microscopy centre that aimed at enabling the country as well as Africa to compete with the world's finest in nanoscience and nanotechnology research (AZoNano, 2014; Douglas 2017).

3.3 Nanotechnology landscape in South Africa

South Africa has been deemed the most technologically advanced country in Africa, according to a 2010 survey. It is also home to a few universities offering research and educational opportunities in nanotechnology (AZoNano, 2014). Despite nanotechnology research being somewhat new in South

Africa, there are isolated projects that have been initiated and running for more than half a decade. South Africa is a middle ground runner when it comes to developing countries and their nanotechnology activity. However, there is limited industry involvement (Singer et al., 2005). According to Pouris (2012), in South Africa, the era of nanoscience and nanotechnology was initiated when the Department of Science and Innovation (DSI) commissioned the South African Nanotechnology initiative (SANi) to develop a South African Nanotechnology Strategy that has accelerated the growth of nanotechnology as a research area in South Africa. The strategy identified several key interventions including creating transparency, public awareness, and acceptance of nanotechnology.

The DSI has been actively involved in this initiative, by allocating resources for the advancement of technology, leading to the adoption of a South African nanotechnology 10-year strategy (Cele, 2009). The SA nanotechnology strategy identified the following success indicators for the medium to long term:

- Nanotechnology has the prospective to positively impact the subjective well-being for traditionally marginalized community groups such as women and people with disabilities. Applications emerging from continued nanotechnology research and development could help address long-standing accessibility issues and better support independence for those facing physical or social challenges.
- Commercial impact of nanotechnology, including local and foreign direct investment and return on investment, and contribution to the treasury.
- The amount and quality of unique South African nanotechnology-based products that are launched.
- The rate of the technology transfer of nanotechnology into the existing industry, Black Economic Empowerment (BEE) and the establishment of Small Medium and Micro Enterprises (SMMEs).
- Impact of nanotechnology-based solutions on community upliftment and quality of life.
- Quantity and quality of South African Nanoscience and Nanotechnology contributions in respected national and international journals, books, and conferences.
- Quantity and quality of intellectual property, (patents, designs, and trademarks) held by South African institutions or companies in nanotechnology (Cele, 2009; Pouris et al., 2012).

So far, most nanotechnology experts in South Africa are academics specializing in material science, with few engineers and limited participation from the industry or individuals outside of academia and industry. However, this can negatively impact the commercialisation of nanotechnology research (Masara et al., 2021).

According to Masara et al. (2021), South Africa produced 11,624 publications, publications on scientific work have risen to 2458 % as of 2019. This highlights that nanotechnology research is primarily driven by academics and researchers in material science, rather than inventors and engineers who tend to focus more on patents. Hence, there is a need to evaluate the strategy so that other diverse nanotechnology and nanomaterials areas outside academia must be targeted. There is persistent question as to whether the 10-year plan has been accomplished, if it has, then has nanotechnology broken barriers into fields such as medicine and/or complementary medicine? The next heading aims to briefly discuss green nanotechnology and nanomedicine and some of the work that has been carried out from a complementary medicine aspect.

3.4 Green nanotechnology

In general, green nanotechnology means synthesising nanoparticles or nanomaterials using biological routes such as those involving microorganisms, plants, or their by-products such as primary and secondary metabolites. Green nanotechnology refers to a technology at nanoscale that serves to i) reduce contaminants and harmful processes through directly counteracting them or through altering the conditions that created them, ii) conserves the environment and natural resources by avoiding the use of toxic constituents, iii) increase human quality in life, by advancements in healthcare, food, and agriculture through nanotechnology (Ranik and Sridevi, 2017). Other specific goals for the advancement of green nanotechnology, globally, were highlighted by Patra and Baek (2014) and included:

- Sustainability - using resources to meet present without depleting so much that there is enough left for future use.

- Waste Reduction - reducing toxic waste production by changing patterns of production and consumption.

- Innovation - developing alternatives to technologies whether fossil fuel or chemical intensive agriculture and healthcare that have been demonstrated to have an impact on human and environmental health.

- Green Chemistry - inventing, developing and applying chemicals and procedures to minimise or eradicate the use and production of harmful and toxic substances.

Green nanotechnology represents a promising and vital intersection of nanoscience and sustainability. The pursuit of innovation in this field opens the door to alternative technologies that not only enhance human quality of life but also protect and preserve the planet's resources for future generations. As research and development continue, green nanotechnology holds the potential to be a transformative force in addressing global challenges in health, agriculture, and environmental protection.

3.4.1 Metal nanoparticles in green nanotechnology

As early as 4000 B.C.E, metals have since been used by the ancients in an array of configurations and for numerous medical conditions. Metals were formulated to work topically, either as solutions or ointments, as well as oral and injection treatments and as such, have found their place in green nanotechnology (Alexander, 2009). For instance, iron, silver, copper and gold have been used to formulate herbal remedies by the use of *Bhasma*, a herbo- metal formulations, which is obtained through incineration where metal undergoes an intricate process of purification followed by the incorporation of some herbal extract (Pal et al., 2014). *Bhasma* formulations has been claimed to be biologically produced nanoparticles, which are prescribed with several other medicines of *Ayurveda* (Bhowmick et al., 2009). Over recent years, metal nanoparticles have drawn specific focus across diverse applied scientific disciplines. With the improvement of scientific research techniques and the advancement of technological expertise, their sizes and shapes have also been well exploited and seem to be comprehensible (Karn and Wong, 2013; de la Guardia, 2014).

The defined sizes, shapes, and orientations of nanoparticles influence their ability to catalyse chemical reactions and impact their capacity to facilitate changes, resulting in the interest of producing nanoparticles with defined properties, for targeted applications. Several approaches have found use in the production of metallic nanoparticles, and these strategies include wet methods, which entail the utilisation of certain hazardous chemical reagents including sodium borohydride and poly-N-vinyl pyrrolidine (PDP). Dry methods involve the synthesis of nanoparticles using aerosol and lithography as well as UV irradiation (Narayanan and Sakthivel, 2010). There exist concerns pertaining to the utilization of hazardous reagents for the method of nanosynthesis as these remain on the interface of the nanoparticle after synthesis, thereby limiting their applications, and eventually pose harm to the environment and toxicity if used in biological or biomedical applications. The realisation of such unfavourable results has demanded new approaches, such as green nanotechnology, in designing innovative nanoparticles, which are human and environmentally friendly (Nath and Banerjee, 2013; de la Guardia, 2014).

3.5 Nanoparticles in medicine and biology

3.5.1 Nanoparticle roles Nanomedicine

The continued use of nanotechnology advances in less invasive treatments, henceforth referred to as nanomedicine, demonstrates promising potential for impacting preventative measures, early diagnosis, reliable diagnosis, and treatment modalities for diseases that were previously impossible to treat. Many scientists worldwide are investigating the use of nanoparticles as a potential remedy for diseases that are characterised by generally slow progression such as cancer as well as various communicable diseases. The notion of "nanorobots" for medical purposes has rapidly emerged in literature and cinema in the last decade. Although nanorobots are not a notion that has been fully developed and fully existent,

more complex nanoparticles have been developed for application in nanomedicine. The nanoparticles can be used to load medicinal compounds, which enhances the dose and efficiency of the medicine. These nanoparticles could easily interact with targeted cells or muscle tissues, in response to factors such as pH levels, temperature of the environment into which they are loaded, and respond to stimuli, pH, temperature, and radiation exposure during cancer treatments (Schaming and Remita, 2015).

Nanomedicine remains an evolving field in Africa relative to more developed areas globally, however, one factor that can constrain progress in research is the expense of necessary infrastructure (Saidi et al., 2018). However, several products have been developed and patented to the benefit of society. For instance, Tsai et al. (2013) developed a polymeric hydrogel composition fabricated for controlled drug delivery, activated by an electrical stimulus. The hydrogel is made from polyvinyl alcohol (PVA) cross-linked with diethyl acetamidomalonate (DAA), and includes an electroactive polymer, such as polyaniline. When the hydrogel is planted at the target site, and electric current is applied to it, a loaded pharmaceutical drug used to treat pain is released. du Toit et al. (2014) also developed an inflammation-responsive nanoparticle based implantable device for the in-situ delivery of one or more pharmaceutically active agents such as antibiotics and an anti-inflammatory drugs. Nanoparticle use has the potential to curb treatment failures, especially when a doctor prescribes treatment drugs in large and prolonged doses, depending largely on the patient's conditions, whereas the same large doses and prolonged treatment can adversely affect the patient's health (Saravanan et al., 2018). The treatment of communicable diseases has posed challenges for several years in Africa, making nanomedicine a much-needed solution. Over the years, the use of nanomedicine has then improved the fight against Tuberculosis, Human immunodeficiency virus (HIV), and malaria. In this way, effective drug delivery methods have been discovered and will continuously be produced to prevent dangerous illnesses or infections for generations to come (Choonara et al., 2011).

Nanoparticles have had numerous remarkable implications in nanomedicine, especially with regard drug discovery and development. Nanotechnology has had a great impact in the design of targeted drugs that promise not to only offer diagnostic methods but are also able to efficiently treat patients with specific regard to their illness. The principal benefits are associated with the structured drug dosage needed for treatment over a particular treatment period, all of which can be decreased for more efficient treatment with fewer side effects. Efficiency is accomplished by targeting nanoparticles incorporated herbs to improve specific drug uptake, which improves both dosage concentrations and bioavailability (Etheridge et al. 2013). Also, nanoparticles, because of their nature, can target and interact with disease receptors. The binding of nanoparticles to receptors takes place through the covalent linking between ligands and antibodies, in this way, the drug is released into the diseased cells or tissues. This approach of targeted drug release triggers a rise of the distribution rate of the medication within the circulatory system (Kamaly et al., 2012). Progress in the development of nanomedicines aimed at passive diffusion

through endothelial tissue, result in better drug permeation across the lungs and intestines, as well as other barriers (Onoue et al., 2014).

3.5.2 Nanoparticle roles in biology

Nanoparticles have enabled innovation across diverse disciplines, notably within biology. Their exceptional properties, such as their excitation due to interaction with light, morphological versatility, and ease of transport through biological membranes, make them incredibly useful for applications ranging from drug delivery to imaging and sensing in biological systems (Wang et al., 2014). Nanoparticles have demonstrated considerable potential for utilization in precise delivery of drugs owing enhanced regulation of release kinetics and the potential to mitigate harmful impacts, relative to traditional therapeutic agents (Patra et al., 2018). In this comprehensive exploration, there are diverse roles nanoparticles play in biological systems, their applications, recent advancements; a brief description of a few of these categories is provided below.

3.5.2.1 Drug Delivery: Nanoparticle-based drug administration systems offer precise targeting, controlled release, and enhanced efficacy compared to conventional drug formulations. Various nanoparticle platforms, including inorganic nanoparticles, liposomes, polymeric nanoparticles, and dendrimers, have been engineered to load drugs for precision treatments, whether in plants or warm-blooded organisms. Surface modifications on nanoparticles enable them to evade the immune system and penetrate biological barriers, enhancing drug delivery to previously inaccessible sites. A study by Khanzada et al. (2021) assessed the impact of nanoparticles synthesised from gold, on various bacteria and found that they had a higher drug loading efficiency compared to conventional drugs used for inflammation, confirming the potential function of gold nanoparticles as competent nanocarriers. On the other hand, Machado et al. (2020) worked on nanoparticles that could load fungicides to combat grapevine disease and fungicide resistance for over four years in cultivated grape species.

3.5.2.2 Imaging and Diagnostics: Nanoparticles serve as contrast media in radiographic diagnostics (Naranthatta et al., 2021), computed tomography (CT), and fluorescence imaging (Narayanan et al., 2012). Functionalised nanoparticles have been used to enable specific labelling of cells or biomolecules, facilitating early detection of neoplasms (Fazal et al., 2014). Formerly, semiconductor nanoparticles, and nanoparticles from inorganic material are among the commonly used imaging agents, providing high sensitivity and spatial resolution for diagnostic purposes (Nune et al., 2009; García-Álvarez et al., 2020).

3.5.2.3 Theranostics and Biomolecule Detection: The integration of diagnostics and therapy, termed theranostics, is made possible by multifunctional nanoparticles capable of both imaging and drug delivery. Theranostic nanoparticles enable real-time monitoring of treatment response and personalised medicine by tailoring therapy based on individual patient characteristics. This approach holds promise for improving treatment outcomes and reducing adverse effects by optimising drug dosing and delivery

(Zavaleta et al., 2018). Nanoparticles also serve as sensitive probes for detecting biomolecules, including proteins, nucleic acids, and small molecules, in biological samples. Surface functionalisation with specific ligands enables selective binding to target molecules, facilitating applications such as biomarker detection for disease diagnosis and monitoring. Nanoparticle-based biosensors offer rapid, cost-effective, and portable platforms for point-of-care testing and early disease detection (Barash et al., 2009; Hong et al., 2012).

Nanoparticles represent a transformative toolset for advancing biological research and healthcare applications. As such, fostering a culture that actively supports exploration, experimentation, and the creation of novel ideas in nanoparticle design, synthesis, and functionalisation guarantee a scientific discourse for unmet medical needs, enhancing treatment outcomes, and ultimately improving human health (Murphy et al., 2015; Waheed et al., 2022).

3.6 A South African perspective on nanomedicine

In 2005, the Nanosciences African Network was founded for the advancement of the involvement of countries on the African continent in the nanotechnology field (Molapisi, 2012). Approximately 27 countries engaged in this network, with a specific focus on nanomedicine and improvement of therapy against common communicable diseases such as Tuberculosis and HIV amongst others (Hayeshi et al., 2012; Saravanan et al., 2018). Furthermore, workshops have been held in South Africa to discuss ground-breaking plans for the inclusion and implementation of nanomedicine training and research, in collaboration with industry and universities. As such, this collaboration substantially boosts the industry to accelerate research, with focus on manufacture therapeutic compounds that will be conjugated with nanoparticles, for the mitigation of transmissible illnesses (Chang et al., 2015; Saravanan et al., 2018). Due to fatalities and the inadequate compliance and regulation regarding treatment of these diseases, there is a need to expedite nanomedicine use. New and existing therapeutic agents, including traditional medicines, and even drug leads that had been disqualified during the development process, can all be reformulated, using nanomedicine for improved performance (Salie and Saidi, 2023).

South Africa, along with Russia, China, Brazil, and India, has been categorised as one of the five big emerging economies (Bezuidenhout et al., 2021; Trivedy and Khatun, 2023). While South Africa is actively involved in nanomedical investigations and nanomedicine development on the African continent, the majority of work in this field has been restricted to foundational wet lab focus research and seldom break into production or commercialisation (Patra et al., 2018; Saidi et al., 2018). Dube and Ebrahim (2017) provided a high-level summary of South Africa's national initiatives pertaining to nanotechnology, including details on applicable government policies, funding sources, laboratory infrastructure, and workforce training programs implemented to advance nanoscale science and engineering capabilities in the country.

This summary analysed South African scientific reports and provided perspectives on prospective approaches to impact prevailing frameworks and investments to facilitate transformation of nanomedicine scientific reports into products and services that create commercial and social value.

3.7 Green synthesis of nanoparticles from plant extracts

A substantial body of literature already exists on various aspects of nanotechnology in general, including advances on research and development on green nanosynthesis using plant extracts. Following the essential necessity for the advancement of environmentally friendly methods for nanoparticle synthesis, scientists have now turned their focus to the use of plant extracts. In recent years, plant extracts have received high interest and advantage over other hazardous methods as a method of nanosynthesis because naturally replenished at a higher rate than they are consumed. In addition, plants extracts are energy-efficient, economically feasible as they are found freely in nature, and exert non-toxic behaviour during synthesis (Geraldes et al., 2016). Scientific studies show that antioxidant and anti-inflammatory agents derived from plants and nanoparticles show increased capacity to scavenge free radicals, thereby reducing generation of oxidants, and are also able to exert high activity against microorganisms (Nasim et al., 2022; Muruganandham et al., 2023; Fatima et al., 2024). Their ability to permeate differently structured tissues makes them highly efficient drug delivery agents (Khanna et al., 2015; Pandiyan et al., 2023). As such, the modification of nanoparticles for direct exposure to infected cells or affected tissues grants them significant advantage as they are able to radically improve in activity once absorbed intestinally, due to their high solubility (Bell et al., 2013).

Several works have been carried out using plant extracts to facilitate the production of nanoparticles from plants extracts due to the ability of their phytochemicals to conduct complete reduction of the precursor metals. The summary in Table 3.1 lists a summary of studies of plant extract-facilitated nanoparticle remedies drawn from African herbalism, homeopathic and nutraceutical systems. Some of these findings can be an appropriate blueprint in the continued designing of green synthesis experiments for other plants.

Table 3.1. Provides a number of studies of some bioactive medicinal plants that have been used for the green synthesis of various nanoparticles.

Plant species	**Plant material and solvent**	**Type of NP's & mechanism**	**Size (nm)**	**References**
Avicennia marina	Seed, aqueous	Au, bioactive compounds	2 – 40	Naidu et al., 2019
Eichhornia crassipes	Shoot, Toluene, Ethanol	Ag, cellulose	2.17	Oluwafemi et al., 2016
Lasienthra africanum	Leaf, aqueous	Ag, bioactive compounds	8 – 35	Elemike et al., 2017
Citrus paradisi	Peel, aqueous	Ag-MgO, bioactive compounds	20 – 100	Ayinde et al., 2018
Aloe arborescens	Leaf, aqueous	Ag, bioactive compounds	40 – 80	Kumara et al., 2018
Iboza Riparia *Ilex mitis*	Leaf Root bark, aqueous	Ag, Saponins, Diterpenes	50 – 156	Sabela et al., 2018
Hibiscus sabdariffa	Flower, aqueous	CeO_2, bioactive compounds	3.9	Thovhogi et al., 2015
Agathosma betulina	Leaves, aqueous	CdO, bioactive compounds	8 – 100	Thema et al., 2015
Plumbago auriculata	Leaf, calyx, aqueous	Ag, bioactive compounds	15 – 30	Singh et al., 2018
Eichhorn crassipes	Shoot, ethanol, toluene	Ag, cellulose	5.69	Machochoko et al., 2013
Codium capitatum	Leaves, aqueous	Ag, bioactive compounds	3 – 44	Kannan et al., 2013
Dovyalis caffra	Fruit, aqueous	Ag, Au; Au-Ag, bioactive compounds	3 – 5 9 – 14	Adeyemi et al., 2019
Moringa oleifera	Flower, aqueous	Pd, bis-phthalate	2 – 18	Anand et al., 2016
Moringa oleifera	Leaf, aqueous	ZnO, bioactive compounds	12 - 30	Matinise et al., 2017
Moringa oleifera	Leaf, ethanol, aqueous	NiO, bioactive compounds	9.69	Ezhilarasi et al., 2016
Agathosma betulina	Leaves, bark, ethanol	Ag, bioactive compounds	5 – 60	Chiguvire, 2015
Hibiscus rosa sinesnsi	Leaves, aqueous	$ZnCr_2O_4$, bioactive compounds	60 – 70	Mayedwa et al., 2020
Asphalathus linearis	Flower, aqueous	ZnO, bioactive compounds	1 – 8.5	Dialo et al., 2015
Asphalathus linearis	Leaf, aqueous	Co_3O_4, bioactive compounds	2 – 7	Dialo et al., 2016

Key: Ag-Silver; Au-Gold; CeO_2-Cerium oxide; CdO- Cadmium oxide; Co_3O_4-Cobalt oxide; NiO-Nickel oxide; Pd-Palladium; ZnO- Zinc oxide; $ZnCr_2O_4$- Zinc chromite.

3.8 Significance of nanoparticles as an herbal medicine delivery system

Herbal medicines have been used since ancient times, especially amongst patients with chronic diseases. It is also known that herbs are quite effective in curing and or treating several chronic diseases. In modern day health systems, individuals taking prescription medications also use them alongside herbal supplements thus, interactions between herbs or dietary supplements requires critical attention to avoid toxic effects (Fatima and Nayeem, 2016). Some herbs, such as *Ginkgo biloba* L. have been reported to cause clinically significant adverse drug reactions. For instance, Gingko products that are administered alongside aspirin, interact, and can cause bleeding. Also, other herbs like St John's wort (*Hypericum perforatum*), while administered alongside anti-depressants cause gastrointestinal disturbances and allergic reactions (Bo et al., 2016). Generally, the safety of herbs mostly depends on experience over a long time. However, at times, observations of its negative effects are not always effective in assisting the limitation of side effects. As such, the first signs of the negative effects take a long time to be recognised (Ansari et al., 2012), due to the notion that herbal medicines are natural; and therefore, are neither toxic nor capable of inducing unintended consequences (Zhang et al., 2015).

In as much as medicinal herbs have always had reputable therapeutic effects, they may also potentially induce negative effects if used erroneously or in doses that are more than required. For example, high dosages of *Tephrosia vogelii* Hook.f root extracts caused hepatic necrosis and vacuolation in rats and were suggested to have the ability to infer similar effects in humans if taken in larger than normal doses (Mlozi et al., 2020). Additionally, lethargy and depression was observed in a study where *Anogeissus leiocarpus*, *Khaya senegalensis*, *Azadirachta indica* and *Tamarindus indica* were administered to rats in single large doses (Tauheed et al., 2021). The probability of negative effects becomes more possible due to negligent, or unregulated use as well as lack of proper herb standardisation. Many international forums have placed focus on these concerns as the center of medicinal plant research. Mechanisms have also been put in place to track adverse effects of herbal medicines; however, they can be inadequate (Ozioma and Chinwe, 2019). Examples of such, include the pharmacovigilance and regulation of herbal medicines by the South African Health Products Regulatory Authority (SAHPRA). These actions make sure that adverse occurrences, interactions, and information about post-marketing surveillance and vigilance of herbal medicines—both past and present—are tracked, examined, and taken appropriate action upon (Keyter et al., 2018). In other countries, spontaneous reporting of adverse drug reactions (ADR) is an effective means of ensuring post consumption surveillance of herbal drugs, by health professionals and pharmaceutical manufacturers to the national regulatory authority (Kim et al., 2015; Worakunphanich et al., 2023).

In cases where proper herb standardisation has occurred, which includes adjusted drug formulations that contain a specific set of compounds with established therapeutic activity (Kunle et al., 2012), there is still a concern about changes that have occurred before and after their processing stages, which may have become significant in modifying the metabolic compounds that constitute the herbs. Most of these changes, result in herbal formulations being poorly soluble, or being largely sized, thus, limiting their performance as therapeutics (Dhiman et al., 2012). Therefore, it is important to introduce communication on available information on risks associated with herbal medicines as a crucial part of pharmacovigilance, which could improve the wellbeing and safety of patients. This calls for improved collaboration between traditional herbal practitioners and modern health care specialists, researchers, and drug regulatory authorities, thus, assist in advancing the study and research of the medicinal plants that are being used (Ahmad and Hashim, 2012; Mothibe and Sibanda, 2019; Ozioma and Chinwe, 2019). Since recent advancements have turned to more efficient herbal drug delivery methods, the use of nanoparticles has gained a wide range of interest (Javed et al., 2020; Dubey et al., 2022). Researchers focusing on nanomedicine have discovered that nanoparticles can provide more effective therapy than synthetic forms of drugs. It would be worthwhile to use the concepts of nanoscience to resolve several issues that have been encountered, as interactions occur on a molecular level (Gopi et al., 2016), some of which are discussed below:

3.8.1 Sufficient bioavailability

According to Ansari et al. (2012), the optimum quantity of drugs (whether herbal or synthetic) does not always reach the blood due to being digested by the highly acidic environment of the stomach or being metabolised by the liver. This leads to a lesser number of drugs being adsorbed; thus, no therapeutic effect is observed. This is described as “bioavailability”, which is the extent and rate at which a substance, such as a drug or nutrient, is absorbed into the bloodstream and becomes available to produce its desired effect in the body. For good bioavailability, drugs must easily be dissolvable into the gastric fluids and permeate across the lipid barriers. (Gomes et al., 2014). Most metabolites, like phenols, are usually hydrophilic as they solubilise efficiently in water, but are usually malabsorbed due to their large molecular weights (Asano et al., 2023). Large-sized molecules cannot be absorbed by simple diffusion; this limits the possibility of passing across lipid-rich membranes in the intestines. In this case, if they were to be used, nano-carriers would carry the maximum drug dosage to the targeted site and would be able to permeate all biological membranes as well as survive acidic gastric fluids (Dhiman et al., 2012; Sobiesiak, 2017).

Recently, alcoholic extracts and tinctures have become readily available which may not be necessarily suitable for delivery as they are, as such, they would need to be packaged into nanoparticles for better delivery during treatment intake. Extracts or herbs have been observed to be bulky, so dose reduction is intended and can only be achieved when the surface area has been reduced to that of a nano size

(Khoza et al., 2012). According to Dhiman et al. (2012), the principal goal in synthesizing nanoparticles as delivery vectors is to have the ability to govern nanoparticle dimension and modify surface morphology. Regulation of nanoparticle size and surface properties during synthesis allows for modulation of drug release kinetics and biodistribution characteristics to optimize targeted delivery and furthermore, presents opportunities to develop more effective nanotherapeutics. Alternatively, in recent years, biodegradable nanoparticles made from different organic materials, have gained traction as potentials in targeted drug delivery, and are related with existing marketed formulations, such as liposome-based Onivyde® and Lipusu® which are used for the treatment of metastatic pancreatic cancer and lung squamous cell carcinoma, respectively (Milano et al., 2022; Zhang et al., 2022). Biodegradable polymeric nanoparticles have attracted considerable attention as potential drug delivery devices and are associated with currently marketed formulations from different pharmaceutical companies (Shan et al., 2022; Thapa and Kim, 2023).

3.8.2 Less Toxicity

At present, there are misunderstandings and predisposition towards the safety of herbal medicine. It has been continuously advertised that because herbal medicine originates from nature, it cannot be toxic, and that people can use it as a long-term drug. However, they are not entirely devoid of potential toxicity; as such, caution is still warranted as all manners of pharmacologic medicine agents can induce undesirable side effects depending on large dosage and course of treatment (Zhang et al., 2015). In contrast, recent advancements in nanotechnology have shown promise in reducing toxicity of nanoparticles. The significant contribution of nanoscience has been used to convert large drug molecules, for dose reduction and targeted delivery, thus, enhancing efficacy and, ultimately, reducing toxicity (Ahmad et al., 2012). For instance, *Rhus coriaria*-ZnO (16 nm sized) nanoparticles possessed remarkably cytotoxic effects on both MCF-7 cells, where the growth of cancer cells experienced a marked decrease at lower concentrations (Mongy and Shalaby, 2024). In an earlier study by Naz et al. (2017), hydrophilic anticancer drugs (ACD), doxorubicin (DOX) and daunorubicin (DNR), were loaded onto silver nanoparticles (5–20 nm) from *Setaria verticillate* with anticancer activity. This was in order to create a novel drug delivery vehicle with significant adsorption capacity and efficiency to eliminate the side effects of the medications already effective for leukemia chemotherapy. Generally, even ionic metals by themselves, can cause toxicity in any system; however, at nanoscale, they produce less toxicity, or close to none (Saraf, 2010; Chakraborty et al., 2016). According to Mamillapalli et al. (2016), the idea of drugs at a nanoscale has potential to minimize toxicity risks through optimized dispersal enabled by morphological modification at the nanoscale and strict dosage management.

3.8.3 Pharmacological activity enhancement

Naturally occurring biologically active molecules comprising diverse structural properties provide an intricate basis for elucidating novel pharmaceutical drugs (Mehra et al., 2022). This is because they

have unique traits such as a rich profile of known and unknown compounds that have significant biological activity and cause little to no adverse reactions. As such, they become remarkable prospects in drug discovery (Patra et al., 2018). Nevertheless, in most cases, such molecules also demonstrate faster metabolic elimination, resulting in the decrease of their expectancy period and increase in treatment times and larger doses, due to the decline in therapeutic efficiency (Yuan et al., 2016; Patra et al., 2018). Presently, enhancing the efficiency of bioactives through nanosynthesis has become a popular function, and is set to grow rapidly (Wang et al., 2015; Patra et al., 2018). According to Patra et al. (2018) there is also evidence that some release mechanisms of natural bioactive compounds may help avoid drug resistance. Since some diseases require longer treatment doses, they may end up developing low responses to certain treatments. Therefore, it seems that scientific advancement of nanotechnology will revolutionise the production of drug formulations based on natural bioactive compounds and nanoparticles, develop technological approaches that can address limitations hindering the widespread of the application and maximisation of their potential (Bonifácio et al., 2014; Lam et al., 2017).

3.9 Recent and present developments in nanotechnology

South Africa has two research units doing ground-breaking work in nanotechnology at the Council for Scientific and Industrial Research (CSIR) and at MINTEK. The National Centre for Nano-Structured Materials at the CSIR was founded by the government in 2007. Its main goal is to create novel materials enabled by nanotechnology for use in the water, industry, and health sectors. The other national center situated at Mintek and concentrates on water nanotechnology, biolabels, nanotechnology-based health solutions and sensors (Makhoba and Pouris, 2017). Four South African Universities, namely, University of Johannesburg, Nelson Mandela University (NMU), University of the Free State (UFS), and the University of the Western Cape (UWC), are also conducting work on nanotechnology. This is fastracted by the funding the establishment of a taught master's degree programme in Nanoscience and nanotechnology (N&N) in the abovementioned universities (Pouris, 2007). As such, South Africa is expected to take off in the growth of a whole new industry (Saidi and Douglas, 2018)

The country’s strategy places a focus on four precedence areas of intermediation:

- Establishing of characterisation centres (national multi-user facilities), such as the CSIR National Centre for Nanostructured Materials which is highly equipped with world-class equipment, and the Department of Science and Innovation nanotechnology innovation centers for sensors and bio-labeling, located at Rhodes University and the University of the Western Cape, respectively
- Developing research and innovation networks (to enhance interdisciplinary, regional, and international collaboration),
- Strengthening of human capacity

- Creation of flagship programs (to demonstrate the advantages of nanotechnology) (Douglas, 2017), such as the establishment of grants in 2008 from the DSI and National Research Foundation (DST/NRF). Such grants facilitated the output of research based on advance materials, viral therapeutics (HIV, Hepatitis), and the development of 1D nanostructures for fast nano electronics. By the year 2012, the projects were still on the research stage due to lack of industry partnerships (Hillie, 2012). However, by 2015, research outputs had exceeded targets and reached the following milestones: 464 postgraduate students had been trained; 92 postdoctoral fellows were supported; 326 collaborations were established; 352 research articles were published in ISI journals; 80 conference proceedings were recorded; and 17 patents were registered that aligned to nanoscience and nanotechnology (Lindsay and Nel, 2024).

Traditional medicine is now an internationally acknowledged legitimate alternative therapeutic approach and represents a viable option for improving health and wellbeing. Continuous research in the traditional medicine field is continually explored, especially because the drug delivery system for such medications is outdated (Tilburt and Kaptchuk, 2008). In South Africa, the current advances in nanotechnology research led to more advanced applications in healthcare practice such as detection, diagnosis (diagnosis and imaging of diseases), monitoring, drug delivery and therapy (Pouris et al., 2012). These advances include prototypes for the prototypes for detection of measles, functionalised gold nanoparticles for detecting human and animal diseases, such as the Mintek Phila·Test HIV 1/2 Rapid Test, test kits for Tuberculosis and malaria and nanoparticle carriers for drug delivery from the CSIR (Tshikhudo et al., 2009; Kalombo, 2013; Mashazi et al., 2013; Sibuyi et al., 2022). Since then, remarkable efforts have been made at research centres working to release outputs with a variety of educational institutes (Mintek, 2019).

This implementation will not only assist in the early identification and diagnosis of diseases efficiently and accurately but will also allow broader inclusion of disadvantaged communities without basic healthcare infrastructure. Tangible products are currently being developed, which further emphasise the future of nanomedicine. This was evidenced by the discoveries of nanofiber scaffolds to spur the growth of nerve cells for the treatment of Parkinson's disease and nanostructured gels for cell regeneration (Pillay et al., 2009; Manjula et al., 2013). Nanoparticle research aimed at directly delivering chemotherapy drugs to cancer cells has been progressing in South Africa for some time, reaching advanced stages of coupling presently on the market doxorubicin with nanoparticles for targeted co-deliver. These efforts have demonstrated significant breakthroughs, particularly in enhancing drug targeting and minimizing side effects (Kalombo, 2013; Mhlanga et al., 2015; Chidanyika, 2016; Maney and Singh, 2017; Harris, 2022). Scientists at CSIR have also engineered nano-biodegradable polymer capsules that contain TB treatment drugs to more efficiently distribute the medication used by white blood cells. Hulda Swai, a former senior scientist at CSIR's Polymer Technology Centre, says the result is a slower release of the medication into the bloodstream, as well as a reduction in the frequency at which TB patients need to take the drug. Researchers at CSIR are presently also working on nano

capsules for the treatment of retroviruses and malaria. Nano-encapsulation can require a 100 to 500 nm diameter coating of the anti-malaria drug chloroquine with liposomes, which can deliver the drug by penetrating cell membranes, making its action on diseased cells more targeted and effective (Swai et al., 2012). Researchers have also developed a method for releasing antiretroviral therapy in the treatment of HIV / Aids by nano-designed drugs (Parboosing et al., 2012). By facilitating drug transport via nanoparticles, this approach may enhance clinical outcomes relative to existing administration methods. Further research appears warranted to evaluate both the pharmaceutical viability and cost-effectiveness of nanoparticle carriers (Patra et al., 2018). Thus, the pattern of introducing nanoparticles for herbal medicinal products can then also be implemented at an industrial scale.

3.10 Conclusions and future perspectives

3.10.1 Conclusions

The use of traditional African medicine is an emerging practice documented in both early practitioners' folklore and books. Currently, considering the abundance and development of synthetic drugs, a huge fraction of inhabitants in nations with poor and moderate incomes still depend on and actively use plant medicines for their health management needs. Extensive research has been going on to come up with innovative ways to expand and improve drug delivery through nanotechnology after targeting bioactive compounds in plants. Green nanotechnology has been one of the most innovative areas of nanotechnology with an extensive variety of applications in pharmaceutics, health/cosmetic and research industries. Using herbs from traditional medicine in conjunction with nanoparticles will continue to foster their capacity for the management and mitigation of various chronic and communicable illnesses. In the direction of nanotechnology, several promising examples of experienced evidence are present among us. Nanotechnology products are currently becoming more common, such as reinforced stain-resistant fabrics, improved cosmetics and healthcare products, and control of environmental pollution. Nanotechnology has been depicted as impractical in terms of research, starting from numerous ends of the world speculations and continuous non-scientific revelations in popular culture that predict self-reproducing nanoparticles taking part in major attacks on humanity and the environment. We continue to be surrounded by these kinds of pessimistic outlooks on new discoveries and developments, which stunts development.

Overall, this analysis shows that nanotechnology has great potential for the delivery of herbal drugs from traditional medicine and nutraceuticals; and that its use for successful disease prevention and health promotion is important and to be expected, considering the extensive health problems the world is faced with. While nanotechnology offers promising approaches to the delivery of herbal drugs and nutraceutical applications, more ground-breaking research is required to resolve the nanomaterials' cost-effectiveness and long-term safety. The scope of traditional medicine in South Africa presently and in the near future is very wide due to cultural significance, widespread use and a huge plant biodiversity;

but the issue of incorporating nanotechnology is still paramount. Possible limitations to this review include:

Limited availability of literature: There might be a scarcity of peer-reviewed literature specifically addressing the intersection of nanotechnology and African traditional medicine. This could restrict the depth and breadth of the review.

Quality of sources: The quality of available sources may vary, with some studies being more rigorous and reliable than others. Some sources may lack empirical evidence or may be based on anecdotal evidence rather than scientific research.

3.10.2 Future prospectives

More than ten years have passed since the implementation of the strategy and the question has inevitably arisen as to how technology has contributed to the growth of the country. Going forward, the existing framework has established an adequate foundation to facilitate amplified research outcomes. This enhanced scientific work could potentially advantage both South African citizens and the international community. Seemingly, the authors argue in a UCT paper by Saidi and Douglas (2017) that quantifying the bearing of nanotechnology on socioeconomic progress and advancement is a tedious task. However, visible gaps have been recognised, and the perspectives on how they can be filled, have been summarised below:

- One of the key recommendations is to harness the potential of traditional herbal medicine by integrating it into mainstream healthcare practices. This integration can strengthen the fight against both communicable and non-communicable diseases, providing an additional layer of treatment options that are rooted in cultural practices and local knowledge.
- Visible gaps can also be filled by including structures that provide an enabling atmosphere for the elevation of capacity building, research, discovery and development, as well as the manufacture of regulated high-standard traditional herbal medicines.
- The practical concepts of herbal medicines should be advanced to align with those of more developed countries, for global alliance to take place. In this way, traditional medicine will continue to attract research probes from pharmaceutical industries, and with the emergence of new approaches such as nanotechnology, new types of therapies can be developed to improve current systems used to administer drugs.
- Realising the potential benefits of nanotechnology and permitting penetration into many different fields of research, such as herbal medicine. Take into consideration the ethics, as well as regulatory affairs of nanomedicine to lessen impacts which may be considered negative on human and public health, thus avoiding a public criticism.

- The introduction and implementation of nanotechnology to members of the public who may benefit from it needs the evaluation of long-term social or ethical impacts as this might pose a huge challenge. Developing mechanisms to involve the public so that their perspectives and concerns on the general safety of nanoparticles in drugs feed back into research and development.
- Achieving meaningful change demands re-evaluating how nanotechnology makes a contribution to the pharmaceutical administration of drugs. Understanding the limitations of nanoparticles, identifying the myths persistent in the field, and facing inconvenient facts. For this approach to be successful, it may require adjustment of the procedure to maximise the usefulness of the nanoparticle to the herbal drug delivery system.

These if achieved, would put the incorporation of nanotechnology into African herbal medicine in an admirable position in the World health care system.

3.11 References

[https://www.azonano.com/article.aspx?ArticleID=3594 (Accessed 16 May 2020)].

Ahmad, S. and Hashim, U., 2012. Nanoherbals in Human Healthcare: A Proposed Research and Development Roadmap I. *ASEAN Journal on Science and Technology for Development*, 29(1), 65 - 75.

Ansari, S.H., Islam, F. and Sameem, M., 2012. Influence of nanotechnology on herbal drugs: A Review. *Journal of advanced pharmaceutical technology & research*, 3(3), 142 - 146.

Asano, D., Takakusa, H. and Nakai, D., 2023. Oral Absorption of Middle-to-Large Molecules and Its Improvement, with a Focus on New Modality Drugs. *Pharmaceutics*, 16(1), 47.

babu Maddinedi, S., Mandal, B.K. and Maddili, S.K., 2017. Biofabrication of size controllable silver nanoparticles–a green approach. Journal of Photochemistry and Photobiology B: Biology, 167, 236-241.

Barash, O., Peled, N., Hirsch, F.R. and Haick, H., 2009. Sniffing the Unique "Odor Print" of Non-Small-Cell Lung Cancer with Gold Nanoparticles. *Small*, 5(22), 2618-2624.

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M. and Rizzolio, F., 2019. The history of nanoscience and nanotechnology: from chemical–physical applications to nanomedicine. *Molecules*, 25(1), 112.

Bell, I.R., Schwartz, G.E., Boyer, N.N., Koithan, M. and Brooks, A.J., 2013. Advances in integrative nanomedicine for improving infectious disease treatment in public health. *European journal of integrative medicine*, 5(2), 126-140.

Beumer, K., 2018. Travelling risks: How did nanotechnology become a risk in India and South Africa?. *Journal of Risk Research*, 21(11), 1362-1383.

Bezuidenhout, H., Mhonyera, G., Van Rensburg, J., Sheng, H.H., Carrera Jr, J.M. and Cui, X., 2021. Emerging market global players: the case of Brazil, China and South Africa. *Sustainability*, 13(21),12234.

Bhowmick, T.K., Suresh, A.K., Kane, S.G., Joshi, A.C. and Bellare, J.R., 2009. Physicochemical characterization of an Indian traditional medicine, Jasada Bhasma: detection of nanoparticles containing non-stoichiometric zinc oxide. *Journal of Nanoparticle Research*, 11, 655-664.

Bhowmick, T.K., Suresh, A.K., Kane, S.G., Joshi, A.C. and Bellare, J.R., 2009. Physicochemical characterization of an Indian traditional medicine, Jasada Bhasma: detection of nanoparticles containing non-stoichiometric zinc oxide. *Journal of Nanoparticle Research*, 11, 655-664.

Bo, L., Baosheng, Z., Yang, L., Mingmin, T., Beiran, L., Zhiqiang, L. and Huaqiang, Z., 2016. Herb-drug enzyme-mediated interactions and the associated experimental methods: a review. *Journal of Traditional Chinese Medicine*, 36(3), 392-408.

Bonifacio, B.V., da Silva, P.B., Ramos, M.A.D.S., Negri, K.M.S., Bauab, T.M. and Chorilli, M., 2014. Nanotechnology-based drug delivery systems and herbal medicines: a review. *International journal of nanomedicine*, 9, 1-15.

Brill, R.H. and Cahill, N.D., 1988. A red opaque glass from Sardis and some thoughts on red opaques in general. *Journal of Glass Studies*, 30, 16-27.

Cele, L.M., Ray, S.S. and Coville, N.J., 2009. Guest editorial: nanoscience and nanotechnology in South Africa. *South African Journal of Science*, 105(7-8), 242-242.

Chakraborty, K., Shivakumar, A. and Ramachandran, S., 2016. Nanotechnology in herbal medicines: A review. International Journal of Herbal Medicine, 4(3), 21-27.

Chenthamara, D., Subramaniam, S., Ramakrishnan, S.G., Krishnaswamy, S., Essa, M.M., Lin, F.H. and Qoronfleh, M.W., 2019. Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials research*, 23(1), 1-29.

Chidanyika, T., 2016. The nano drug delivery system in South Africa. NPEP Newsletter. https://www.npep.co.za/newsletter. Accessed 15 March 2020.

Choonara, Y.E., Pillay, V., Ndesendo, V.M., du Toit, L.C., Kumar, P., Khan, R.A., Murphy, C.S. and Jarvis, D.L., 2011. Polymeric emulsion and crosslink-mediated synthesis of super-stable nanoparticles as sustained-release anti-tuberculosis drug carriers. *Colloids and Surfaces B: Biointerfaces*, 87(2), 243-254.

De Jong, W.H. and Borm, P.J., 2008. Drug delivery and nanoparticles: applications and hazards. *International journal of nanomedicine*, 3(2), 133-149.

de la Guardia, M., 2014. The challenges of green nanotechnology. *BioImpacts: BI*, 4(1), 1-2.

Dhiman, A., Nanda, A. and Ahmad, S., 2012. Novel herbal drug delivery system (NHDDS): the need of hour. In *International Conference on Environment, Chemistry and Biology,* 49 (34):171-175.

Du Toit, L.C., Pillay, V., Choonara, Y.E., Govender, T. and Carmichael T.R., 2014. A Drug Delivery Device. U.S. Patent US20140023692 A1.

Dube, A. and Ebrahim, N., 2017. The nanomedicine landscape of South Africa. *Nanotechnology Reviews*, 6(4), 339-344.

Dubey, S.K., Parab, S., Achalla, V.P.K., Narwaria, A., Sharma, S., Jaswanth Gowda, B.H. and Kesharwani, P., 2022. Microparticulate and nanotechnology mediated drug delivery system for the delivery of herbal extracts. *Journal of Biomaterials Science, Polymer Edition*, 33(12), 1531-1554.

Etheridge, M.L., Campbell, S.A., Erdman, A.G., Haynes, C.L., Wolf, S.M. and McCullough, J., 2013. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine: nanotechnology, biology, and medicine*, 9(1), 1-14.

Fatima, K., Asif, M., Farooq, U., Gilani, S.J., Bin Jumah, M.N. and Ahmed, M.M., 2024. Antioxidant and Anti-inflammatory Applications of Aerva persica Aqueous-Root Extract-Mediated Synthesis of ZnO Nanoparticles. *ACS omega*, 9(14), 15882-15892.

Fatima, N. and Nayeem, N., 2016. Toxic effects as a result of herbal medicine intake. *Toxicology-new aspects to this scientific conundrum*, London, UK: InTech Open, 193-207.

Fazal, S., Jayasree, A., Sasidharan, S., Koyakutty, M., Nair, S.V. and Menon, D., 2014. Green synthesis of anisotropic gold nanoparticles for photothermal therapy of cancer. *ACS applied materials & interfaces*, 6(11), 8080-8089.

Feynman, R.P., 1960. There's plenty of room at the bottom. *Engineering and Science*, 23(5), 22–36.

Feynman, R.P., 1992. There's plenty of room at the bottom [data storage]. *Journal of microelectromechanical systems*, 1(1), 60-66.

García-Álvarez, R., Chen, L., Nedilko, A., Sánchez-Iglesias, A., Rix, A., Lederle, W., Pathak, V., Lammers, T., Von Plessen, G., Kostarelos, K. and Liz-Marzán, L.M., 2020. Optimizing the geometry of photoacoustically active gold nanoparticles for biomedical imaging. *ACS photonics*, 7(3), 646-652.

Geraldes, A.N., da Silva, A.A., Leal, J., Estrada-Villegas, G.M., Lincopan, N., Katti, K.V. and Lugão, A.B., 2016. Green nanotechnology from plant extracts: synthesis and characterization of gold nanoparticles. *Advances in Nanoparticles*, 5(03), 176-185.

Gomes, A., Ghosh, S., Sengupta, J., Datta, P. and Gomes, A., 2014. Herbonanoceuticals: a new step towards herbal therapeutics. *Medicinal Aromatic Plants*, 3(3), 1-9.

Gopi, S., Amalraj, A., Haponiuk, J.T. and Thomas, S., 2016. Introduction of nanotechnology in herbal drugs and nutraceutical: a review. *Journal of Nanomedicine and Biotherapeutic Discovery* 6(2), 143-150.

Harris, R.A., 2022. The PEGylated and non-PEGylated interaction of the anticancer drug 5-fluorouracil with paramagnetic Fe3O4 nanoparticles as drug carrier. *Journal of Molecular Liquids*, 360, 119515.

Hayeshi, R., Semete, B., Kalombo, L., Katata, L., Lemmer, Y., Melariri, P., Nyamboli, B. and Swai, H., 2012. Nanomedicine in the development of drugs for poverty-related diseases. *Drug Discovery in Africa: Impacts of Genomics, Natural Products, Traditional Medicines, Insights into Medicinal Chemistry, and Technology Platforms in Pursuit of New Drugs*, 407-429.

Hillie, K.T., 2012. Strategic management of innovation and entrepreneurial framework applied to the South African nanotechnology flagship projects (Doctoral dissertation, Massachusetts Institute of Technology).

Hong, Y., Huh, Y.M., Yoon, D.S. and Yang, J., 2012. Nanobiosensors based on localized surface plasmon resonance for biomarker detection. *Journal of Nanomaterials*, 111-111.

Hulla, J.E., Sahu, S.C. and Hayes, A.W., 2015. Nanotechnology: History and future. *Human & experimental toxicology*, 34(12), 1318-1321.

Jasmin, B.A. and Furkan, R., 2016. Nanotechnology and Its Impact on Medicine. *Inosr Applied Sciences*, 2(1), 7-13.

Javed, M.N., Dahiya, E.S., Ibrahim, A.M., Alam, M.S., Khan, F.A. and Pottoo, F.H., 2020. Recent advancement in clinical application of nanotechnological approached targeted delivery of herbal drugs. *Nanophytomedicine: Concept to Clinic*, 151-172.

Kalombo, L., Council for Scientific and Industrial Research CSIR, 2013. Nanoparticle carriers for drug administration and process for producing same. U.S. Patent 8,518,450.

Kamaly, N., Xiao, Z., Valencia, P.M., Radovic-Moreno, A.F. and Farokhzad, O.C., 2012. Targeted polymeric therapeutic nanoparticles: design, development, and clinical translation. *Chemical Society Reviews*, 41(7), 2971-3010.

Kanwar, R., Rathee, J., Salunke, D.B. and Mehta, S.K., 2019. Green nanotechnology-driven drug delivery assemblies. *ACS omega*, 4(5), 8804-8815.

Karn, B. and Wong, S.S., 2013. Sustainable nanotechnology and the environment: advances and achievements. *American Chemical Society*, 1- 10.

Karunamoorthi, K., Jegajeevanram, K., Vijayalakshmi, J. and Mengistie, E., 2013. Traditional medicinal plants: a source of phytotherapeutic modality in resource-constrained health care settings. Journal of Evidence-Based Complementary & Alternative Medicine, 18(1), 67-74.

Kaul, S., Gulati, N., Verma, D., Mukherjee, S. and Nagaich, U., 2018. Role of nanotechnology in cosmeceuticals: a review of recent advances. *Journal of pharmaceutics*, 3420204.

Keyter, A., Banoo, S., Salek, S. and Walker, S., 2018. The South African regulatory system: past, present, and future. *Frontiers in pharmacology*, 9,1407.

Khanna, P., Ong, C., Bay, B.H. and Baeg, G.H., 2015. Nanotoxicity: an interplay of oxidative stress, inflammation and cell death. *Nanomaterials*, 5(3), 1163-1180.

Khanzada, B., Akthar, N., Bhatti, M.Z., Ismail, H., Alqarni, M., Mirza, B., Mostafa-Hedeab, G. and Batiha, G.E.S., 2021. Green synthesis of gold and iron nanoparticles for targeted delivery: an in vitro and in vivo study. *Journal of Chemistry*, 1-16.

Khoza, P.B., Moloto, M.J., Sikhwivhilu, LM., 2012. The effect of solvents, acetone, water, and ethanol, on the morphological and optical properties of ZnO nanoparticles prepared by microwave. *Journal of Nanotechnology*, 195106.

Khumalo, G.P., 2018. An inventory of the most popular medicinal barks sold on Johannesburg muthi markets and the antimicrobial activity of selected extracts and isolated chemical compounds. Magister Scientiae. University of Johannesburg (South Africa).

Khumalo, G.P., Van Wyk, B.E., Feng, Y. and Cock, I.E., 2023. Toxicity and phytochemical properties of southern African medicinal plants used traditionally to treat pain and inflammatory ailments. South African Journal of Botany, 160,102-122.

Kim, M., Han, C.H., Kim, M. and Han, C.H., 2015. Analysis of herbal-drug-associated adverse drug reactions using data from spontaneous reporting system in electronic medical records. *Journal of Korean Medicine*, 36(1), 45-60.

Kumari, A., Kumar, V. and Yadav, S.K., 2012. Nanotechnology: a tool to enhance therapeutic values of natural plant products. *Trends in Medical Research*, 7(2), 34-42.

Kunle, O.F., Egharevba, H.O. and Ahmadu, P.O., 2012. Standardization of herbal medicines-A review. *International journal of biodiversity and conservation*, 4(3), 101-112.

Lam, P.L., Wong, W.Y., Bian, Z., Chui, C.H. and Gambari, R., 2017. Recent advances in green nanoparticulate systems for drug delivery: efficient delivery and safety concern. *Nanomedicine*, 12(4), 357-385.

Lin, P.C., Lin, S., Wang, P.C. and Sridhar, R., 2014. Techniques for physicochemical characterization of nanomaterials. *Biotechnology advances*, 32(4), 711-726.

Lindsay, R. and Nel, J., 2024. Nanoscience teaching and research program in South Africa. *Frontiers in Nanotechnology*, 6, 1401598.

Machado, T.O., Beckers, S.J., Fischer, J., Müller, B., Sayer, C., de Araújo, P.H., Landfester, K. and Wurm, F.R., 2020. Bio-based lignin nanocarriers loaded with fungicides as a versatile platform for drug delivery in plants. *Biomacromolecules*, 21(7), 2755-2763.

Makhoba, X. and Pouris, A., 2017. Bibliometric analysis of the development of nanoscience research in South Africa. *South African Journal of Science*, 113(11-12), 1-9.

Mamillapalli, V., 2016. Nanoparticles for herbal extracts. *Asian Journal of Pharmaceutics (AJP)*, 10(2), 54 -60.

Maney, V. and Singh, M., 2017. An in vitro assessment of novel chitosan/bimetallic PtAu nanocomposites as delivery vehicles for doxorubicin. *Nanomedicine*, 12(21), 2625-2640.

Manjula, B., Varaprasad, K., Sadiku, R. and Raju, K.M., 2013. Preparation and characterization of sodium alginate–based hydrogels and their in vitro release studies. *Advances in polymer technology*, 32(2).

Masara, B., van der Poll, J.A. and Maaza, M., 2021. A nanotechnology-foresight perspective of South Africa. *Journal of Nanoparticle Research*, 23,1-22.

Masara, B., van der Poll, J.A. and Maaza, M., 2021. The "Nanotechnology Innovation Diamond", a model for successful nanoscience research and development. *Journal of Nanoparticle Research*, 23(3), 60.

Mashazi, P., Vilakazi, S. and Nyokong, T., 2013. Design and evaluation of an electrochemical immunosensor for measles serodiagnosis using measles-specific Immunoglobulin G antibodies. Talanta, 115, 694-701.

Mehra, A., Chauhan, S., Verma, P. and Kumar, N., 2022. Latest advances of phytomedicine in drug delivery systems for targeting metabolic disorders. In Drug Delivery Systems for Metabolic Disorders. *Academic Press*. Pp 469-479.

Mhlanga, N., Sinha Ray, S., Lemmer, Y. and Wesley-Smith, J., 2015. Polylactide-based magnetic spheres as efficient carriers for anticancer drug delivery. *ACS applied materials & interfaces*, 7(40), 22692-22701.

Milano, G., Innocenti, F. and Minami, H., 2022. Liposomal irinotecan (Onivyde): Exemplifying the benefits of nanotherapeutic drugs. *Cancer Science*, 113(7), 2224-2231.

Mintek. 2019. Annual Intergrated Report. 1-183.

Mlozi, S.H., Mmongoyo, J.A. and Chacha, M., 2020. The in vivo toxicity evaluation of leaf and root methanolic extracts of Tephrosia vogelii Hook. f using animal model. *Clinical Phytoscience*, 6,1-9.

Molapisi, J., 2012. Nanotechnology development in South Africa. *In* International Symposium on Assessing the Economic Impact of Nanotechnology, 27-28.

Mongy, Y. and Shalaby, T., 2024. Green synthesis of zinc oxide nanoparticles using *Rhus coriaria* extract and their anticancer activity against triple-negative breast cancer cells. *Scientific Reports*, 14(1),13470.

Mothibe, M.E. and Sibanda, M., 2019. African traditional medicine: South African perspective. *Traditional and Complementary Medicine*,1-27.

Murphy, C.J., Vartanian, A.M., Geiger, F.M., Hamers, R.J., Pedersen, J., Cui, Q., Haynes, C.L., Carlson, E.E., Hernandez, R., Klaper, R.D. and Orr, G., 2015. Biological responses to engineered nanomaterials: needs for the next decade. *ACS central science*, 1(3), 117-123.

Muruganandham, M., Al-Otibi, F.O., Alharbi, R.I., Sivasubramanian, K., Raja, R.K., Velmurugan, P. and Basavegowda, N., 2023. Antibacterial, antifungal, antioxidant, and cytotoxicity activities of the aqueous extract of *Syzygium aromaticum*-mediated synthesized novel silver nanoparticles. *Green Processing and Synthesis*, 12(1), 20230188.

Namdari, M., Eatemadi, A., Soleimaninejad, M. and Hammed, A.T., 2017. A brief review on the application of nanoparticle enclosed herbal medicine for the treatment of infective endocarditis. *Biomedicine & Pharmacotherapy*, *87*, 321-331.

Naranthatta, S., Janardhanan, P., Pilankatta, R. and Nair, S.S., 2021. Green synthesis of engineered CdS nanoparticles with reduced cytotoxicity for enhanced bioimaging application. *ACS omega*, 6(12), 8646-8655.

Narayanan, K.B. and Sakthivel, N., 2010. Biological synthesis of metal nanoparticles by microbes. *Advances in colloid and interface science*, *156*(1-2), 1-13.

Narayanan, S., Sathy, B.N., Mony, U., Koyakutty, M., Nair, S.V. and Menon, D., 2012. Biocompatible magnetite/gold nanohybrid contrast agents via green chemistry for MRI and CT bioimaging. *ACS applied materials & interfaces*, 4(1), 251-260.

Nasim, I., Shamly, M., Jaju, K., Vishnupriya, V. and Jabin, Z., 2022. Antioxidant and anti-inflammatory activity of a nanoparticle based intracanal drugs. *Bioinformation*, 18(5), 450.

Nath, D and Banerjee, P., 2013. Green nanotechnology–a new hope for medical biology. *Environmental toxicology and pharmacology* 36(3), 997-1014.

Naz, M., Nasiri, N., Ikram, M., Nafees, M., Qureshi, M.Z., Ali, S. and Tricoli, A., 2017. Eco-friendly biosynthesis, anticancer drug loading and cytotoxic effect of capped Ag-nanoparticles against breast cancer. *Applied Nanoscience*, 7, 793-802.

Nune, S.K., Gunda, P., Thallapally, P.K., Lin, Y.Y., Laird Forrest, M. and Berkland, C.J., 2009. Nanoparticles for biomedical imaging. *Expert opinion on drug delivery*, 6(11), 1175-1194.

Onoue, S., Yamada, S. and Chan, H.K., 2014. Nanodrugs: pharmacokinetics and safety. *International Journal of Nanomedicine,* 9, 1025–1037.

Ozioma, E.O.J and Chinwe, O.A.N., 2019. Herbal medicines in African traditional medicine. *Herbal Medicine,* 10, 191-214.

Pal, D., Sahu, C.K. and Haldar, A., 2014. Bhasma: the ancient Indian nanomedicine. *Journal of advanced pharmaceutical technology & research*, 5(1), 4-12.

Pandey, A. and Pandey, G., 2013. Usefulness of nanotechnology for herbal medicines. *Plant Archives,* 13(2), 617-621.

Pandiyan, I., Arumugham, M., Srisakthi, D. and Shanmugam, R., 2023. Anti-inflammatory and Antioxidant Activity of *Ocimum tenuiflorum*-and *Stevia rebaudiana*-Mediated Silver Nanoparticles: An In Vitro Study. *Cureus*, 15(12).

Parboosing, R., Maguire, G.E., Govender, P. and Kruger, H.G., 2012. Nanotechnology and the treatment of HIV infection. *Viruses*, 4(4), 488-520.

Patil, R.Y., Patil, S.A., Chivate, N.D. and Patil, Y.N., 2018. Herbal drug nanoparticles: advancements in herbal treatment. *Research Journal of Pharmacy and Technology,* 11(1), 421-426.

Patra, J.K. and Baek, K.H., 2015. Green nanobiotechnology: factors affecting synthesis and characterization techniques. *Journal of Nanomaterials*, 219-219.

Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.D.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S. and Habtemariam, S., 2018. Nano based drug delivery systems: recent developments and future prospects. *Journal of nanobiotechnology*, 16(1), 1-33.

Pillay, S., Pillay, V., Choonara, Y.E., Naidoo, D., Khan, R.A., du Toit, L.C., Ndesendo, V.M., Modi, G., Danckwerts, M.P. and Iyuke, S.E., 2009. Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain. *International journal of pharmaceutics*, 382(1-2), pp.277-290.

Pouris, A., 2007. Nanoscale research in South Africa: A mapping exercise based on scientometrics. *Scientometrics*, 70(3), 541-553.

Pouris, A., Pouris, A. and Buys, A., 2012. Nanotechnology and biotechnology research in South Africa: Technology management lessons from a developing country. *Technology Management for Emerging Technologies,* 1, 346–357.

Prasad, R., Bhattacharyya, A. and Nguyen, Q.D., 2017. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. *Frontiers in microbiology*, *8*, 1014.

Reeve, J., 2019. The british museum. In *Culture, Education and the State*. Routledge Publishers, London, UK. Pp. 65-93.

Roger, K. and El Amri, N., 2022. Controlling nanoparticle formation from the onset of nucleation through a multi-step continuous flow approach. *Journal of Colloid and Interface Science*, 608, 1750-1757.

Saidi, T. and Douglas, T.S., 2017. Nanotechnology in South Africa-Challenges in evaluating the impact on development. *South African Journal of Science,* 113(7-8), 1-2.

Saidi, T., 2018. Perceived risks and benefits of nanomedicine: a case study of an anti-tuberculosis drug. *Global Health,* 1(1), 6.

Salie, F. and Saidi, T., 2023. Nanomedicine drug delivery in South Africa: a retrospective study on research, funding and collaboration. *Frontiers in Pharmacology*, 14, 1317137.

Saraf, S., 2010. Applications of novel drug delivery system for herbal formulations. *Fitoterapia,* 81(7), 680-689.

Saravanan, M., Ramachandran, B., Hamed, B. and Giardiello, M., 2018. Barriers for the development, translation, and implementation of nanomedicine: an African perspective. *Journal of Interdisciplinary Nanomedicine*, 3(3), 106-110.

Schaming, D. and Remita, H., 2015. Nanotechnology: from the ancient time to nowadays. *Foundations of Chemistry*, 17, 187-205.

Shan, X., Gong, X., Li, J., Wen, J., Li, Y. and Zhang, Z., 2022. Current approaches of nanomedicines in the market and various stage of clinical translation. *Acta Pharmaceutica Sinica B*, 12(7), 3028-3048.

Sibuyi, N.R.S., Moabelo, K.L., Meyer, S., Skepu, A., Onani, M.O., Madiehe, A.M. and Meyer, M., 2022. Nanotechnology-based strategies for treatment of obesity, cancer and anti-microbial resistance: highlights of the department of science and innovation/mintek nanotechnology innovation Centre biolabels research node at the university of the western Cape. *Applied Sciences*, 12(20),10512.

Singer, P.A., Salamanca-Buentello, F. and Daar, A.S., 2005. Harnessing nanotechnology to improve global equity. *Issues in Science and Technology*, 21(4), 57-64.

Sobiesiak, M., 2017. Phenolic compounds-natural sources, importance, and applications. *IntechOpen*, 66537.

Taniguchi, N., Arakawa, C. and Kobayashi T., 1974. On the basic concept of nanotechnology. In Proceedings of the International Conference on Production Engineering, Tokyo, Japan, 26–29 August 1974.

Tauheed, A.M., Mamman, M., Ahmed, A., Sani, N.I.A., Suleiman, M.M., Sulaiman, M.H. and Balogun, E.O., 2021. Acute, sub-acute, sub-chronic and chronic toxicity studies of four important Nigerian ethnomedicinal plants in rats. *Clinical Phytoscience*, 7, 1-12.

Thapa, R.K. and Kim, J.O., 2023. Nanomedicine-based commercial formulations: Current developments and future prospects. *Journal of Pharmaceutical Investigation*, 53(1), 19-33.

Tilburt, J.C. and Kaptchuk, T.J., 2008. Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization*, 86 (8), 594-599.

Trivedy, A. and Khatun, M., 2023. Importance of BRICS as a regional politics and policies. *GeoJournal*, 88(5), 5205-5220.

Tsai, T., Pillay, V., Choonara, Y.E. and Du Toit, L.C., University of the Witwatersrand, 2013. Polymeric hydrogel compositions which release active agents in response to electrical stimulus. U.S. Patent Application 13/989,435.

Tshikhudo, R.T., der Van (Lingen, E.), Mdluli, P. and Sosibo, N., 2009. Gold Nanotechnology at Mintek. World Gold Conference 2009*, The Southern African Institute of Mining and Metallurgy*, 247 - 252.

Waheed, S., Li, Z., Zhang, F., Chiarini, A., Armato, U. and Wu, J., 2022. Engineering nano-drug biointerface to overcome biological barriers toward precision drug delivery. *Journal of Nanobiotechnology*, 20(1), 395.

Wang, E.C. and Wang, A.Z., 2014. Nanoparticles and their applications in cell and molecular biology. *Integrative biology*, 6(1), 9-26.

Wang, N. and Feng, Y., 2015. Elaborating the role of natural products-induced autophagy in cancer treatment: achievements and artifacts in the state of the art. *BioMed Research International*, 934207.

Worakunphanich, W., Suwankesawong, W., Youngkong, S., Thavorncharoensap, M., Anderson, C. and Toh, L.S., 2023. Thai stakeholders' awareness and perceptions of the patient adverse event reporting system for herbal medicines: a qualitative study. *International Journal of Clinical Pharmacy*, 45(2), 491-501.

Yuan, H., Ma, Q., Ye, L. and Piao G., 2016. The traditional medicine and modern medicine from natural products. *Molecules,* 21(5), 559.

Zavaleta, C., Ho, D. and Chung, E.J., 2018. Theranostic nanoparticles for tracking and monitoring disease state. *SLAS Technology: Translating Life Sciences Innovation*, 23(3), 281-293.

Zhang, J., Onakpoya, I.J., Posadzki, P. and Eddouks, M., 2015. The safety of herbal medicine: from prejudice to evidence. *Evidence-based complementary and alternative medicine*, 316706.

Zhang, J., Pan, Y., Shi, Q., Zhang, G., Jiang, L., Dong, X., Gu, K., Wang, H., Zhang, X., Yang, N. and Li, Y., 2022. Paclitaxel liposome for injection (Lipusu) plus cisplatin versus gemcitabine plus cisplatin in the first-line treatment of locally advanced or metastatic lung squamous cell carcinoma: A multicenter, randomized, open-label, parallel controlled clinical study. *Cancer Communications*, 42(1), 3-16.

CHAPTER 4

Phytochemical analysis and LC-MS metabolite profiling of *Lannea discolor* (Sond.) Engl. methanolic extracts

Abstract

Lannea discolor, commonly known as “dikbas", grows in the Limpopo region, distributed towards Zimbabwe and Zambia and it is often used in traditional medicine. Total phenols, flavonoids, and radical scavenging activities were determined in the leaves, stem, and roots methanol extracts. The chemical composition of phenolics (38.46 -266.75 mg GAE/g of dry mass), and flavonoids (82.28 - 324.23 mg QE/g of dry mass) was determined for the leaves, stem, and roots. The plant extracts were subsequently evaluated for their capacity to scavenge free radicals and the strongest activities were obtained from the stem and root extracts (IC_{50}= 0.16211 and 0.008441 µg/ml correspondingly) in comparison to the leaf extracts (IC_{50}=1.095126 µg/ml). Methanol extracts were used for the analysis of secondary metabolites using conventional phytochemical tests and LC-MS. Herein, a maximum of 77 compounds were tentatively identified by comparing the molecular formulae with those already published in various chemical databases. Moreover, metabolite databases such as KnapSack, PubChem and ChemSpider, and have been used to further validate the identity of the metabolites. Amongst the identified compound classes, flavonoids, fatty acids, phenolics and terpenoids were found to be predominantly occurring in the extracts. Specific phytochemical compounds such as Quinic acid, Gallic acid, and Quercetin derivatives were picked up after LC-MS. Furthermore, compounds not previously identified in *Lannea* such as Anacardoside, Decaffeoylacteoside, Brevifolincarboxylic acid, Tanacetol A, Laciniatoside I and Insuloside. The activities of the extracts were tested against *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus*, *Pseudomonas aeruginosa and Bacillus subtilis* and they demonstrated obvious antibacterial effects, with the stem and root extracts displaying remarkable activity with zones of 12 to 18 mm and MIC values of 1.56 – 12.5 mg/ml across all the microorganisms tested. The overall results demonstrated that *L. discolor* has significant potential source of diverse and novel molecules with high radical scavenging capacity and anti-bacterial activities, which could be an interesting material for advanced therapeutic purposes.

Keywords: Antibacterial, Antioxidant activity, flavonoids, *Lannea discolor*, LC-MS, phenolics

4.1 Introduction

Many medicinally used plants have exhibited their importance as a source of phytochemical components that possess therapeutic potential. They remain as a meaningful source of new pharmaceutical prospects. There has always been a push to find new drug candidates, and owing to this reason medicinal plants provide a lot of potential for research in the ongoing attempt to find new lead compounds for treating human illnesses (Newman et al., 2020). Natural compounds from plants are a viable alternative to synthetic medicines, with new studies focusing on the health-improving properties of phytochemicals (Stavrianidi, 2020; Tiwari et al., 2020; Atanasov et al., 2021). Accordingly, phytochemicals demonstrating countless biological properties have recently obtained substantial investigation interest (Atanasov et al., 2021). Liquid chromatography mass spectrometry (LC-MS) has facilitated the identification and structural analysis of diverse plant-derived chemicals on a scale not previously possible. Through continuous refinement of separation science and mass analysis, LC-MS promises to further improve our understanding of the multifaceted phytochemical profiles contained in plants around the world (Volpi and Bergonzini, 2006; Gardana et al., 2007; Falcão et al., 2010; Pellati et al., 2011). Because mass spectrometry has a relatively high sensitivity, it allows for the discovery of new minor phytochemicals that are difficult to uncover using traditional methods such as reagent-based phytochemical screening and thin layer chromatography. Additionally, further techniques include the characterisation of unknown substances without the use of chemical standards and the elucidation of structural information (Cuyckens and Claeys, 2004; Atanasov et al., 2021).

Liquid chromatography is as intricate as a separation system that separates liquid components according to their affinities, while mass spectrometry detects masses on a mass-to-charge basis. These two concepts are coupled together to come up with the accurate analysis of phytochemical compounds (Pang et al., 2016). LC-MS not only allows the organization of intricate groups of phytochemical compounds, but also gives information about the previously hypothesized compounds (Ignat et al., 2011; Atanasov et al., 2021). There are also several advantages to using LC-MS, such as assigning each peak to a specific molecular weight, providing the molecular formulae to the specific weights, and the quantitative data (Hollander et al., 2019). Nonetheless, comparing the amounts of thousands of metabolite peaks in one sample group to another in an untargeted manner has become rather conventional and would assist in acquiring an overwhelming chemical profile of a plant. For instance,

a number of compounds were tentatively identified in *Lannea stuhlmannii* and *Lannea humilis* bark and highlighting the first pioneer study on their compounds and confirming their traditional use (Sobeh et al., 2018). Furthermore, LC-MS analysis was carried out to generate information on the phytochemical compound in *Lannea microcarpa* berry extracts, wherein compounds from different phenolic compounds where identified, further corroborating the significance of the plant (Bello et al., 2022).

Lannea discolor is locally named as "muvhumbu" and has had an array of ethno-medical applications amongst the Venda speaking people of the Limpopo province. The plant contains a diverse range of phytochemicals which are accountable for its pharmacological activities (Clarkson *et al*., 2004; Kazembe *et al*., 2012; Maroyi, 2013). It is useful in the treatment of gastrointestinal infections, abscesses, and female reproductive issues, sexually transmitted infections, and wound care in different southern African countries (Hutchings et al., 1996; Van Wyk et al., 1997; Maroyi, 2013; Mølgaard et al., 2001; Fowler, 2002; Steenkamp, 2003; Bruchi et al., 2011; Maroyi, 2013; Chinsembu et al., 2015; Thomford et al., 2015). The presence of alkaloids, coumarins, flavonoids, polyphenols, tannins, triterpenes and saponins have been reported on a basis of both qualitative phytochemical screening as well as mass spectroscopic techniques, in different plants parts from other *Lannea* plants, apart from *L. discolor* (Table 6.1). There is a paucity of scientific information concerning the identity of *L. discolor's* chemical constituents. In this regard, this study was concerned with the (i) validation of the presence of phytochemicals, as well as the tentative identification of the constituents in the methanolic extracts of *L. discolor*, (ii) determination of biological activities of the extracts, (iii) the search of possible correlations between chemical compositions and bioactivities.

Table 4.1. Compounds historically isolated from some plants in the *Lannea* genus.

Plant species	Isolated compounds	Biological activities	References
Lannea acida	6,7,2,2-dimethylchromeno-8_, _-dimethylallyl Lanceolatin B and 7,2'-dimethoxy-4',5'-methylenedioxyflavone	Anti-diarrhoeal and anti-inflammatory, cytotoxic and anti-Mycobacterium tuberculosis H37Rv, oestrogenic activities and anti-osteoporotic potential	Sultana and Ilyas, 1986a; 1986b
Lannea coromandelica	, (2*R*,3*R*) -(+)-4',7-di-*O*methyldihydrokaempferol and (2*R*,3*R*) -(+)-4'-*O*-methyldihydroquercetin, quercetin derivatives	Antimicrobial, hypotensive, sporicidal, anti-diarrhoeal, hepatoprotective and antioxidant activities	Islam and Tahara, 2000; Nair *et al*., 1963; Sankara and Nair, 1971; Subramanian and Nair, 1971; Yun, 2012; Kaur et al., 2013; Rao et al., 2014; Majumder and Alam, 2018
Lannea edulis	vestitol, 5-methoxyvestitol, lotisoflavan,	*in vitro* mutagenic and antioxidant activity	Queiroz *et al*., 2003; Banda et al., 2018
Lannea grandis	Lanosterol, epicatechin, cyanidin	Anti-diarrheal and Sedative activity	Govindac *et al*., 1971. Sulochana *et al*., 1967; 1970. Sulochana and Sastry, 1968; Akhter et al., 2020
Lannea microcarpa	*Vitexin*, myricetin derivatives	Antibacterial, antioxidant, and anti-inflammatory activities	Pale *et al*., 1998; Picerno *et al*., 2006
Lannea nigratiana	epicatechin gallate, gallic acid, phenol glucoside, benzoic acids, lanneanol	antibacterial, cytotoxic activity	Kone et al., 2004; Kapche *et al*., 2007
Lannea welwitschii	hydroquinones, lanneaquinol, hydroquinones	antibacterial, antioxidant, antidiarrhoeal, analgesic and anti-inflammatory activity	Groweiss *et al*., 1997; Olatokunboh et al., 2010; Agyare et al., 2013; Osafo and Boakye, 2017
Lannea velutina	Catechin, procyanidin B-1	antimicrobial, larvicidal, molluscidal, antioxidant and 15-lipoxygenase inhibitory activities	Maiga *et al*., 2007; Bouare et al., 2013; Malu et al., 2022
Lannea alata	Lupeol, Lanneaflavonols, myricetin derivatives, betmidin, sitosterol	antibacterial and antioxidant activity	Okoth, 2014; Mbaoji and Nweze, 2020
Lannea rivae	Phenol glucosides, myricetin derivatives, epicatechin gallate, taraxerone, E-lutein,	cytotoxicity in human tumour cell lines; antibacterial and anti-inflammatory activity	Okoth, 2014; Yaouba et al., 2018;
Lannea schweinfurthii	3-[tridecyl] phenol, 3-[heptadecyl] phenol Lupenone, epicatechin, catechin, Rutin	antimicrobial and in vitro acetylcholinesterase inhibitory activity	Okoth, 2014; Yaouba et al., 2018
Lannea schimperi	3-[12'(*E*)-pentadecenyl] phenol, 5-[12'(*E*)-pentadecenyl]	anticoccidial activities	Okoth, 2014; Mikail et al., 2016; Mohammed and Mikail, 2019

4.2 Materials and Methods

L. discolor organs were collected in Vuwani, Limpopo, (23°09′42.9″ S 30°25′22.6″ E) in the summer of November 2018. The material was separately air-dried at ambient temperature, then cut into small pieces, ground to a coarse powder, and stored in sealed containers.

4.2.1 Preparation of plant extracts

Previously ground, the material was subjected to methanolic extraction at ambient temperature with consistent agitation for 9 days. The extractants were filtered with Whatman #1 filter at 3-day intervals for maximum extraction yield evaporated to complete dryness with a rotary evaporator and stored for further use.

4.2.2 Qualitative Phytochemical screening

Phytochemical groups were determined in the different samples using standard qualitative methods modified according to Thakuria et al. (2018).

(a) Tannins and phenols

A few drops of a 10% ferric chloride solution were mixed with 2 ml of the extract's solution. The appearance of a dark blue color indicated the existence of phenols and tannins.

(b) Alkaloids

An orange-red precipitate was generated when 1 ml of Dragendorff's reagent was added to 2 ml of extract, signifying the presence of alkaloids.

Wagner's reagent was combined in equal parts with two milliliters of each extract. The occurrence of reddish-brown precipitate signifies the existence of alkaloids.

(c) Terpenoids

Concentrated sulfuric acid (3 ml) was carefully added to the mixture of extract (5 ml) and chloroform (2 ml) to produce a layer. A reddish brown interface indicated the presence of terpenoids.

(d) Flavonoids

Two millilitres of extract was treated with concentrated sulfuric acid and observed for the formation of green to blackish colour.

(e) Coumarin test

A millilitre of 10% sodium hydroxide was added to 2 ml of extract. The development of a yellow color indicates the presence of coumarins.

(f) Saponins

Plant extract (1 ml) was diluted with 20 ml of distilled water, and the mixture was shaken in a graduated cylinder for fifteen minutes. A 1-centimetre-thick layer of foam revealed the presence of saponins.

(g) Cardiac Glycosides

For the Keller-Kiliani test, a drop of Ferric chloride, concentrated sulfuric acid, and glacial acetic acid were each added to 3 ml of extracts. A reddish brown layer at the junction of two liquids revealed the presence of cardiac glycosides.

(h) Carbohydrates

In a test tube, 1 ml each of Fehling's A and B were combined with 1 ml of extract and brought to a boil for five to ten minutes in a water bath. A brick red precipitate was observed for the presence of carbohydrates.

(i) Fats and oils

An ample amount of undissolved extract was sandwiched between two sheets of filter paper. Oil stains on the paper indicated the presence of fats and oils.

4.2.3 Quantitative Phytochemical screening

4.2.3.1 Estimation of total phenolic contents

The Folin–Ciocalteu reagent assay was conducted in triplicate to determine the phenolic content of each extract, using gallic acid as a standard (Herald et al., 2011; Akwu et al., 2021). An aliquot of 150 µl and 120 µl of 0.7 M Na_2CO_3 was added to 30 µl of each extract (240 µg/ml) from the different plant parts. The solutions were allowed to stand for approximately 30 minutes at ambient temperature.. A SpectraMax M3 spectrophotometer was used to determine absorbance at 765 nm and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight using the following formula.

$$C_{tp} = C * \frac{V}{m} \qquad (1)$$

Where, Total phenolic content (mg/g) in a GAE (gallic acid) equivalent is denoted as C_{tp}

C is Concentration of gallic acid as derived from the standard curve in mg/ml

V is the volume of *L. discolor* extract in **ml**

m is the mass of extract in **grams**

4.2.3.2 Estimation of total flavonoid contents

The total flavonoid content of *L. discolor* extracts was determined using the aluminium chloride colorimetric method adapted from Olajuyigbe and Afolayan (2011). An aliquot of 100 µl of each extract was added into wells of a 96-well plate. 100 µl of 1% aluminium chloride solution was added to each

well. Quercetin was used to generate a standard curve. The plate was made to stand at room temperature for an hour after which absorbance was measured at 420 nm using a microplate spectrophotometer. The total flavonoid content was calculated based on the quercetin standard curve and was expressed as quercetin equivalents (mg QE/g) of dry mass. The tests were carried out in triplicates.

4.2.4 Secondary metabolite profiling by Liquid Chromatography Mass Spectrophotometry

Metabolite extraction was carried out by dissolving 2 g of ground leaves, stems and root bark in 20 ml of 80% methanol (LC-MS grade). The mixture was centrifuged at 5000 xg for 20 minutes and the supernatant was decanted into a clean tube. The supernatants were further diluted with LC-MS grade methanol, to an absolute volume of 20 ml, filtered further using a 0.2 µm nylon filter into a 0.2 ml glass insert with a conical bottom, and fitted into a 2 ml vial. Mass spectrometry was used to monitor analyte elations in negative ionization using conditions previously described by Madala and Kabanda (2021). The compounds were identified by comparing the calculated m/z ranges of 100 – 1000 against the corresponding molecular formulae to those found in previous literature reports, and by searching the Knapsack Family (www.knapsackfamily.com), ChemSpider (www.chemspider.com), and Pubchem (www.pubchem.ncbi.nlm.nih.gov) databases.

4.2.5 Antioxidant Assay

4.2.5.1 *In vitro* antioxidant DPPH scavenging activity

The 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was determined using a modified version of the method outlined by Bhakya et al. (2016). Ascorbic acid served as a standard control. Briefly, 0.3 mM DPPH solution was mixed into 100 µl aliquots of each test sample in a 96-well plate. The plate was incubated in darkness for 30 minutes. The absorbance of each sample were then taken at 517 nm using a DPPH solution without any test sample as the blank control.

$$\text{Dpph Scavenging activity (\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

where $Abs_{control}$ is the absorbance of DPPH and methanol

Abs_{sample} is the absorbance of DPPH radical + methanol extract (or ascorbic acid standard)

The IC_{50} was calculated from the inhibition curves, which were plotted from the log(s) of the sample concentrations and the percentage inhibition.

4.2.6 Antimicrobial Assays

The following microorganisms were selected for testing: *Staphylococcus aureus* (ATCC 35923), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 15213), *Klebsiella pneumoniae* (ATCC 700603) were selected because some of the infections they cause can be treated with *L. discolor* based on a study by Mabona et al. (2013) . The bacteria were cultured on Mueller Hinton agar (MHA).

4.2.6.1 Sensitivity test

Bacterial inoculums were prepared by mixing a loop of bacteria into Muller Hinton broth (BMH) (10 ml) and adjusting the density to a 0.5 McFarland standard. Antibacterial activity was evaluated using the agar well diffusion method modified from Mamphiswana et al. (2013) and Kpegba et al. (2019). Mueller Hinton agar plates were plated with bacteria using a swab technique. Wells 8 mm in diameter were punched in the agar and filled with 50 µl of extract solution at a concentration of 50 mg/ml. The plates were allowed to stand 30 minutes to enable diffusion into the agar before incubation at 34°C for 24 hours. The diameter of inhibition zones around each well was measured to assess sensitivity according to Ponce et al. (2003). Sterile distilled water served as a negative control while gentamicin was used as a positive control for the bacteria. Tests were performed in triplicate.

4.2.6.2 Determination of Minimal Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) values of the extracts were determined using a sterile 96-well plate with resazurin as an indicator for cell growth. One hundred microliters of Mueller-Hinton broth were loaded into the first row of the plate followed by 100 µl of extract at a 10% dimethyl sulfoxide concentration, and serial dilutions were performed. Ten microliters of bacterial inoculum were added to each well, followed by 10 µl of resazurin solution and incubation overnight at 34 °C. Color changes in the wells were observed visually, where a change from blue to pink or clear indicated non-inhibition of bacterial growth. The MIC values were determined as the lowest concentration of extract resulting in color change. All experiments were performed in triplicates. Gentamicin (10 µg/ml) was used as a positive control.

4.3 Results and Discussion

4.3.1 Qualitative phytochemical screening

L. discolor leaves, stem and root bark have been widely used as a remedial treatment in Southern Africa; however, the active constituent in the plant that is responsible for its medicinal effectiveness remains to be determined. This study aimed to establish the pharmacological rationale for employing traditional remedies such as *L. discolor* extract in treating various ailments. This was based on preliminary studies reporting potent bioactive compounds of *L. discolor* extracts identified from the methanol extracts (Chakuma et al., 2015; Kabongo-Kayoka et al., 2016). The presence of various phytochemical compounds, which are extracted by solvents of diverse polarities, determine the medicinal properties of plants. For this study, methanol was used since it has been shown to extract metabolites of wider polarity (Chigayo et al., 2016; Altemimi et al., 2017; Newton et al., 2021). The reagent based phytochemical analysis of the extracts of the vegetative organs of *L. discolor* (Table 4.2) revealed the occurrence of various metabolites. However, the tests performed are basic and preliminary in nature; and therefore required the validation of other advanced instrumental analysis. Furthermore, the data also suggests the importance of a particular test employed also as a decisive factor for confirming the occurrence

compounds. Basic phytochemical screening of the extracts for their major compounds is vital as the active principles of many drugs are these significant metabolites found in plants. The occurrence of metabolites was observed in all the plant parts assessed. Similarly, the methanolic extract of the leaves of *L. welwitschii*, has demonstrated similar phytochemicals (Agyare et al., 2013). The detection of tannins, flavonoids, saponins and phenols agrees with the findings of Idowu and Idowu (2012) on *L. welwitschii*, and with Kone et al. (2011) on *L. barteri*. Consistent with the ethnopharmacological significance ascribed to related species, preliminary investigations indicate that the leaves and stem bark may exhibit therapeutic potential for the topical management of wound due to compounds that exert antimicrobial and anti-inflammatory activities (Maroyi, 2018). Coumarins are more pronounced only in the leaf extract but are barely detected in the bark. This phenomenon was similarly observed in research conducted by Idowu et al. (2020). It is hypothesized that these phytochemicals are potentially accountable for the documented biological properties reported in previous studies (Mabona et al., 2013; Chakuma et al., 2015; Kabongo-Kayoka et al., 2016).

Table 4.2. Showing the presence of different phytochemicals after reagent based preliminary phytochemical screening of methanol extracts of the leaves, stem bark and root of *L. discolor*.

	Leaves	**Stem**	**Root**
TANNINS and PHENOLS • **Ferric Chloride**	++ (Dark green – blackish color)	++ (Bluish – Blackish color)	++ (Blue – Black)
ALKALOIDS • **Dragendorff's**	++ (Deep green with orange precipitate)	++ (Deep brown coloration)	++ (Brownish precipitate)
• **Wagner's**	++ (Brownish precipitate)	++ (Brown precipitate)	++ (Red – brown precipitate)
TRITERPENOIDS • **Salkowski's**	++ (Brown Reddish ring at interface)	++ (Brownish ring at interface)	++ (Brownish ring at interface)
FLAVANOIDS • **Conc. Sulphuric Test**	++ (Deep green precipitate)	++ (Reddish -Brownish precipitate)	++ (Brown – Blackish precipitate)
• **Ammonia Test**	++ Yellowish – orange coloration	++ (Deep brown coloration)	++ (Dark brown coloration)
COUMARINS	++ (Yellow coloration)	++ (Yellow coloration)	++ (Yellow coloration)
SAPONINS • **Foam Test**	++ (Persistent foam)	-	-
CARDIAC GLYCOSIDES • **Keller-Killiani Test**	++ (Green – blue coloration)	++ (Bluish coloration)	++ (Deep greenish precipitate)
CARBOHYDRATES • **Fehling's Test**	++ (Brick Brown precipitate)	++ (Brick red precipitate)	++ Reddish precipitate
FATS & OILS	++	++	++

Present = ++; absent = -

4.3.2 Quantitative phytochemical screening

4.3.2.1 Effect of Extraction Parameters on Total Phenolic and Flavonoid Content

There was a consistent difference in the phenolic content between the plant parts extracted. The TPC within the extracts was extrapolated utilizing the regression equation derived from the standard calibration curve (Appendix Figure A1) of gallic acid (Y =0.0041x+ 0.1849 (R^2 = 0.9833) and expressed in gallic acid equivalents (GAE). Total phenolic content varied widely from 38.46±0.15 to 266.75 ±0.15 mg/g GAE. Interestingly, *L. discolor* showed a relatively high content of phenolic compounds. The TPC of the extracts differed from one extract to another, while the highest amount of phenolics was found in both the stem and root bark (172.4874±0.156 and 266±0.46 mg GAE/g of dried extract). TPC of the extracts showed the following order: roots > stem> leaves and are summarised in Table 6.3.

The observed results were consistent with those of Chakuma et al., (2015), where the TPC of the stem extract was higher than that of the roots. *L. discolor* leaves showed a TPC of 27±0.09 mg GAE/g in a study by Mwamatope et al. (2020), which is lower than that reported in this study for all the extracts tested. The elevated concentrations of phenolic compounds may account for the utilization of this species in addressing certain illnesses and their corresponding oxidative stress (Sherfi et al., 2016). Variation observed in the abundance of phenolic compounds amongst the plant parts could be because, other plants may have an abundance of specific metabolites at different vegetative stages (Mpofu et al., 2006; Sreelatha, and Padma, 2009). The variations could also be attributed to the season of harvesting as well as geographical areas, as it has been suggested that the presence and abundance of phenolics and certain phytochemical too, may also be determined by climates and seasons in which collection took place (Chepel et al., 2020; Mwamatope et al., 2020). There is constantly a heterogeneous distribution of phenols within the plant, making them distinct. The variability in this case was observed according to the plant part used in extraction (Hussein et al., 2019); thus, the vast variation between samples. A previous phytochemical study on several *Lannea* species documented the high phenolic content at a range between 79.46– 292.78 GAE/g. The results now provide evidence to the incident that genus *Lannea* is rich in an array of phenolics (Wamuyu et al., 2020).

A standard curve was generated using the compound quercetin (Appendix Figure A2). The total flavonoid content was then quantified by equation (Y = 0.0038x + 0.0063 (R^2 = 0.9947) obtained from said standard curve and expressed in quercetin equivalents (QE). The leaf extract (Table 4.3) contained the lowest at (82.28± 0.20 mg QE/g), followed by the root (324.23±0.20), then the stem (385.26±0.20 mg QE/g). Maximum flavonoids were found to be abundant mostly in the stem extract, compared to leaf and root as shown in Table 4.3. The results of the extracts revealed high total flavonoid content, consistently like that reported in literature (Kumar and Jain, 2015). Flavonoids are secondary metabolites with antioxidant activity and are the most prevalent group of compounds and are extensively distributed throughout the *Lannea* species (Okoth et al., 2014; Okoth et al., 2016; Ogunsina,

2020). Plants that contain flavonoids have been used as protectants against cardiac diseases and are also associated with the treatment of malignant tumours and growths (Sherfi et al., 2016). In a study by Guerrero et al. (2012), several flavonoids were evaluated in *in vitro* and were found to have the ability to inhibit Angiotensin converting enzyme (ACE), confirming their significance in the relaxation of arteries to lower blood pressure, thus curbing cardiovascular complains. Several studies on flavonoids and their derivatives have shown vast biological activities (Montoro et al., 2005; Ekalu et al., 2020). This result ties well with previous studies wherein significant amounts of flavonoid were found in plants of the genus *Lannea* (Mwamatope et al., 2020; Njinga et al., 2021). When comparing our findings to those of previous studies, it must be emphasised that, since different extraction methods were used, variation of the total flavonoid content differs greatly, depending on genetic diversity, biological, environmental, seasonal and climatic variations (Mpofu et al., 2006; Uma et al., 2015; Aryal et al., 2019; Alizadeh et al., 2022).

Table 4.3 Quantification of Total Polyphenol and Flavonoid Contents of *L. discolor*.

Samples (extracts)		Total Phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Methanol	Leaves	38.4663± 0.156	82.2892±0.203
	Stem	172.4874±0.156	385.2632±0.203
	root	266.7561±0.156	324.2368±0.203

All values were expressed as the mean of triplicates ± standard deviation (SD).

4.3.2.2 LC-MS Metabolite Profile of *Lannea discolor* Extracts

LC–MS is a powerful tool for natural product analysis, and its sensitivity approach allows for the discovery of new minor constituents that are difficult to obtain through conventional methods. Tandem mass spectrometry can be used to acquire more detailed structural information, allowing the characterisation of unknown compounds even without the use of standards (Cuyckens and Claeys, 2004), however, this was not specifically investigated in the present study. The chemical evaluation of phytochemicals conveniently derives a plant's chemical profile, thus reinforcing the quest for novel compounds that may be used to advance drug discovery (Lu et al., 2008). Chemical profiling has been shown to be a valuable tool in phytochemical studies and can be used to authenticate a plant sample to determine the accuracy of its identity (Li et al., 2008; Rasheed et al., 2012). In drug discovery, LC-MS is highly commendable, as it assists in determining the phytochemical contents of the plant medicine, furthermore, it has significant uses in the quality control of plants from which medicine is derived. (Riswanto et al., 2022). Data regarding the *L. discolor* phytochemical profile has not been sufficiently reported in literature. Therefore, studies regarding the phytochemical profile are required as they can contribute to research aimed at analysing the relation between phytochemicals and diseases. In addition, they can facilitate the elucidation of new products and with wider benefits (Farag et al., 2012; Schulze-

Kaysers et al., 2015). Considering purported benefits to human beings, and the drift for natural phytochemicals, there is a need for steady exploration for the sources of certain phytochemicals acquired within the utilisation of *L. discolor*. Consequently, the vegetative organs were extracted, and their phytochemical compounds identified using LC-MS. Studies are available for targeted assays of various compounds from extracts of *L. discolor* but extracts were never subjected to LC-MS to attain a comprehensive phytochemical profile. In the present study, *L. discolor* extracts underwent LC-MS analysis for the tentative identification of major phytochemical compounds in these preparations. A total of 77 metabolites were tentatively identified by matching the accurate masses spectral data with the database established as well as some known and previously identified compounds. However, some of the compounds have been described previously (Kapche et al., 2007; Engels et al., 2012; Okoth et al., 2016).

The compounds separated by the Liquid Chromatography were identified based on their molecular formula and molecular weights. The LC–MS analysis of these extracts was done in negative ion electro-spray ionisation mode as it was found to be better for identification of a greater number of compounds. Using multiple online databases, including KNApSAcK (http://kanaya.naist.jp/KNApSAcK/) and Pubchem ((www.pubchem.ncbi.nlm.nih.gov) and Chemspider (https://www.chemspider.com/), in-house libraries based on standards, as well as the mass spectra information within the clustered mass peaks. Figures 4.1 - 4.3 show base peak chromatograms (BPC) obtained during the analysis of *L. discolor* extracts. The molecular structures of various major peaks of compounds from *L. discolor* are shown in Figures 4.4 and 4.5. While the phytochemicals, which contribute to the activities of the plant are shown in Tables 4.4 – 4.6. Selected metabolites were manually annotated and tentatively identified as far as was possible by comparing the accurate mass and molecular formulae with metabolites previously found on the online databases as well as in literature.

The LC-MS investigation of *L. discolor* exposed the presence of an array of recently identified compounds that are commonly found in angiosperms, Anacardiaceae family and *Lannea* genus at large. By assessment of the retention times, molecular formulae, and mass to charge ratio (m/z), a total of 77 compounds were discovered in all plant samples, which includes 10 phenols, 21 flavonoids, 4 iridoids, 21 fatty acids, 4 carboxylic acids, 9 terpenoids, 2 tannins, 1 coumestan, 4 carboxylic acids, 3 lignan glycosides and 1 lactone. The first compound to emerge was Quinic acid for all the plant parts, while the last compound to emerge was Oleic acid, and Maslinic acid for the leaf, stem, and root extract respectively. This observation is typical for many plants extracts in a reverse column chromatography system where polar compounds elute earlier, and non-polar compounds elute late. The extracts produced peaks mainly within the 0.5 to 48 min range of the 53-minute-long chromatogram with several pronounced peaks. This distribution of peaks reveals that the extract is composed different phytochemical compounds of different polarities. Furthermore, it validates the successful extraction and separation of the different phytochemical metabolites within the extracts. The masses obtained through

the observed m/z ratios were compared to an online database using the software and from there, several compounds were tentatively identified from the *L. discolor* sample. Of the compounds detected, Quinic acid, Gallic acid, Quercetin glucuronide, O-Methoxy myricetin 3-O-α-L-rhamnopyranoside, Myricetin 3-O-glucuronide, Quercetin-3-galactoside, Myrecitin-3-xyloside, Oleic acid, Epicatechin, Procyanidin B2 and Linoleic acid are widely reported in different plants as well as in family Anacardiaceae (Farag, 2008). An occurrence of other compounds which are not found in *Lannea* were detected, such as iridoids, and terpenoids and lignin glycosides.

The major peak in the leaves at m/z 631 and retention time 11.50 was highly intense but was absent in the stem and root bark. According to literature data the structure of the corresponding compound is Myricetin-O-(O-galloyl)-hexoside, which is a flavonoid possibly responsible for some of the biological properties described for most plants (Saldanha et al., 2013). Other major peaks were established to be flavonoids, which are likely to be responsible for inferring anti-tumor, antileukemic, antiviral and antibacterial activities (Saddiqa et al., 2017; Nitiéma et al., 2019; Shabir et al., 2021). Several intense peaks observed at *m/z* 533, and *m/z* 311 in the stem were also perceived to be present in the root bark along retention times 1.56 and 46.50. The structures of the compound corresponding to these peaks were tentatively identified as Quinic acid which is classified as a tannin (Sobeh et al., 2018; Costa et al., 2020), and Octadeca-9-ene-1,18-dioic-acid which is classified as a fatty acid. Quinic acid occurs in most plants and are advantageous for the derivation of other tannins. The tannin-containing plants are largely used as astringents against diarrhoea, and diuretics, and have become increasingly important for the development of novel pharmaceuticals against modern deadly illnesses, making it an exceptional drug candidate (Mangla et al., 2021).

Multiple peaks at *m/z* 577, *m/z* 575 and *m/z* 865 were observed in both the stem and root bark and indicated Procyanidin. Similarly, a series of Procyanidins have been found in the stem and root bark of *L. discolor* (Maiga et al., 2007, Sobeh et al., 2018; Ogunsina, 2020). While no additional exhaustive phytochemical analyses have been conducted on *L. discolor* itself, prior investigations have documented the presence of proanthocyanidins in other *Lannea* species. (Sultana and Ilyas, 1986; Islam et al., 2002; Queiroz et al., 2003; Okoth, et al., 2013; Okoth et al., 2016). Procyanidins belong to a group of flavonoids and have been particularly investigated due to their potential in immunity boosting effects observed in *in vitro* and in animal models (Patanè et al., 2023). Most prominently, they have shown an important capability to scavenge reactive oxidative and nitrogen species (Faraone et al., 2020). It can be suggested that procyanidin in *L. discolor* bark and root explain the high flavonoid content and its activity as an active in mitigating infections (Mabona et al., 2013; Chakuma et al., 2015). The compound procyanidin may also be of relevance to the ethno-pharmacological use of these plants to counter wound infections, bacteria caused gastric complains, and diarrhoea (Cires et al., 2017; Maltarud, 2017).

The commonly detected compounds include a myriad of phenols and phenolic glycosides. Precursor ion at *m/z* 169 in the leaves appeared at 1.29 minutes and was indicated to be Gallic acid and its occurrence has been reported widely *Lannea* species, *Lannea corommandelica*, *Lannea microcarpa* (Alam et al 2017; Mangla et al., 2021). Noticeably a precursor iron m/z 447 at retention time 1.56 in the stem and root extracts and allocated the name Anacardoside, which was first isolated and identified in *Semecarpus anacardium*, by Gil (1995) and has been branded a major active constituent in common traditional medicine, with beneficial biological and pharmacological activities (Lv et al., 2019). Among the characteristic phenols as observed at family and genus level, peaks m/z 329 and m/z 343 which indicated ellagic acid (m/z 300) derivatives, 3,3'-di-O-methylellagic acid and 3,3'4'-tri-O-methylellagic acid were observed in the leaf extract and have also observed in *Lannea edulis* (Kougan et al., 2013; Schulze-Kaysers et al., 2015). The presence of these two compounds seems to be previously reported only in one *Lannea species*, and presently in *L. discolor*. Protocatechuic acid, observed at *m/z* 153 in the stem bark, is a widely distributed compound in plants as well as in Anacardiaceae, and is considered an active component in western complementary medicine (Umadevi et al., 1988; Schulze-Kaysers et al., 2015; Jain et al., 2020). Protocatechuic acid was confirmed to have the ability to reduce inflammation in *L. coromandelica* and *L. acida* (Yun et al., 2014; Owusu et al., 2017). Phenols have been assumed to be characteristic of the Anacardiaceae, particularly genus *Rhus* and *Lannea*. Some of these phenolic compounds observed in the family have demonstrated exceptional activities against bacteria, cancer cells, malaria and oxidative stress within cells (Sherfi et al., 2016), subsequently validating their activity and possible significance as chemo-markers at either family or genus level (Okoth et al., 2016). Decaffeoylacteoside (m/z=461.1667; Rt=11.08 min) has also been isolated from *Aquilaria malaccensis* and observed to possess antioxidant capacity (Azhar et al 2021). Decaffeoylacteoside has been utilised in traditional Chinese medicine to decrease heat from blood, thus improving circulation, and to disintegrate agglomerated blood cells (Wagner et al., 2004). Brevifolincarboxylic acid is a known phenolic acid that has been isolated from a few plants and has been stated to infer anticancer and analgesic activities (Jaing et al., 2008; Lee at al., 2021).

The flavonoid compounds identified in the plant, either in combination or singly may be accountable for the antioxidant and hepato-protective effects possessed by the compounds in the various extracts (Mbaoji et al., 2020). They are also cancer prevention agents and may have potential in the mitigation of degenerative infections (Achika et al., 2017). Flavonoid constituents present in the leaves, Quercetin glucuronide, Myricetin-O-(O-galloyl)-hexoside, Myricetin 3-O-glucuronide Quercetin-3-galactoside Myrecitin-3-xyloside, and quercetin - 3 - O - β - D – xylopyranoside can infer antioxidant activity through inducing the movement and proliferation of neural stem cells, thereby treating diseases such as Alzheimer's (Ho et al., 2013; Costa et al., 2020; Muñoz-Ramirez et al., 2020). Additional compounds, namely Kaemferol hexoside, Apigenin, Amentoflavone, have been identified as flavonol glycosides and reported to have antioxidant, anti-hepatotoxic, antibacterial activity (Kwang et al., 2013; Vallverdū-

Queralt et al., 2015; Mousa et al., 2019; Geduk et al., 2021; Elmongy et al., 2022). Patuletin glucoside, found in the leaves is reported to be a rare flavonoid found in *Tagetes patula* observed to have significant healing properties against inflammation and arthritis when tested in a rodent model study by Jabeen et al., (2016). According to the previous studies the pharmacological activity of the extracts of *L. discolor* revealed a high amount of phenolic flavone which is responsible for antifungal, antibacterial, analgesic, nematicidal, anthelmintic and cytotoxic properties (Mølgaard et al., 2001; Clarkson et al., 2004; Mabona et al., 2013 and Kabongo-Kayoka et al., 2016).

The fatty acids present include linoleic, octadecanoic and hexadecanoic acids and their derivatives. These compounds are said to assist in prostaglandins which may be responsible for cardiovascular and anti-inflammatory activity in gynaecological complains (Jandacek et al., 2017; Mohale et al., 2020). Fatty acids also found in *Lannea microcarpa*, namely, oleic acid, palmitic, linoleic acid, and stearic acid such as the ones observed in this study, which are the most frequently used in cosmetic products as they provide moisture for the skin, aid in the healing process of dermatoses and sunburns (Bazongo et al., 2014; Ouilly et al., 2017; Warra, 2019). The same have been identified in *Lannea coromandelica* bark (Yun et al., 2014) and were observed to be similarly prevalent in the stem and root bark of *L. discolor*. These have been said to have potential in the production of cosmetics and to be significant in the food manufacturing industry (Warra, 2019; Mahdavi et al., 2021). Confirming the use and potential of *L. discolor* for traditional cosmetics. Some of the components such as hexadecanoic acid have also been reported in the methanolic leaf extract of *Lannea kerstingii* (Ibibia et al., 2016). In *L. discolor*, the n-hexadecanoic acid (palmitic acid) at *m/z* 255 (Rt = 48.547) and its isomer, 16-Hydroxy-hexadecanoic acid at m/z 271 (Rt= 46.21) found in the leaves has been documented to possess anti-inflammatory and antitoxic activity (Aparna et al., 2012; Ibibia et al., 2016; Ravi et al., 2017). Oleic acid, detected at m/z 281 (Rt = 48.73) has been documented to be and anti-inflammatory and antioxidant agent and has been named as one of the agents used to reduce harmful triglycerides (Olufunmilayo et al., 2017). The fatty acids observed and annotated in *L. discolor* have been reported to possess antibacterial, antimycobacterial, antioxidant, hypocholesterolemic, anti-inflammatory and cancer-preventive activity (Ahmad, et al., 2022) and have also been observed to appear in most kernel bearing plants of the Anacardiaceae family. These studies have also reported antioxidant and antibacterial activities for *Lannea nigritiana*, *Mangifera indica* and *Lannea rivae*, due to the identified compounds (Abiodun et al., 2020; Owolabi et al., 2020; Silva et al., 2020; Mahdavi et al., 2021).

All the plant organs contain a variety of terpenes triterpenes, sesquiterpenes and terpenes for which a wide variety of pharmacological and therapeutic activities have been documented (Soltane et al., 2021). Commonly known Maslinic acid at *m/z* 471 has potential as an anti-tumor, anti-inflammatory, cytotoxic and anti-malarial agent (Moneriz et al., 2011; Lozano-Mena et al., 2014; Yap et al., 2015). Maslinic acid has also been showed to have cytotoxic activity in some cancer cells and induces the apoptosis and inhibits the spread of the cancer cells (Tong et al., 2004; Zhu et al., 2023). The compound has also been

reported to be a potential anti-covid drug as it impairs the viral replication of SARS-CoV-2 (Soltane et al., 2021). Pulicanol (*m/z* 311), a sesquiterpenoid derivative has also been reported to show cytotoxicity on the human leukemia cell line HL-60 (Triana et al., 2005). 6-Deoxydestigloylswietenine has been isolated earlier from the leaves of *Rhododendron alutaceum* and further identified as dihydrobuddlenol B (Li et al., 2012), which was previously isolated from the bark of *Prunus jamasakura* (Yoshinari et al., 1990). Other terpenoids, such as ent-17-Nor-3,8-dioxo-13Z-labden-15-oic acid which was originally isolated from *Ayapana amygdalina* (Bohlmann et al., 1979), has also been observed in the stem extracts of *L. discolor*. In the leaf and stem extract, Tanacetol A, previously identified in higher plants of *Tanacetum vulgare* was tentatively identified (Appendino et al., 1983), and is reported to possess antimicrobial, *in vivo* antitumor, antioxidant and anti-inflammatory activity in other forms (Stranska-Zachariasova et al., 2017).

Though some compounds may be new findings in the genus *Lannea*, they have been identified and isolated in other species outside the family. An extensive phytochemical screening was carried out on the *Lannea* genus in a study by Umadevi et al., (1998) where in iridoid glucosides were perceived to be absent. However, in this study, they were detected and furthermore, tentatively identified in the stem bark. Particularly, iridoid glucosides, Laciniatoside I and Insuloside have been initially isolated from *Pterocephalus hookeri* and *Cephalaria gaziphashensis* (Sarikahya et al., 2012; Wu et al., 2014) and *Fraxinus insularis* (Tanahashi et al., 1998). Furthermore, Melittoside was reported, from *Melittis melissophyllum* (Venitti et al., 2016) and *Stachys* sp. (Meremeti et al., 2002). Iridoid glucosides that were detected in the stem bark are said to be characteristic for the treatment of cancer and inflammations and neuroprotective activity (Wu et al., 2013). In other folk medicine, they are used as bitter tonics for hypertension and as sedatives, (Tundis et al., 2008) thus validating the use of the plant in southern Africa for convulsions and epilepsy (Sobeicki, 2002). More of these include 6, alpha-Hydroxyforsythide dimethyl ester, which has been isolated in earlier studies, from *Eremostachys glabra* (Delazar et al., 2004).

Numerous bioactive compounds similar to the ones in this study have been identified and isolated from different *Lannea* species. Okoth et al. (2014) identified and isolated the highly antioxidant arabinofuranoside and rhamnopyranoside from the root of *Lannea alata*. Rhamnopyranoside was also identified in the root of *L. discolor*, thus, validating the use the *L. discolor* roots as an antibacterial agent (Steenkamp, 2003). Other *Lannea species* such as *Lannea microcarpa*, *Lannea schweinfurthii*, are composed of many chemical compounds such as epi-catechin, gallic acid, myricetin in the root extracts (Yaouba et al., 2018; Mangla et al., 2021). Catechin derivatives, Epicatechin, Epicatechin gallate, and Catechin 3-O-alpha-L-rhamnoside were generally observed to be present in the stem and root extracts. Catechins have garnered attention for their noteworthy aptitude to sequester diverse free radicals through disparate pathways. Indirectly, catechins may enact antioxidant impacts through upregulating the concentrations of intrinsic antioxidants such as glutathione reductase, and catalase, thus

demonstrating exceptional antioxidant potential (Sobeh et al., 2017). Known widely in Chinese medicine, Sophoracoumestan A was identified in the root extracts and has been previously isolated from the extract of *Psoralea corylifolia*. This compound is known as a coumestan that acts as a phytoestrogen and has been deemed significant as an effective invigorant against, menstruation disorder and uterine haemorrhage, similarly, *L. discolor* roots have been used to treat excessive menstruation (Chakuma et al., 2015).

The identification of these metabolites corroborates prior accounts denoting that numerous plants within the same family as *L. discolor* comprise a considerable proportion of analogous phytochemical constituents. Their appearance is much expected as past studies have attributed the plants properties, predominantly to them (Achika et al., 2017).

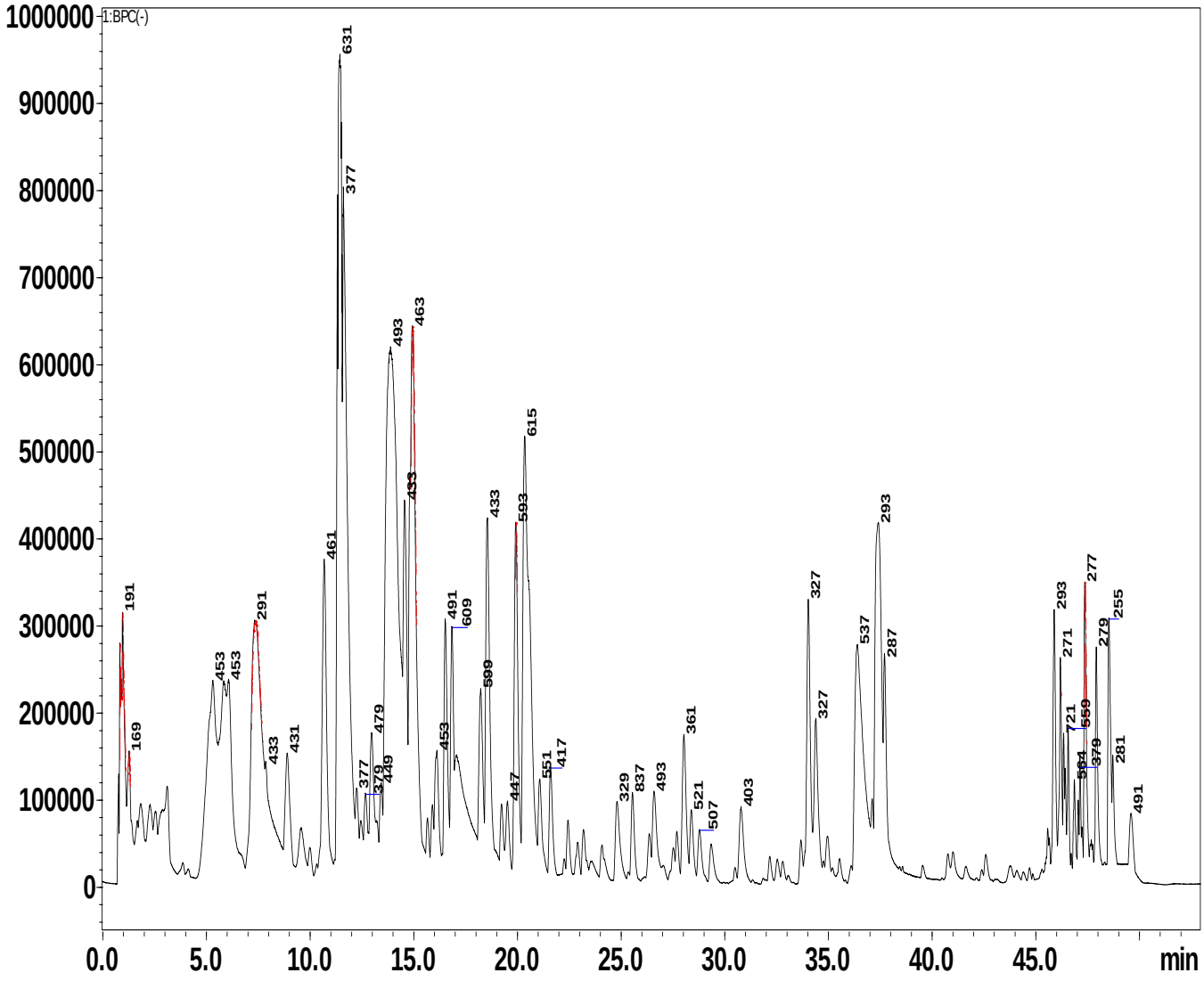


Figure 4.1. Peaks and the corresponding compounds detected in the leaves of *L. discolor* in the negative mode of ionisation.

Table 4.4. Compounds tentatively detected in the leaf extract of *L. discolor*.

Retention time	[M-H]- (m/z)	Molecular formula	Tentative assignment
0.74	191. 05611	$C_7H_{12}O_6$	Quinic acid (Tannin)
1.297	169.01407	$C_7H_6O_5$	Gallic acid (Phenolic acid)
4.95	477.06946	$C_{21}H_{18}O_{13}$	Quercetin glucuronide (flavonoid)
5.050	453.20000	$C_{19}H_{34}O_{12}$	Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside] (glycoside)
5.183	453.2000	$C_{19}H_{34}O_{12}$	Butyl (S)-3-hydroxybutyrate [arabinosyl-(1->6)-glucoside] (glycoside)
7.83	291.01559	$C_{13}H_8O_8$	Brevifolincarboxylic acid (phenolic)
10.712	461.16862	$C_{20}H_{30}O_{12}$	Decaffeoylacteoside (phenolic)
13.66	477.10590	$C_{22}H_{22}O_{12}$	0-Methoxy myricetin 3-O-α-L-rhamnopyranoside (flavonoid)
14.39	493.064484	$C_{21}H_{18}O_{14}$	Myricetin 3-O-glucuronide (flavonoid)
15.922	463.09060	$C_{21}H_{19}O_{12}$	Quercetin-3-glucoside (flavonoid)
14.84	449.21003	$C_{20}H_{18}O_{12}$	Myrecitin-3-xyloside (flavonoid)
18.59	433.07954	$C_{20}H_{18}O_{11}$	quercetin - 3 - O - β - D – xylopyranoside (flavonoid)
20.13	447.09528	$C_{21}H_{20}O_{11}$	Kaempferol hexoside (flavonoid)
21.53	593.15451	$C_{20}H_{34}O_{20}$	Apigenin-6,8-C-diglucoside (flavonoid)
22.28	587.14300	$C_{28}H_{28}O_{14}$	Dalmaisione A (Flavonoid)
23.21	475.09017	$C_{22}H_{20}O_{12}$	Kaempferol-6″-methyl-glucuronide (flavonoid)
24.93	329.03132	$C_{16}H_{10}O_8$	3,3'-di-O-Methylellagic acid (phenol)
25.3	300.99981	$C_{14}H_6O_8$	Ellagic acid (phenol)
25.36	493.23080	$C_{22}H_{38}O_{12}$	Patuletin glucoside (flavonoid)
26.72	343.04670	$C_{17}H_{12}O_8$	3,3',4'-tri-O-methylellagic acid (phenol)
34.055	327.71922	$C_{18}H_{32}O_5$	9S,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid (fatty acid)
36.49	537.08511	$C_{30}H_{18}O_{10}$	Amentoflavone (biflavonoid)
36.52	329.23432	$C_{18}H_{34}O_5$	Tianshic acid 9S,12S,13S-Trihydroxy-10E-octadecenoic acid (fatty acid)
37.53	293.17692	$C_{17}H_{26}O_4$	Tanacetol A (sesquiterpenoid)
37.72	287.22380	$C_{16}H_{32}O_4$	10,16-dihydroxy hexadecanoic acid - palmitic acid (fatty acid)
42.617	311.18718	$C_{17}H_{28}O_5$	Pulicanol(sesquiterpenoid)
45.51	313.23970	$C_{18}H_{34}O_4$	9,10-Epoxy-18-hydroxy-octadecanoic acid (epoxy fatty acid)
45.91	293.21336	$C_{18}H_{30}O_3$	Colneleic acid (fatty acid)
46.21	271.22881	$C_{16}H_{32}O_3$	16-Hydroxy-hexadecanoic acid-palmitic acid (fatty acid)
47.38	277.21836	$C_{18}H_{30}O_2$	9,12,15-octadecatrienoic acid, (Z, Z, Z)- (fatty acid)
47.412	283.26510	$C_{18}H_{36}O_2$	Octadecanoic acid (fatty acid)
47.93	279.23381	$C_{18}H_{32}O_2$	Linoleic acid 9,12-Octadecadienoic Acid (Z, Z)-(fatty acid)
48.54	255.23397	$C_{16}H_{32}O_2$	n-hexadecanoic acid Palmitic acid (fatty acid)

48.73	281.24958	$C_{18}H_{34}O_2$	Oleic acid, 11-Octadecenoic acid (fatty acid)

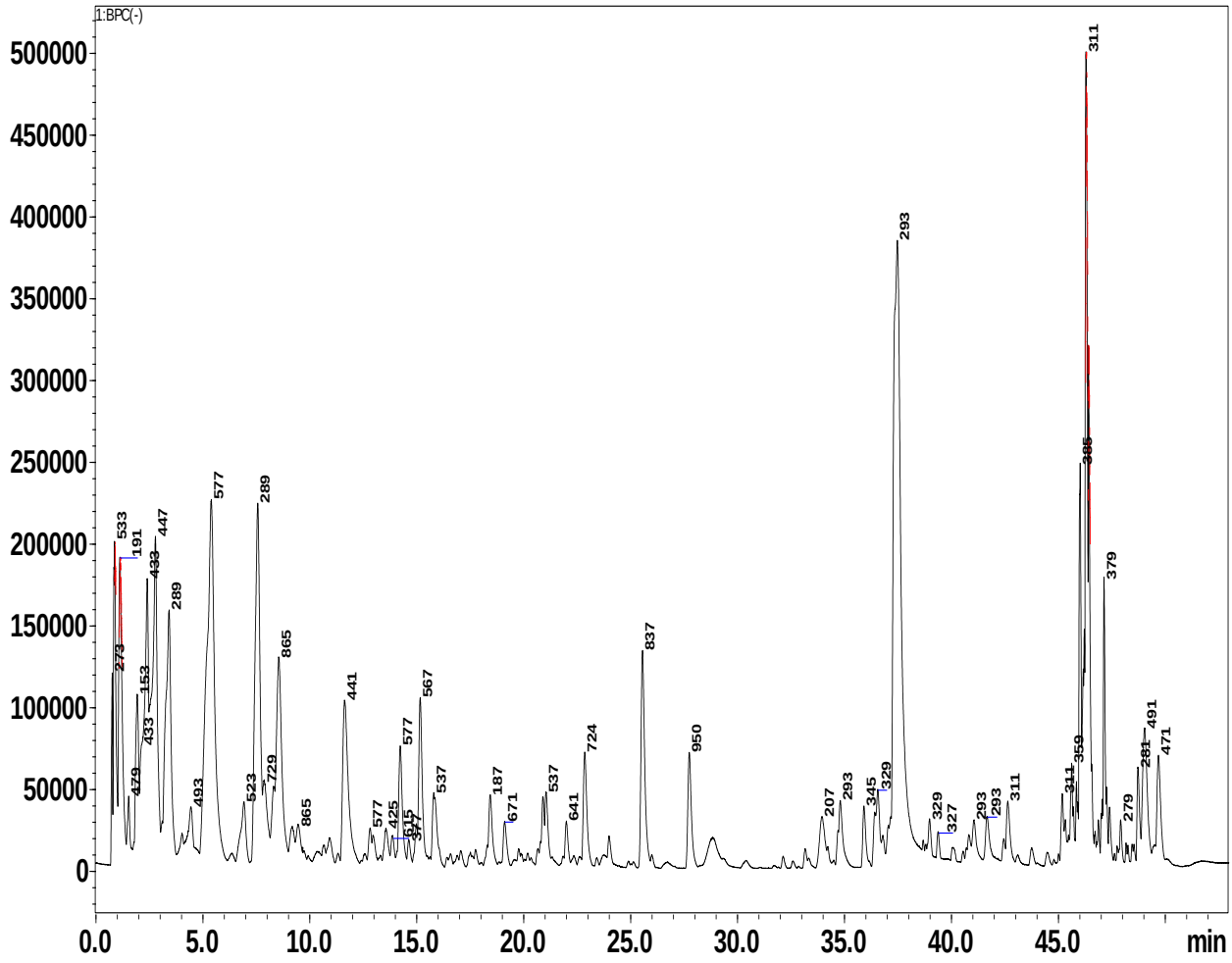


Figure 4.2. Peaks and the corresponding compounds detected in the stem bark of *L. discolor* in the negative mode of ionisation.

Table 4.5. Compounds tentatively detected in the stem bark extract of *L. discolor*.

Retention time	**[M-H]- (m/z)**	**Molecular formula**	**Tentative assignment**
0.895	533.17394	$C_{19}H_{34}O_{17}$	Quinic acid (tannin)
0.917	377.08662	$C_{18}H_{18}O_9$	Methoxymatteucin (flavanone)
1.147	191.01980	$C_6H_8O_7$	Citric acid (tricarboxylic acid)
1.552	479.14198	$C_{19}H_{28}O_4$	ent-17-Nor-3,8-dioxo-13Z-labden-15-oic acid (Triterpenoid)
1.558	433.13653	$C_{18}H_{26}O_{12}$	6 alpha-Hydroxyforsythide dimethyl ester (iridoid)
1.958	153.01901	$C_7H_6O_4$	Protocatechuic acid, (phenol)

2.591	447.15243	$C_{19}H_{28}O_{12}$	Anacardoside (phenol)
3.428	289.07221	$C_{15}H_{14}O_6$	Epicatechin, (flavonoids)
4.563	493.15800	$C_{20}H_{30}O_{14}$	Butanedioic acid (carboxylic acid)
5.517	577.13748	$C_{30}H_{26}O_{12}$	Procyanidin B4 (flavonoids)
7.015	523.16917	$C_{21}H_{32}O_{15}$	Melittoside (iridoid)
7.532	289.07229	$C_{15}H_{14}O_6$	Epicatechin, (flavonoids)
11.213	585.22180	$C_{27}H_{38}O_{14}$	Laciniatoside I (bis-iridoid glucoside)
11.573	441.08402	$C_{22}H_{18}O_{10}$	Epicatechin gallate (flavonoid)
12.847	577.13690	$C_{30}H_{26}O_{12}$	Procyanidin B2 (Flavonoid)
13.588	425.08907	$C_{22}H_{18}O_9$	Epiafzelechin 3-O-gallate (flavonoid)
14.255	577.13635	$C_{30}H_{26}O_{12}$	Procyanidin B2 (flavonoid)
15.01	781.27458	$C_{40}H_{46}O_{16}$	Insuloside (Iridoids and secoiridoidS)
15.188	567.21065	$C_{27}H_{36}O_{13}$	Citrusin B (lignan glycosides)
15.822	537.19961	$C_{26}H_{34}O_{12}$	(+)-Cycloolivil 4'-O-beta-D-glucopyranoside (lignan glycosides)
18.506	187.09737	$C_9H_{16}O_4$	Oxalic acid (carboxylic acid)
18.891	575.12169	$C_{30}H_{24}O_{12}$	Procyanidin dimer type A (flavonoid)
19.231	671.23748	$C_{34}H_{40}O_{14}$	R-Myricanol 5-O-beta-D-glucopyranoside (phenol)
22.32	641.24814	$C_{30}H_{42}O_{15}$	Sabphenoside D (Phenolic)
28.48	343.21339	$C_{18}H_{32}O_2$	Linoleic acid, (Z,Z)-9,12-Octadecadienoic acid (fatty acid)
34.134	207.13947	$C_{13}H_{20}O_2$	Prosopidione (terpenoid diketone)
34.563	419.26540	$C_{21}H_{40}O_8$	Fusartricin (sesquiterpenoid)
36.519	329.23404	$C_{18}H_{34}O_5$	Tianshic acid (fatty acid)
37.524	293.17656	$C_{17}H_{26}O_4$	Tanacetol A (sesquiterpenoid)
39.489	327.21828	$C_{18}H_{32}O_5$	9S,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid (fatty acid)
42.583	311.22346	$C_{18}H_{32}O_4$	Octadeca-9-ene-1,18-dioic-acid (fatty acids)
46.015	315.25489	$C_{18}H_{36}O_4$	9,10-Dihydroxystearic acid (fatty acid)
46.045	385.26097	$C_{21}H_{38}O_6$	Rangiformic acid (carboxylic acid)
46.0577	285.20797	$C_{16}H_{30}O_4$	1,16-Hexadecanedioic acid (fatty acid)
46.19	293.21297	$C_{18}H_{30}O_3$	9-Oxo-10(E),12(E)-octadecadienoic acid (fatty acid)
46.237	295.22845	$C_{18}H_{32}O_3$	12-oxo-10Z-octadecenoic acid (fatty acid)
46.317	311.22362	$C_{18}H_{32}O_4$	Octadeca-9-ene-1,18-dioic-acid (fatty acid)

46.468	311.20267	$C_{21}H_{28}O_2$	5,6-Didehydro-O-methylsugiol (diterpenes)
47.415	379.23544	$C_{18}H_{36}O_8$	Hexahydroxyoctadecanoic acid (fatty acid)
48.015	279.23327	$C_{18}H_{32}O_2$	Linoleic acid, (Z,Z)-9,12-Octadecadienoic acid (fatty acid)
48.015	279.30873	$C_{18}H_{32}O_2$	Linoleic acid, (Z,Z)-9,12-Octadecadienoic acid (fatty acid)
49.231	281.24914	$C_{18}H_{34}O_2$	Oleic acid (fatty acid)
49.705	471.34957	$C_{30}H_{48}O_4$	Maslinic acid (triterpene)

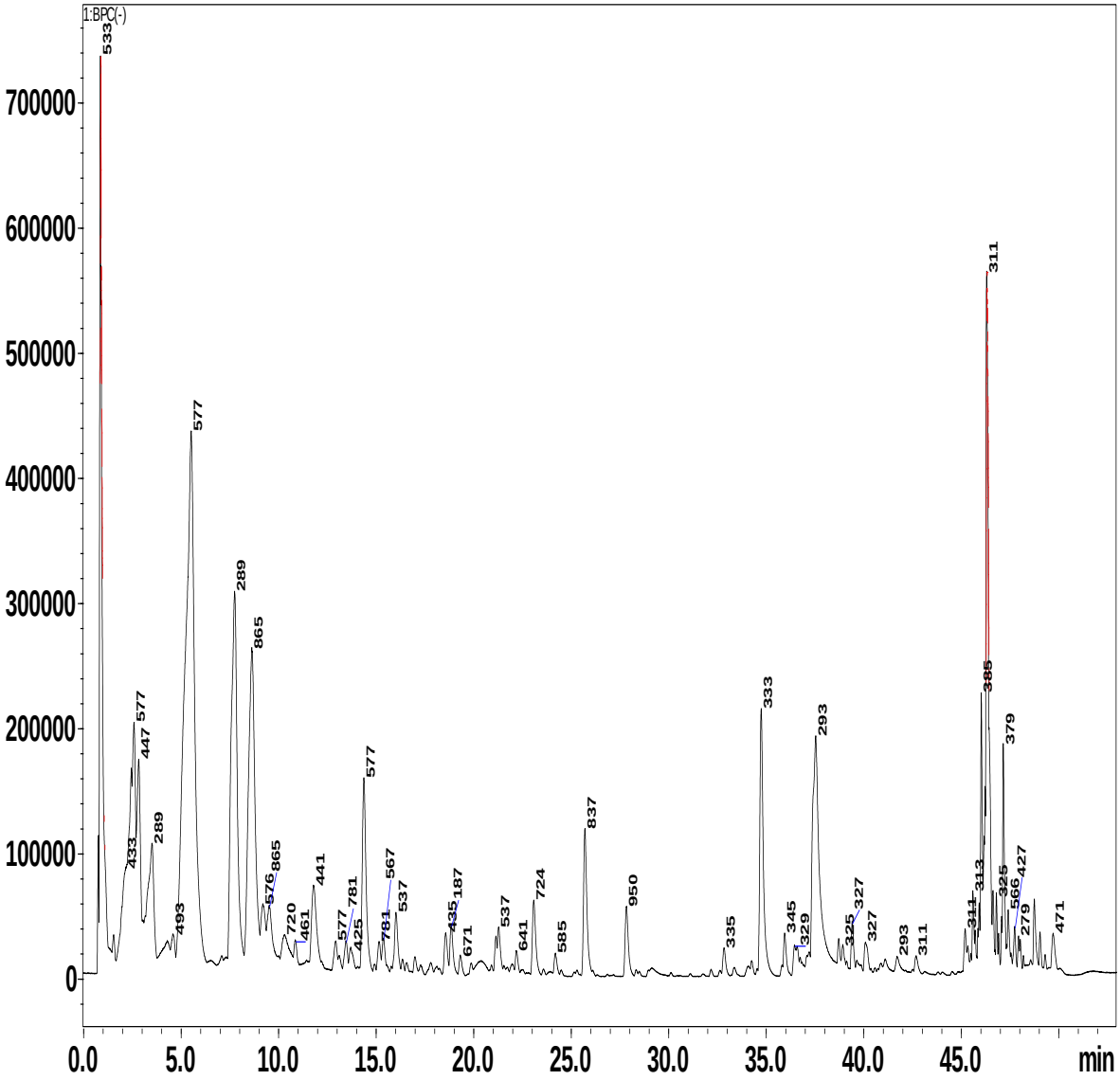


Figure 4.3. Peaks and the corresponding compounds detected in the root bark of *L. discolor* in the negative mode of ionisation.

Table 4.6. Compounds tentatively detected in the root bark extract of *L. discolor.*

Retention time	[M-H]- (m/z)	Molecular formula	Tentative assignment
0.89	533.17467	$C_{19}H_{34}O_{17}$	Quinic acid (tannin)
1.57	337.08669	$C_{18}H_{18}O_9$	Methoxymatteucin, (dimethylflavanones)
2.510	433.13629	$C_{18}H_{26}O_2$	(Z)-Octadec-12-ene-8,10-diynoic acid (fatty acid)
2.54	577.13709	$C_{30}H_{26}O_{12}$	Proanthocyanidin B (Flavonoid)
2.59	447.15222	$C_{19}H_{28}O_{12}$	Anacardoside (Phenol)
3.527	289.07216	$C_{15}H_{14}O_6$	Epicatechin (Flavonoid)
5.53	577.13757	$C_{30}H_{26}O_{12}$	Procyanidin B (Flavonoid)
7.57	289.07245	$C_{15}H_{14}O_6$	Epicatechin (Flavonoid)
8.581	865.20234	$C_{45}H_{38}O_{18}$	Procyanidin C1 (Flavonoid)
11.13	461.16770	$C_{20}H_{30}O_{12}$	Decaffeoylacteoside (phenol)
11.58	441.08371	$C_{22}H_{18}O_{10}$	Epicatechin gallate (Flavonoid)
13.12	577.13722	$C_{30}H_{26}O_{12}$	Procyanidin B2 (Flavonoid)
14.51	577.13724	$C_{30}H_{26}O_{12}$	Procyanidin B2 (Flavonoid)
16.01	567.21016	$C_{27}H_{36}O_{13}$	Citrusin B (lignan glycosides
16.538	537.19916	$C_{26}H_{34}O_{12}$	(+)-Cycloolivil 4'-O-beta-D-glucopyranoside (lignan glycosides)
19.13	435.13095	$C_{21}H_{24}O_{10}$	Catechin 3-O-alpha-L-rhamnoside (Flavonoid)
19.523	187.09742	$C_9H_{16}O_4$	Oxalic acid (dicarboxylic acid)
21.16	509.20513	$C_{25}H_{34}O_{11}$	3-[4-[1-(4-Hydroxy-3-methoxyphenyl)-1,3-dihydroxyisopropoxy]-2-hydroxyphenyl] propyl alpha-L-rhamnopyranoside (fatty acyl glycosides)
21.41	537.19918	$C_{26}H_{34}O_{12}$	(+)-Cycloolivil 4'-O-beta-D-glucopyranoside ((lignan glycosides)
22.32	641.24814	$C_{30}H_{42}O_{15}$	Sabphenoside D (Phenolic)
24.218	585.23575	$C_{31}H_{38}O_{11}$	6-Deoxydestigloylswietenine (triterpenoid)
33.17	335.09287	$C_{20}H_{16}O_5$	Alphinumisoflavone 2,3-Dihydro-2,3-dihydroxy-9-(4-hydroxy-3-methoxyphenyl)-1H-phenalen-1-one (flavonoid)
34.768	333.07761	$C_{20}H_{14}O_5$	Sophoracoumestan A (coumestan)
35.972	345.22920	$C_{18}H_{34}O_6$	sorbitan monolaurate (fatty acid)
36.528	329.23375	$C_{18}H_{34}O_5$	Tianshic acid (fatty acid)
37.552	293.17634	$C_{17}H_{26}O_4$	9, 12-Octerdecanoic acid (z, z, z)-(fatty acid)
39.515	325.20264	$C_{18}H_{30}O_5$	Albocycline M-3 (Lactone)
40.028	327.21840	$C_{18}H_{32}O_5$	9S,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid (Fatty acids)
41.513	293.17651	$C_{17}H_{26}O_4$	9, 12-Octerdecanoic acid (z, z, z)- (fatty acid)
42.72	311.18680	$C_{17}H_{28}O_5$	Pulicanol (sesquiterpenes)

45.53	311.22325	$C_{18}H_{32}O_4$	Octadeca-9-ene-1,18-dioic-acid (fatty acid)
45.627	313.23902	$C_{18}H_{34}O_4$	9,13-dihydroxy-11-octadecenoic acid (carboxylic acid)
46.085	315.25455	$C_{18}H_{36}O_4$	9,10-Dihydroxystearic acid (fatty acid)
46.096	385.26079	$C_{21}H_{38}O_6$	(+)-Rangiformic acid (carboxylic acid)
46.132	293.21266	$C_{18}H_{30}O_3$	9-Oxo-10 (E), 12 (E)-octadecadienoic acid (fatty acid)
46.312	295.22835	$C_{18}H_{32}O_3$	12-oxo-10Z-octadecenoic acid (fatty acid)
46.555	311.22372	$C_{18}H_{32}O_4$	Octadeca-9-ene-1,18-dioic-acid (fatty acid)
47.415	379.23544	$C_{18}H_{36}O_8$	Hexahydroxyoctadecanoic acid (fatty acid)
48.052	279.23371	$C_{18}H_{32}O_2$	Linoleic acid, (Z,Z)-9,12-Octadecadienoic acid (fatty acid)
48.068	423.32839	$C_{29}H_{44}O_2$	alpha-Tocotrienol (vitamin E)
48.765	281.24925	$C_{18}H_{34}O_2$	Oleic acid (Z)-9-Octadecenoic acid (fatty acid)
49.716	471.34960	$C_{30}H_{48}O_4$	Maslinic acid (triterpene)

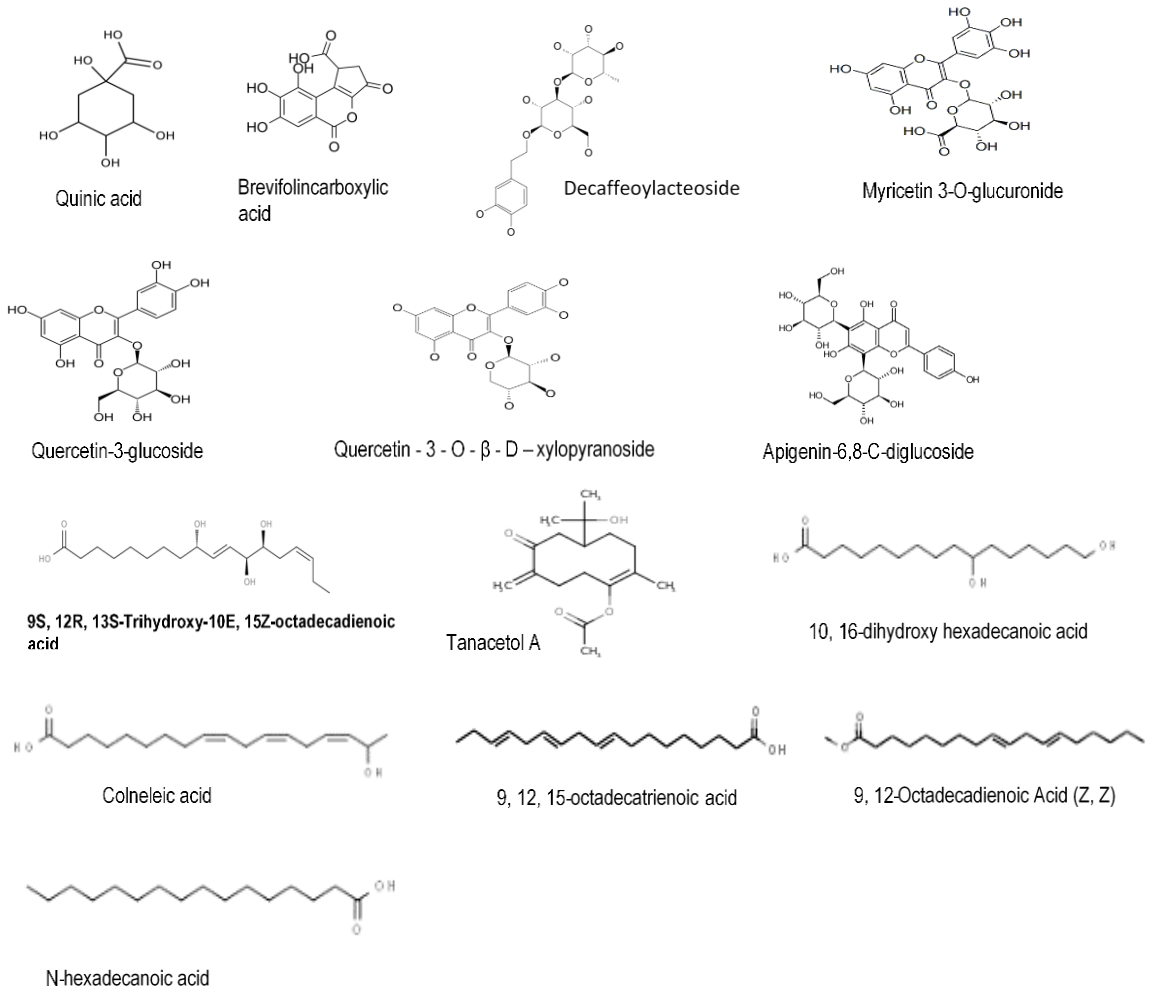


Figure 4.4. The molecular structures of major peaks of isolated compounds from *L. discolor* leaves.

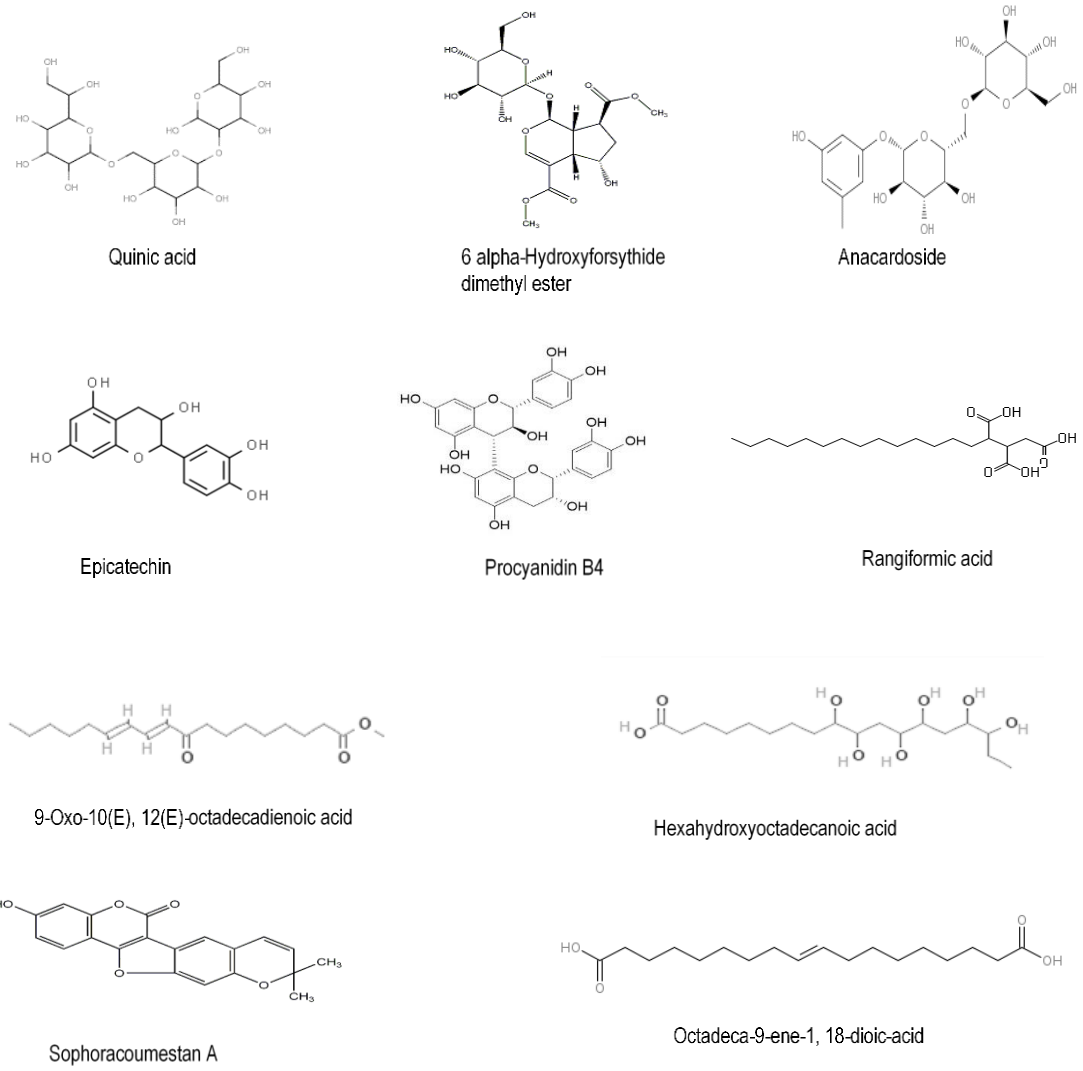


Figure 4.5. The molecular structures of major peaks of isolated compounds from *L. discolor* stems and root bark.

4.3.2.3 Chemo-taxonomical relevance of the LC-MS results

It may be challenging to differentiate or associate certain plants through their physical characteristics, as such, chemotaxonomy can be used to confirm their place based on chemical composition or key characteristics of a certain group of phytochemicals. Advanced technological means are usually employed to classify certain plants into either genus or family, since such tools give a clear description and identity after a thorough comparative analysis (Umoh, 2020). As seen from the results presented herein, this species produces flavonoids, phenolic and fatty acid derivatives, are seen in other plants from family Anacardiaceae (Sameh et al., 2018). Flavonoids and phenolic compounds comparable to those found in this study have been isolated and identified in several studies. For example, in the Anacardiaceae family, phenols, myricetin and other types of flavonoids have been identified as having

chemotaxonomic qualities, and their separation and identification in other *Lannea* species confirms their importance within the family. These flavonoids are expected in these species, as seen in *L. discolor*, implying that most plants in *Lannea* may have followed a shared a similar synthesis pathway. (Okoth et al., 2014). This brings about the indication that the co-occurrence of some of these compounds within the species of the family Anacardiaceae might have chemotaxonomic implications, at least at family level. Thus, members of this plant family appear to be predestined to serve as sources of biologically active phenolic compounds. Indeed, some species belonging to the Anacardiaceae have an extensive history of use by human beings as food and for technological and medicinal purposes (Schulze-Kaysers et al., 2015; Sameh et al., 2018).

4.3.3 Biological activities

4.3.3.1 Antioxidant activity of *Lannea discolor* extracts.

The antioxidant activity of the vegetative parts of *L. discolor* were evaluated using a solution of DPPH reagent. The presence of antioxidant constituents could be confirmed by the reduction of the visibility of the normal purple color of DPPH (Dar et al., 2017). The antioxidant activity results of the crude methanolic plant extract are presented in Table 4.7. The values acquired were compared to ascorbic acid, a known effective antioxidant. Compounds within the plant extract are able to produce free radicals which subsequently react with DPPH, gradually changing its colour (Nabeelah et al., 2020). In this study, extracts from three separate plant parts all demonstrated the ability to decolorize DPPH. Their percent inhibition decreased in relation to decreasing concentration.

Although there exist various assays for determining the antioxidant activity of phytochemical compounds, the DPPH technique is most preferred due the easiness of the technique as well as the specificity (Miguel 2010). The percent inhibition ability for DPPH free radical scavenging was found to be plant part dependent. All extracts showed high radicals scavenging activities range between 77.31% – 79.55% and, comparing with Ascorbic acid which showed radicals scavenging activity of 64.55 – 89.25%, the IC_{50} value was found to be 2.5733 µg/ml, for the Ascorbic acid, a value that was observed to be higher than that obtained in the each of the methanol extracts, suggesting it has lower antioxidant activity than the plant extracts.

According to literature, a steep gradient denotes efficiency of the extract in its ability to scavenge the DPPH (Molole et al., 2020). Subsequently, the root bark had the highest ability in scavenging the DPPH, compared to the stem, leaves and ascorbic acid standard. In principle, the DPPH radicals were reduced by the ability of *L. discolor* root extract to donate hydrogen (Baliyan et al., 2022). The bark and root extracts of the studied plant demonstrated the ability to donate hydrogen atoms, as evidenced by their capacity to reduce the stable 2,2-diphenyl-1-picrylhydrazyl radical. The results agree with those reported by Chakuma et al., (2015) where the highest antioxidant activity was also observed to contain the highest phenolic content. Phenolic compounds exhibit antioxidant properties due to their ability to

donate electrons, owing to the exceptional antioxidant activity within the phenolic rich stem and root bark of *L. discolor*. Most diseases are caused by free radicals, as they contain atoms that are damaging to the human cells (Chaudhary et al., 2023). Antioxidant activity may be present due to flavonoid myricetin derivatives, which are acknowledged to typically have strong antioxidant activity (Okoth et al., 2013), observed by the relationship between the TFC and TPC and the antioxidant activity.

According to literature, a low IC_{50}, indicates significant antioxidant activity (Wright et al., 2017). In the stem and root extracts, IC_{50}= 0.16211 and 0.008441 µg/ml were significantly lower than the value in the leaf extract (IC_{50}=1.095126), meaning their antioxidant activity was more than that of the leaf extract. These results concurred with the TPC and TFC values, where the lowest content was obtained in the leaves. Seemingly, the TPC of the stem and root bark could also have influenced the observed results. A similar conclusion was reached by Bello et al., (2022), wherein, the high TPC was observed to positively influence the antioxidant ability of *Lannea microcarpa*. This presence study further confirms that the presence of phenolic and flavonoid compounds are a significant aspect in the antioxidant capacities of plants (Ouattara et al., 2011). Furthermore, the IC_{50} values are not significantly different ($P \geq 0.05$); this demonstrates that distribution of antioxidant agents in *L. discolor* may not be far off from each other with regards to abundance.

Table 4.7: Antioxidant activity of methanolic extracts of *L. discolor* using 2, 2-diphenyl1-pycrilhydrazil hydrate.

Sample		**IC_{50} ± SD µg/ml (DPPH)**
Methanol	Leaves	1.095126 ± 0.030
	Stem	0.162110 ± 0.020
	Root	0.008441 ± 0.015
Standard (ascorbic acid)		2.573369 ± 0.045

4.3.3.2 Antibacterial activities

The preliminary antibacterial assay demonstrates that the L. discolor extracts possess variable inhibitory activity against the tested bacterial species. The methanolic leaf extracts showed prominent activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, and *K. pneumoniae* (Table 4.8). There was insignificant inhibition of *E. coli* and *S. aureus* observed, as indicated by zones of inhibition of 7 mm and 4 mm respectively. However, all microorganisms tested were sensitive to the stem and root bark extracts, as judged by the criteria of Ponce et al. (2003), with zones of inhibition ranging from 12-18 mm. As defined by Ponce et al. (2023), an extract is considered significantly active if it generates a zone of inhibition exceeding 12 mm.

Table 4.9 shows the MIC capability of the extracts on the bacteria. It was observed that higher extract concentrations exhibited greater inhibitory activity. The extracts exhibited significant growth inhibition on all the test organisms compared to the control drug (Gentamicin). Colorimetric MIC endpoints were interpreted as the lowest sample concentration that remained blue (indicating no growth) or the first

dilution that changed from blue to a slightly pink (equivalent to prominent growth inhibition) after the addition of Resazurin. The concentration of 1.56 mg/ml, followed by 3.12 mg/ml is the minimum at which the growth of *P. aeruginosa* and *B. subtilis* was inhibited when tested against leaf and stem and root bark extracts. The concentration of 3.12 mg/ml and 6.25 mg/ml is the minimum at which the growth of *B. subtilis* was inhibited. For *K. pneumoniae*, 6.25 mg/ml, was recorded as the minimum extract concentration for the all the organs extracted, while 12.5 (stem and roots), 25 and 50 mg/ml (leaves) was the minimum concentration for the growth inhibition of *E. coli* and *S. aureus*.

The phytochemical constituents such as tannins, flavonoids and phenolic acids, have been reported to possess antibacterial activity (Patra, 2012). It would therefore suffice to say since the extracts of *L. discolor* contain these phytochemicals, they influenced antibacterial activity against strains of *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. The obtained activities of the different plant parts demonstrate that the stem and root bark extract had the most activity against the bacteria. The low MIC values (0.39 mg/ml) observed for Gentamicin as a positive control for all the microorganisms, confirmed its pre-eminence over the plant extracts. Amongst the crude extracts obtained from the organs of *L. discolor*, the stem and root exhibited a high antibacterial activity with MIC values of the range of 1.56 - 12.5 mg/ml) respectively compared to the leaf extract (3.12 – 50 mg/ml), indicating the relative polarity of the antimicrobial constituents and selected activity of the 3 plant parts. It is also probable that this is because extracts had only either a specific antibacterial compound present, or compounds present in extraordinary amounts to bring about the desired activity. Previous work by Mabona et al. (2013) on leaf extracts of *L. discolor* showed compelling activities with MIC values 1.0 mg/ml and 2.0 mg/ml for *P. aeruginosa* and *S. aureus* respectively. Contrastingly, in the present study, the leaf extracts were moderately active against *E. coli* (50 mg/ml), *S. aureus* (25 mg/ml), but significantly active against *B subtilis* (6.25 mg/ml), *P. aeruginosa* (3.12 mg/ml), and *K. pneumoniae* (6.25 mg/ml). This may be because active compounds may be more soluble and more concentrated in the stem and root extracts (Feng et al., 2019). A similar pattern of results was obtained in *L. welwitshii* leaf extracts, wherein the antibacterial activity was recorded at MIC values of 5 mg/ml for *E. coli* and *S. aureus* as well as 2.5 mg/ml and 10 mg/ml for *B. subtilis* and *P. aeruginosa* respectively (Agyare et al., 2013). In conjunction with the aforementioned, methanol has been deemed one of the most suitable extractants for the screening of antimicrobial components in plants, validating the significant activity of methanol extracts against the tested microorganisms (Eloff, 1998; Kotze and Eloff, 2002). The richness of the stem and root bark in phenolic and flavonoid compounds fortifies its high antimicrobial activity. The antibacterial result in the present study exhibits similarities to other results obtained from *Lannea* species, which revealed that plants in the *Lannea* genus possesses potent antibacterial properties due to its high phytochemical content has significant antibacterial activities due to the rich phytochemical content (Mabona et al., 2013; Okoth, 2014; Lall et al., 2018). Rafiu et al. (2019) reported significant activity of *Lannea egregia* stem bark extracts, where the microorganisms *S. aureus*, *P. aeruginosa*, and *E. coli*

showed sensitivity to the extracts at MIC values 6.25 mg/ml and 0.0008 mg/ml respectively. Ogunsina et al., 2020 established the antibacterial activity of *Lannea acida* methanolic stem that exhibited MIC values ranging from 20 to 50 mg/ml with MIC of 20 mg/ml for *S. aureus* and *K. pneumoniae*, 30 and 50 mg/ml for *B. subtilis, S. aureus, E. coli, K. pneumoniae*, and *E. coli.* The significant activity observed in the stem and root bark implies that most biological activity is associated with the stem and root bark, as they are often used traditionally across the genus and family (Grace et al., 2003).

Recently, there are a significant number of bacteria that cause common infections such as pneumonia, urinary tract infections that have become resistant to conventional antibiotics in all regions of the world. Highly resistant bacteria such as those in *Enterobacteriaceae* (mostly *Klebsiella pneumoniae*) and other multidrug-resistant bacteria have been the leading cause of most hospital-acquired infections (Kahn et al., 2017). Therefore, even the slightest growth inhibitory effect of *L. discolor* stem-bark extract on the *Staphylococcus aureus* and *Klebsiella pneumoniae* in this study signifies that the plant can be used for to tackle infectious diseases caused by other drug resistant bacteria such as Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumoniae*. This is solely due to the presence of various derivatives of highly active phenols and flavonoids that are characteristic of *Lannea* species (Islam and Tahara, 2000; Islam et al., 2002; Okoth et al., 2013). Sani et al., (2019) studied the effect of stem-bark extracts of the highly active *Lannea microcarpa* to resolve the antibiotic resistance in some selected clinically isolated bacterial strains; *Staphylococcus aureus, Klebsiella pneumoniae*. When compared, the antibacterial activity of *L. discolor* stem-bark extract on the similar bacterial strains in this study is higher than the activities of all the plant extracts tested by Sani et al., (2019).

It is said that flavonoids are synthesised by plants in order to fight against various microorganisms, as such flavonoids have become a big part of the battle against pathogenic bacteria and are considered efficient antibacterial agents (Cowan, 1999). The antibacterial compounds act by different mechanisms: by preventing the synthesis of the bacterial peptidoglycan wall, by impeding bacterial protein and nucleid acid synthesis, by unsettling the structure of the bacterial membrane or by blocking the metabolic pathways by inhibition of major enzymes (Khameneh et al., 2019). Secondary compounds, especially flavonoids and tannins, are recognized for their toxicity to microorganisms. The mechanism of toxicity may be related to the inhibition of hydrolytic enzymes (proteases and carbohydrolases) and the ability of phytochemical compounds to inactivate microbial virulence factors, and proteins within the cell envelope. Some flavonoids would affect intracellular replication and thus reduce the infectious properties of bacteria. Pare et al. (2019), observed significant polyphenol and flavonoid content in *Lannea velutina*, which was assumed to be the cause of good antioxidant potential as well as antibacterial activity. The findings could explain, to some extent, the usage of *L. discolor* in many communities as herbal remedies and the abundance of antibacterial compounds in its phytochemical profile. They could also indicate that bark extracts offer more bacterial activity than leaf extracts as they

are typical of exhibiting high activity in biological studies on the genus *Lannea* (Outtara et al., 2011; Chakuma et al., 2015).

Table 4.8. Sensitivity of methanolic extracts on sensitive microorganism's inhibition zones (mm) ±of three replicates.

	Zones of inhibition (mm)		
	Methanol extract		
Organisms	leaf	stem	root
S. aureus	7	12	12
B. subtilis	12	15	17
P. aeruginosa	13	17	18
E. coli	4	13	14
K. pneumoniae	13	15	16
Gentamicin	30	30	30
Sterile DH2O	ND	ND	ND

Values expressed in mean ± standard deviation. Unit (mm). (ND) not determined Not sensitive (−) for diameters less than 8 mm; sensitive (+) for diameters 9–14 mm; very sensitive (++) for diameters 15–19 mm and extremely sensitive (+++) for diameters larger than 20 mm.

Table 4.9. MIC values in mg/ml of *L. discolor* after 24 incubation extract against different bacterial microorganisms.

	MIC values (mg/ml)		
	Methanol extract		
Organisms	leaf	stem	root
S. aureus	25	12.5	12.5
B. subtilis	6.25	3.12	3.12
P. aeruginosa	3.12	1.56	1.56
E. coli	50	12.5	12.5
K. pneumoniae	6.25	6.25	6.25
Gentamicin	0.39	0.39	0.39

MIC = minimum inhibitory concentration (mg/mL). Gentamicin was the positive control for bacteria.

4.4 Conclusion

The present research reported a broad identification of the metabolites in the *L. discolor* extracts and the investigation of the cause of their biological properties. The chemical constituents of medicinal plants products are known to vary considerably in terms of content, physical properties, and biochemical properties. This leads to dissimilar pharmacological effects. The active components of many medicinal plant species remain unknown, and pharmaceutical activity may arise from synergistic interactions between major and minor chemical constituents. Therefore, high sensitivity and selectivity analytical techniques are needed to develop comprehensive chemical profiles. Such profiling would allow for evaluation of medicinal plant and herbal product efficacy, safety, and batch-to-batch consistency.

The difference in chromatographic profiles and identified components to some extent, explained the different bioactivities of these extracts. Phytochemical profiling by conventional phytochemical testing and quantification by determination of total phenolic and flavonoid content was conducted and revealed the presence of a myriad of compound classes. The total phenolic and flavonoid content of the stem and

root bark was the highest when compared to the leaf, in all samples. The LC-MS of extracted plant parts of *L. discolor* affirmed the presence of pharmacologically active compounds phenolics, flavonoids and fatty acids. These extracts were able to scavenge DPPH and inhibit the growth of certain microorganisms at specific concentrations, hence, validation of the use of *L. discolor* is due to the high amounts of phenolic and flavonoids in the plant. The study elucidated the significance of information utilization for the validation of known compounds or the delineation of unknown constituents that may necessitate consideration during the progression of novel pharmaceutical formulations. As such, since *L. discolor* is a valuable source of bioactive compounds, the establishment of a detailed phytochemical profile was fundamental.

4.5 References

Abiodun, O. O., Nnoruka, M. E. and Tijani, R. O., 2020. Phytochemical constituents, antioxidant activity, and toxicity assessment of the seed of *Spondias mombin* L. (Anacardiaceae). *Turkish Journal of Pharmaceutical Sciences*, 17(3), 343.

Achika, J., Oyewale, A. O. and James, D. H., 2017. Chemical investigation and antioxidant activity of fractions of *Lannea humilis* (Oliv.) Engl. *Journal of the Turkish Chemical Society Section A: Chemistry*, 4(2), 563-572.

Achika, J., Racheal, A.Y.O., Oyewale, A. and James, D., 2017. Chemical investigation and antioxidant activity of fractions of Lannea humilis (Oliv.) Engl. *Journal of the Turkish Chemical Society Section A: Chemistry*, 4(2), 563-572.

Agyare, C., Bempah, S.B., Boakye, Y.D., Ayande, P.G., Adarkwa-Yiadom, M. and Mensah, K.B., 2013. Evaluation of antimicrobial and wound healing potential of *Justicia flava* and *Lannea welwitschii*. *Evidence-Based Complementary and Alternative Medicine*, 2013(1), 632927.

Ahmad, I., Ahmad, S., Rao, H., Shaukat, U., Shahzad, M. N., Rehman, M. S., Abdul, B.M., Adeel A. and Ahmad, B., 2022. GC–MS profiling, phytochemical and biological investigation of aerial parts of *Leucophyllum frutescens* (Berl.) IM Johnst. (Cenizo). *South African Journal of Botany*, 148, 200-209.

Akhter, S., Shahadat, S., Homa, Z., Kuddus, M.R., Chowdhury, M.M.U. and Ibrahim, M., 2020. Phytochemical and Pharmacological Investigations of *Lannea grandis* (Dennst.) Engl. Leaves Extracts. *Journal of Drug Delivery and Therapeutics*, 10(5-s), 85-89.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A., 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C. and Haridas, M., 2012. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design*, 80(3), 434-439.

Appendino, G., Gariboldi, P. and Nano, G. M., 1983. Tanacetols A and B, non-volatile sesquiterpene alcohols, from *Tanacetum vulgare*. *Phytochemistry*, 22(2), 509-512.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M. and Supuran, C. T., 2021. Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, 20(3), 200-216.

Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P. and Chang, C.M., 2022. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa*. *Molecules*, 27(4), 1326.

Banda, M., Nyirenda, J., Muzandu, K., Sijumbila, G. and Mudenda, S., 2018. Antihyperglycemic and antihyperlipidemic effects of aqueous extracts of *Lannea edulis* in alloxan-induced diabetic rats. *Frontiers in pharmacology*, 9, 1099.

Bazongo, P., Bassolé, I. H. N., Nielsen, S., Hilou, A., Dicko, M. H. and Shukla, V. K., 2014. Characteristics, composition, and oxidative stability of *Lannea microcarpa* seed and seed oil. *Molecules*, 19(2), 2684-2693.

Bello, O. M., Nok, E. A., Musa, B., Musharaf, A. and Abiodun, O. B., 2022. LC-MS Analysis, Total Polyphenol Content and Antioxidant Activity of Ethanolic Fractions of Skin (Epicarp) and Seed Pulp (Mesocarp) of Green and Red Wild Africa Berries. *Social Science Research Network,* 4021868.

Bohlmann, F., Knoll, K. H., King, R. M. and Robinson, H., 1979. Neue α-santalen-und labdan-derivate aus Ayapana amygdalina. *Phytochemistry*, 18(12), 1997-2002.

Bouare, S., Traore, N., Sidibe, L., Fofana, B., Chalard, P., Figueredo, G. and Chalchat, J.C., 2012. Composition chimique de l'huile essentielle des fleurs de L*annea velutina* (Anacardiaceae) du Mali. *International Journal of Biological and Chemical Sciences*, 6(5), 2274-2279.

Bruschi, P., Morganti, M., Mancini, M. and Signorini, M. A., 2011. Traditional healers and laypeople: a qualitative and quantitative approach to local knowledge on medicinal plants in Muda (Mozambique). *Journal of Ethnopharmacology*, 138(2), 543-563.

Cecilia, M. K., Abrha, A. H., Hlengilizwe, N., Hintsa, A. T., Umer, M., Nixwell, M. F. and Sharifi-Rad, J., 2020. Untargeted profiling of field cultivated bush tea (*Athrixia phylicoides* DC.) based on metabolite analysis. *Cellular and Molecular Biology*, 66(4), 104-109.

Chakuma, N., Chipurura, B., Muchuweti, M., Chitindingu, K., Bhebhe, M. and Chagonda, L., 2015. Total phenolic content, free radical scavenging and antioxidant potential of *Lannea discolor* (Sond.) Engl bark and root extracts. *Journal of Biologically Active Products from Nature*, 5(1), 71-77.

Chaudhary, P., Janmeda, P., Docea, A.O., Yeskaliyeva, B., Abdull Razis, A.F., Modu, B., Calina, D. and Sharifi-Rad, J., 2023. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. *Frontiers in chemistry*, 11, 1158198.

Chigayo, K., Mojapelo, P.E.L., Mnyakeni-Moleele, S. and Misihairabgwi, J.M., 2016. Phytochemical and antioxidant properties of different solvent extracts of *Kirkia wilmsii* tubers. *Asian Pacific Journal of Tropical Biomedicine*, 6(12), 1037-1043.

Cires, M.J., Wong, X., Carrasco-Pozo, C. and Gotteland, M., 2017. The gastrointestinal tract as a key target organ for the health-promoting effects of dietary proanthocyanidins. *Frontiers in Nutrition*, 3, 57.

Clarkson, C., Maharaj, V. J., Crouch, N. R., Grace, O. M., Pillay, P., Matsabisa, M. G. and Folb, P. I., 2004. In vitro antiplasmodial activity of medicinal plants native to or naturalised in South Africa. *Journal of ethnopharmacology*, 92(2-3), 177-191.

Cuyckens, F. and Claeys, M., 2004. Mass spectrometry in the structural analysis of flavonoids. *Journal of Mass spectrometry*, 39(1), 1-15.

Dar, R.A., Brahman, P.K., Khurana, N., Wagay, J.A., Lone, Z.A., Ganaie, M.A. and Pitre, K.S., 2017. Evaluation of antioxidant activity of crocin, podophyllotoxin and kaempferol by chemical, biochemical and electrochemical assays. *Arabian Journal of Chemistry*, 10, S1119-S1128.

Delazar, A., Byres, M., Gibbons, S., Kumarasamy, Y., Modarresi, M., Nahar, L., Shoeb, M. and Sarker, S. D. (2004). Iridoid Glycosides from *Eremostachys glabra*. *Journal of natural products*, 67(9), 1584-1587.

Elmongy, E. I., Negm, W. A., Elekhnawy, E., El-Masry, T. A., Attallah, N. G., Altwaijry, N. and El-Sherbeni, S. A., 2022. Antidiarrheal and Antibacterial Activities of Monterey Cypress Phytochemicals: In Vivo and In Vitro Approach. *Molecules*, 27(2), 346.

Falcão, S. I., Vale, N., Gomes, P., Domingues, M. R., Freire, C., Cardoso, S. M. and Vilas-Boas, M., 2013. Phenolic profiling of Portuguese propolis by LC–MS spectrometry: Uncommon propolis rich in flavonoid glycosides. *Phytochemical Analysis*, 24(4), 309-318.

Fowler, D. G., 2002. Traditional ila plant remedies from Zambia. *Kirkia*, 35-48.

Galati, E.M., Miceli, N., Galluzzo, M., Taviano, M.F. and Tzakou, O., 2004. Neuropharmacological Effects of Epinepetalactone from *Nepeta sibthorpii* Behavioral and Anticonvulsant Activity. *Pharmaceutical Biology*, 42 (6), 391-395.

Gardana, C., Scaglianti, M., Pietta, P. and Simonetti, P., 2007. Analysis of the polyphenolic fraction of propolis from different sources by liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 45(3), 390-399.

Gedük, A. Ş. and Atsız, S., 2022. LC-MS/MS phenolic composition of peach (*Prunus persica* L. Batsch) extracts and an evaluation of their antidiabetic, antioxidant, and antibacterial activities. *South African Journal of Botany*, 147, 636-645.

Guerrero, L., Castillo, J., Quinones, M., Garcia-Vallve, S., Arola, L., Pujadas, G. and Muguerza, B., 2012. Inhibition of angiotensin-converting enzyme activity by flavonoids: structure-activity relationship studies. *PloS one*, 7(11), e49493.

Hien, T. T. T., Quang, T. H., Nhiem, N. X., Tai, B. H., Phi, V. P., Hien, N. T. T. and Van Kiem, P., 2018. Phenolic glycosides from the aerial parts of *Buddleja macrostachya* Benth. *Vietnam Journal of Chemistry*, 56(4), 466-472.

Ho, L., Ferruzzi, M. G., Janle, E. M., Wang, J., Gong, B., Chen, T. Y. and Pasinetti, G. M., 2013. Identification of brain-targeted bioactive dietary quercetin-3-O-glucuronide as a novel intervention for Alzheimer's disease. *The FASEB Journal*, 27(2), 769-781.

Hollender, J., Van Bavel, B., Dulio, V., Farmen, E., Furtmann, K., Koschorreck, J. and Tornero, V., 2019. High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management. *Environmental Sciences Europe*, 31(1), 1-11.

Hwang, J. H., Choi, H., Woo, E. R. and Lee, D. G., 2013. Antibacterial effect of amentoflavone and its synergistic effect with antibiotics. *Journal of microbiology and biotechnology*, 23(7), 953-958.

Ibibia, E.T., Olabisi, K.N. and Oluwagbemiga, O.S., 2016. Gas chromatography-mass spectrometric analysis of methanolic leaf extracts of *Lannea kerstingii* and *Nauclea diderrichii*, two medicinal plants used for the treatment of gastrointestinal tract infections. *Asian Journal of Pharmaceutical and Clinical Research*, 9(4):179-82.

Imam, M. Z. and Moniruzzaman, M., 2014. Antinociceptive effect of ethanol extract of leaves of *Lannea coromandelica*. *Journal of ethnopharmacology*, 154(1), 109-115.

Islam, M.T., Ito, T., Sakasai, M. and Tahara, S., 2002. Zoosporicidal activity of polyflavonoid tannin identified in *Lannea coromandelica* stem bark against phytopathogenic oomycete *Aphanomyces cochlioides*. *Journal of Agricultural and Food Chemistry*. 50, 6697–6703.

Jabeen, A., Mesaik, M. A., Simjee, S. U., Bano, S. and Faizi, S., 2016. Anti-TNF-α and anti-arthritic effect of patuletin: A rare flavonoid from Tagetes patula. *International immunopharmacology*, 36, 232-240.

Jandacek, R. J., 2017. Linoleic acid: a nutritional quandary. *Healthcare,* 5 (2), 25.

Jensen, S. R., Çalış, İ., Gotfredsen, C. H. and Søtofte, I., 2007. Structural revision of some recently published iridoid glucosides. *Journal of natural products*, 70(1), 29-32.

Ji, X., Huang, B., Wang, G., and Zhang, C. (2014). The ethnobotanical, phytochemical and pharmacological profile of the genus Pinellia. *Fitoterapia*, *93*, 1-17.

Kabongo-Kayoka, P. N., Eloff, J. N., Obi, C. L. and McGaw, L. J., 2016. Antimycobacterial activity and low cytotoxicity of leaf extracts of some African Anacardiaceae tree species. *Phytotherapy Research*, 30(12), 2001-2011.

Kapche, G. D., Laatsch, H., Fotso, S., Kouam, S. F., Wafo, P., Ngadjui, B. T. and Abegaz, B. M., 2007. Lanneanol: a new cytotoxic dihydroalkylcyclohexenol and phenolic compounds from *Lannea nigritana* (Sc. Ell.) Keay. *Biochemical Systematics and Ecology*, 35(8), 539-543.

Kaur, R., Jaiswal, M.L. and Jain, V., 2013. Protective effect of *Lannea coromandelica* Houtt. Merrill. against three common pathogens. *Journal of Ayurveda and integrative medicine*, 4(4), 224.

Kazembe, T., Munyarari, E. and Charumbira, I., 2012. Use of traditional herbal medicines to cure malaria. *Bulletin of Environment, Pharmacology and Life Sciences*, 1(4), 63-85.

Koné, W.M., Atindehou, K.K., Terreaux, C., Hostettmann, K.U.R.T., Traore, D. and Dosso, M., 2004. Traditional medicine in North Côte-d'Ivoire: screening of 50 medicinal plants for antibacterial activity. *Journal of ethnopharmacology*, 93(1), 43-49.

Kpegba, K., Eloh, K., Evenamed, K.S., Afanyibo, Y.G., Elomri, A., Simalou, O., Boyode, P., Agbonon, A. and Seguin, E., 2019. A Comparative Study of the Chemical Composition of the Extracts from Leaves, Stem, Bark, and Root Bark of *Cassia sieberiana*: Antibacterial Activities. *Oriental Journal of Chemistry*, *35*(6).

Lall, N., de Canha, M.N., Reid, A., Oosthuizen, C.B., Langhansova, L., Mahore, J., Winterboer, S., Hamilton, C., Kumar, V., Gasa, N. and Twilley, D., 2018. Antibacterial and anticancer activity of ethnomedicinal plants used in the Jongilanga community, Mpumalanga. *International Journal of Pharmacognosy and Phytochemical Research,* 10, 25-37.

Lee, B. Z., Kim, C. S., Jeong, S. K., Lee, S., Lee, I. S. and Hong, K., 2021. Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. rhynchophylla. *Natural Product Research*, 35(22), 4380-4387.

Li, H. Z., Song, H. J., Li, H. M., Pan, Y. Y. and Li, R. T., 2012. Characterization of phenolic compounds from *Rhododendron alutaceum*. *Archives of pharmacal research*, 35(11), 1887-1893.

Lozano-Mena, G., Sánchez-González, M., Juan, M. E. and Planas, J. M., 2014. Maslinic acid, a natural phytoalexin-type triterpene from olives—a promising nutraceutical? *Molecules*, 19(8), 11538-11559.

Lv, T. T., Ju, C. G., Liu, B. N., Ren, X. H., Shan, G. S. and Jia, T. Z., 2020. Determination of orcinol glucoside by LC-MS in *Curculigo orchioides* and its application to a pharmacokinetic study. *Revista Brasileira de Farmacognosia*, 29, 744-748.

Mabona, U., Viljoen, A., Shikanga, E., Marston, A. and Van Vuuren, S., 2013. Antimicrobial activity of southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach to combination studies and the isolation of a bioactive compound. *Journal of Ethnopharmacology*, 148(1), 45-55.

Mahdavi, M., Sharifnaa, F., Salimpour, F., Esmaeili, A. and Larypoor, M., 2021. Evaluation of nuts morphology and composition of fatty acids in certain Iranian *Pistacia vera* L. (Anacardiaceae) cultivars. *Acta agriculturae Slovenica*, 117(3), 1-10.

Mahomoodally, M.F., Atalay, A., Picot, M.C.N., Bender, O., Celebi, E., Mollica, A. and Zengin, G., 2018. Chemical, biological, and molecular modelling analyses to probe into the pharmacological potential of *Antidesma madagascariense* Lam.: a multifunctional agent for developing novel therapeutic formulations. *Journal of Pharmaceutical and Biomedical Analysis*, 161, 425‑435.

Majumder, R., Jami, S.I., Alam, M.E.K. and Alam, M.B., 2013. Antidiarrheal activity of *Lannea coromandelica* Linn. Bark extract. *American-Eurasian Journal of Scientific Research*, 8(3), 128-134.

Malú, Q., Lima, K., Malmir, M., Pinto, R., da Silva, I.M., Catarino, L., Duarte, M.P., Serrano, R., Rocha, J., Lima, B.S. and Silva, O., 2022. Contribution to the preclinical safety assessment of *Lannea velutina* and *Sorindeia juglandifolia* leaves. *Plants*, 12(1), 130.

Mamphiswana, N.D., Mafeo, T.P., Mashinya, F. and Mashela, P.W., 2013. Anti-phytopathogenic bacterial activity of selected plant extracts against four biovars of *Ralstonia solanacearum*. In *African Crop Science Conference Proceedings*, 11, 399-402.

Mangla, B., Kohli, K. and Rabiu, S., 2021. Review of medicinal uses, phytochemistry, pharmacological properties, extraction methods and toxicology of *Lannea microcarpa* (African Grapes). *Current Traditional Medicine*, 7(1), 125-137.

Maroyi, A., 2013. Traditional use of medicinal plants in south-central Zimbabwe: Review and perspectives. *Journal of Ethnobiology and Ethnomedicine*. 9, 1–18.

Maroyi, A., 2018. *Lannea discolor*: Its botany, ethnomedicinal uses, phytochemistry and pharmacological properties. *Asian Journal of Pharmaceutical and Clinical Research*, 11, 49-54.

Mbaoji, F. N. and Nweze, J. A., 2020. Antioxidant and hepatoprotective potentials of active fractions of *Lannea barteri* Oliv. (Anarcadiaceae) in rats. *Heliyon*, 6(6), e04099.

Mbaoji, F.N. and Nweze, J.A., 2020. Antioxidant and hepatoprotective potentials of active fractions of *Lannea barteri* Oliv.(Anarcadiaceae) in rats. *Heliyon*, 6(6).

Meremeti, A., Karioti, A., Skaltsa, H., Heilmann, J. and Sticher, O., 2004. Secondary metabolites from Stachys ionica. *Biochemical systematics and ecology*, 32(2), 139-151.

Mikail, H., Yusuf, M. and Hussain, G., 2016. *In vitro* anticoccidial activity of methanolic leaves extract of *Lannea schimperi* against oocysts of *Eimeria tenella. IOSR Journal of Pharmaceutical and Biological Sciences*, 11, 35-38.

Mikail, H.G., Abubakar, I.A., Mohammed, B.R., Yusuf, M., Hussain, G. and Fatihu, M.Y., 2019. Effects of methanolic leaf extract of *Lannea schimperi* on some organs histopathology in experimentally induced coccidiosis in broiler chickens. *Journal of Veterinary Science and Animal Husbandry*, 6(6), 602.

Mølgaard, P., Nielsen, S. B., Rasmussen, D. E., Drummond, R. B., Makaza, N. and Andreassen, J., 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. Journal of Ethnopharmacology, 74(3), 257-264.

Molole, G.J., Gure, A. and Abdissa, N., 2022. Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. *BMC chemistry*, 16(1), 48.

Moneriz, C., Mestres, J., Bautista, J. M., Diez, A. and Puyet, A., 2011. Multi-targeted activity of maslinic acid as an antimalarial natural compound. *The FEBS journal*, 278(16), 2951-2961.

Mousa, A. A., El-Gansh, H. A. I., Eldaim, M. A. A., Mohamed, M. A. E. G., Morsi, A. H. and El Sabagh, H. S., 2019. Protective effect of *Moringa oleifera* leaves ethanolic extract against thioacetamide-induced hepatotoxicity in rats via modulation of cellular antioxidant, apoptotic and inflammatory markers. *Environmental Science and Pollution Research*, 26(31), 32488-32504.

Mpofu, A., Sapirstein, H.D. and Beta, T., 2006. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry*, 54(4), 1265-1270.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E. and Nyirenda, C., 2020. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*, 9, p.e00481.

Nabeelah, B.S., Montesano, D., Albrizio, S., Zengin, G. and Mahomoodally, M.F., 2020. The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations. *Antioxidants*, 9(8), 709.

Nagai, T., Shimizu, Y., Shirahata, T., Sunazuka, T., Kiyohara, H., Ōmura, S. and Yamada, H., 2010. Oral adjuvant activity for nasal influenza vaccines caused by combination of two trihydroxy fatty acid stereoisomers from the tuber of *Pinellia ternata*. *International immunopharmacology*, 10(6), 655-661.

Newman, D. J. and Cragg, G. M., 2020. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of natural products*, 83(3), 770-803.

Newton, J.M., Betts, E.L., Yiangou, L., Ortega Roldan, J., Tsaousis, A.D. and Thompson, G.S., 2021. Establishing a metabolite extraction method to study the metabolome of blastocystis using nmr. *Molecules*, 26(11), 3285.

Nitiéma, M., Soleti, R., Koffi, C., Belemnaba, L., Mallegol, P., Ouédraogo, N., and Andriantsitohaina, R. (2019). Ethyl Acetate Fraction of *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) Trunk Barks Corrects Angiotensin II-Induced Hypertension and Endothelial Dysfunction in Mice. *Oxidative medicine and cellular longevity*, 9464608.

Ogunsina, O. I., 2020. Evaluation of antiplasmodial and Immunomodulatory effect of methanolic stem bark extract of *Lannea acida.* (Doctoral dissertation), Adekunle Ajasin University, Nigeria.

Okoth, A. D., 2014. Phytochemistry and bioactive natural products from *Lannea alata, Lannea rivae, Lannea schimperi* and *Lannea schweinfurthii* (Anacardiaceae). (Doctoral dissertation) University of Kwazulu-Natal, South Africa.

Okoth, D.A., 2014.Phytochemistry and Bioactive Natural Products from *Lannea alata*, *Lannea rivae*, *Lannea schimperi* and *Lannea schweinfurthii* (Anacardiaceae). Ph.D. Thesis, University of Kwazulu-Natal, Pietermaritzburg, South Africa, 201.

Okoth, D.A., Akala, H.M., Johnson, J.D. and Koorbanally, N.A., 2016. Alkyl phenols, alkenyl cyclohexenones and other phytochemical constituents from Lannea rivae (chiov) Sacleux (Anacardiaceae) and their bioactivity. *Medicinal Chemistry Research*, 25, 690-703.

Okoth, D.A., Chenia, H.Y. and Koorbanally, N.A., 2013. Antibacterial and antioxidant activities of flavonoids from *Lannea alata* (Engl.) Engl. (Anacardiaceae). *Phytochemistry Letters* 6, 476–481.

Olatokunboh, A.O., Mofomosara, S.H. and Ekene, O.A., 2010. Evaluation of the antidiarrhoeal effect of *Lannea welwitschii* Hiern (Anacardiaceae) bark extract. *African Journal of Pharmacy and Pharmacology*, 4(4), 165-169.

Olufunmilayo, L. A., Oshiobugies, M. J. and Iyobosa, A. I., 2017. Gas Chromatography-Mass Spectrometry (GCMS) analysis of phytocomponents in the root, stem bark and leaf of *Vernonia amygdalina*. *World Journal of Pharmaceutical Research*, 6(2), 35-49.

Ono, M., Oishi, K., Abe, H., Masuoka, C., Okawa, M., Ikeda, T., and Nohara, T. (2006). New iridoid glucosides from the aerial parts of *Verbena brasiliensis*. *Chemical & pharmaceutical bulletin*, *54*(10), 1421–1424.

Osafo, N. and Boakye, Y., 2016. A Review: Ethnomedicinal, Phytochemical and Pharmacological Investigations of *Lannea welwitschii* (Hiern) Engl. *Journal of Advances in Medical and Pharmaceutical Sciences*, 11(4), 1-10.

Ouattara, L., Koudou, J., Zongo, C., Barro, N., Savadogo, A., Bassole, I.H.N., Ouattara, A.S. and Traore, A.S., 2011. Antioxidant and antibacterial activities of three species of *Lannea* from Burkina Faso. *Journal of Applied Sciences*, 11, 157–162.

Ouilly, J. T., Bazongo, P., Bougma, A., Kaboré, N., Lykke, A. M., Ouédraogo, A. and Bassolé, I. H., 2017. Chemical Composition, Physicochemical Characteristics, and Nutritional Value of *Lannea kerstingii* Seeds and Seed Oil. *Journal of analytical methods in chemistry*, 2840718.

Owolabi, M. S., Ogundajo, A. L., Dosoky, N. S. and Setzer, W. N., 2020. Chemical composition and antimicrobial potential of essential oils of leaf and stem bark of *Haematostaphis barteri* Hook. f. (Anacardiaceae). *Journal of Essential Oil Bearing Plants*, 23(3), 583-593.

Owusu, G. and Ofori-Amoah, J., 2017. Anti-inflammatory and analgesic effects of an aqueous extract of *Lannea acida* stem bark. *British Journal of Pharmaceutical Research*, 16(6), 1-8.

Pang, B., Zhu, Y., Lu, L., Gu, F. and Chen, H., 2016. The applications and features of liquid chromatography-mass spectrometry in the analysis of traditional Chinese medicine. *Evidence-Based Complementary and Alternative Medicine*, 3837270.

Patanè, G.T., Putaggio, S., Tellone, E., Barreca, D., Ficarra, S., Maffei, C., Calderaro, A. and Laganà, G., 2023. Catechins and Proanthocyanidins Involvement in Metabolic Syndrome. *International Journal of Molecular Sciences*, 24(11), 9228.

Patra, A.K., 2012. An overview of antimicrobial properties of different classes of phytochemicals. *Dietary phytochemicals and microbes*, 1-32.

Pellati, F., Orlandini, G., Pinetti, D. and Benvenuti, S., 2011. HPLC-DAD and HPLC-ESI-MS/MS methods for metabolite profiling of propolis extracts. *Journal of Pharmaceutical and Biomedical Analysis*, 55(5), 934-948.

Ponce, A. G., Fritz, R., Del Valle, C. and Roura, S. I., 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT-Food Science and Technology*, 36(7), 679-684.

Queiroz, E. F, Kuhl, C., Terreaux, C., Mavi, S. and Hostettmann, K., 2003. New dihydroalkylhexenones from *Lannea edulis*. *Journal of Natural Products,* 66, 578–580.

Rafiu, B.O., Adesanya, E.O. and Sonibare, A.M., 2019. Phytochemical screening, antimicrobial and antioxidant studies of *Lannea egregia* Engl. and K. Krause (Anacardiaceae) stem bark. *Journal of Medicinal Plants for Economic Development*, 3 (1),1-9.

Rahman, M. S., Begum, B., Chowdhury, R., Rahman, K. and Rashid, M. A., 2008. Preliminary cytotoxicity screening of some medicinal plants of Bangladesh. *Dhaka University Journal of Pharmaceutical Sciences*, 7(1), 47-52.

Rahman, M., Khatun, A., Uddin, S. J. and Shilpi, J. A., 2016. Comparative effect of *Lannea coromandelica* (Houtt.) Merr. leaves and stem barks on acetic acid induced pain model in mice and chromagenic reagents: exploring the analgesic potential and phytochemical groups. *Pharmacologyonline*, 1(1), 146-152.

Rao, P. S., Subramanayam, G. and Sridhar, P. R., 2019. Quercetin 3-galactoside from *Azadirachta indica*. *Journal of Advances in Molecular Biology*, 3(1), 1-8.

Rasheed, N.M.A., Nagaiah, K., Goud, P.R. and Sharma, V.U.M., 2012. Chemical marker compounds and their essential role in quality control of herbal medicines. *Annals of Phytomedicine,* 1(1): 1-8.

Ravi, L. and Krishnan, K., 2017. Research Article Cytotoxic Potential of N-hexadecanoic Acid Extracted from *Kigelia pinnata* Leaves. *Asian Journal of Cell Biology*, 12, 20-27.

Resida, E., Kholifah, S. N. and Roza, R. M., 2021. Chemotaxonomic study of Sumatran wild mangoes (*Mangifera* spp.) based on liquid chromatography mass-spectrometry (LC-MS). *SABRAO Journal of Breeding & Genetics*, 53(1): 27-43.

Riswanto, F. D. O., Windarsih, A., Lukitaningsih, E., Rafi, M., Fadzilah, N. A. and Rohman, A., 2022. Metabolite Fingerprinting Based on 1H-NMR Spectroscopy and Liquid Chromatography for the Authentication of Herbal Products. *Molecules*, 27(4), 1198.

Saddiqa, A., Çakmak, O. and Usman, M., 2017. Isocoumarins and 3, 4-dihydroisocoumarins, amazing natural products: a review. *Turkish Journal of Chemistry*, 41(2), 153-178.

Saleem, H., Htar, T. T., Naidu, R., Nawawi, N. S., Ahmad, I., Ashraf, M. and Ahemad, N., 2019. Biological, chemical, and toxicological perspectives on aerial and roots of *Filago germanica* (L.) huds: Functional approaches for novel phyto-pharmaceuticals. *Food and Chemical Toxicology*, 123, 363-373.

Saleem, H., Zengin, G., Locatelli, M., Abidin, S. A. Z. and Ahemad, N., 2021. Investigation of phytochemical composition and enzyme inhibitory potential of *Anagallis arvensis* L. *Natural Product Research*, 1-6.

Sameh, S., Al-Sayed, E., Labib, R. M. and Singab, A. N., 2018. Genus Spondias: A phytochemical and pharmacological review. *Evidence-Based Complementary and Alternative Medicine*, 5382904.

Sarikahya, N. B. and Kirmizigül, S., 2012. Novel biologically active glycosides from the aerial parts of *Cephalaria gazipashensis*. *Turkish Journal of Chemistry*, 36(2), 323-334.

Schulze-Kaysers, N., Feuereisen, M. M. and Schieber, A. 2015. Phenolic compounds in edible species of the Anacardiaceae family–a review. *RSC Advances*, 5(89), 73301-73314.

Sequeda-Castañeda, L. G., Celis-Zambrano, C. A. and Torrenegra, R. D., 2021. Bioassay-guided fractionation in Anacardium excelsum (bert. & balb. ex kunth) skeels (Anacardiaceae). *Pharmacologyonline*, 1, 426-446.

Sermakkani, M. and V. Thangapandian., 2012. GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2): 90-94.

Shabir, G., Saeed, A. and El-Seedi, H. R., 2021. Natural isocoumarins: Structural styles and biological activities, the revelations carry on. *Phytochemistry*, 181, 112568.

Sherfi, T. K., Ibrahim, A. A., Ahmed, A. A. A. and Omer, G. E. H. A. N., 2016. Phyto-Constituents and Antioxidant Activity of *Rhus abyssinica*, *Heeria insignis* and *Lannea schimperi* (Anacardiaceae family). *European Academic Research*, 4(8), 6493-6509.

Silva, E. P. D., David, J. M., David, J. P., Garcia, G. H. T. and Silva, M. T., 2020. Chemical composition of biological active extracts of *Tapirira guianensis* (Anacardiaceae). *Química Nova*, 43, 1216-1219.

Sobeh, M., Mahmoud, M. F., Abdelfattah, M. A., El-Beshbishy, H. A., El-Shazly, A. M. and Wink, M., 2017. Hepatoprotective and hypoglycemic effects of a tannin rich extract from *Ximenia americana* var. caffra root. *Phytomedicine*, 33, 36-42.

Sobeh, M., Mahmoud, M.F., Hasan, R.A., Abdelfattah, M.A.O., Sabry, O.M., Ghareeb, M.A., El-Shazly, A.M. and Wink, M., 2018. Tannin-rich extracts from *Lannea stuhlmannii* and *Lannea humilis* (Anacardiaceae) exhibit hepatoprotective activities *in vivo via* enhancement of the anti-apoptotic protein Bcl-2. *Scientific Reports* 8, 9343.

Soltane, R., Chrouda, A., Mostafa, A., Al-Karmalawy, A. A., Chouaïb, K., Dhahri, A. and Ali, M. A., 2021. Strong inhibitory activity and action modes of synthetic maslinic acid derivative on highly pathogenic coronaviruses: COVID-19 drug candidate. *Pathogens*, 10(5), 623.

Sreelatha, S. and Padma, P.R., 2009. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. *Plant foods for human nutrition*, 64, 303-311.

Srinivasa Rao, V., Wilkin Einstein, J. and Das, K., 2014. Hepatoprotective and antioxidant activity of *Lannea coromandelica* Linn. on thioacetamide induced hepatotoxicity in rats. *International letters of natural sciences*, 3.

Stavrianidi, A., 2020. A classification of liquid chromatography mass spectrometry techniques for evaluation of chemical composition and quality control of traditional medicines. *Journal of Chromatography. A* **1609**, 460501.

Stranska-Zachariasova, M., Kurniatanty, I., Gbelcova, H., Jiru, M., Rubert, J., Nindhia, T. G. T. and Ruml, T., 2017. Bioprospecting of *Turbinaria macroalgae* as a potential source of health protective compounds. *Chemistry & biodiversity*, 14(2), e1600192.

Sultana, S. and Ilyas, M., 1986. A flavanone from *Lannea acida*. *Phytochemistry*, 25, 963–964.

Tanahashi, T., Takenaka, Y., Nagakura, N., Inoue, K., Kuwajima, H. and Chen, C. C., 1998. Four secoiridoid glucosides from *Fraxinus insularis*. *Phytochemistry*, 49 (5), 1333-1337.

Thomford, N. E., Dzobo, K., Chopera, D., Wonkam, A., Skelton, M., Blackhurst, D. and Dandara, C., 2015. Pharmacogenomics implications of using herbal medicinal plants on African populations in health transition. *Pharmaceuticals*, 8(3), 637-663.

Tiwari, P., Pandey, R., Singh, R. and Sharma B., 2020. Role of Natural products as alternative of synthetic steroidal drugs. *Advances in Pharmaceutical Biotechnology: Recent Progress and Future Applications*, 77-89.

Tong, X., Lin, S., Fujii, M. and Hou, D. X., 2004. Molecular mechanisms of echinocystic acid-induced apoptosis in HepG2 cells. *Biochemical and biophysical research communications*, 321(3), 539-546.

Triana, J., López, M., Pérez, F. J., González-Platas, J., Quintana, J., Estévez, F. and Bermejo, J., 2005. Sesquiterpenoids from *Pulicaria c anariensis* and Their Cytotoxic Activities. *Journal of natural products*, 68(4), 523-531.

Tundis, R., Loizzo, M. R., Menichini, F., Statti, G. A. and Menichini, F., 2008. Biological and pharmacological activities of iridoids: recent developments. *Mini reviews in medicinal chemistry*, 8(4), 399-420.

Umadevi, I., Daniel, M. and Sabnis, S. D., 1988. Chemotaxonomic studies on some members of Anacardiaceae. *Proceedings: Plant Sciences*, 98(3), 205-208.

Umoh, O.T., 2020. Chemotaxonomy: The role of phytochemicals in chemotaxonomic delineation of taxa. *Asian Plant Research Journal*, 5(1), 43-52.

Uysal, S., Aumeeruddy-Elalfi, Z., Zengin, G., Aktumsek, A., Mocan, A., Custodio, L., Neng, N.R., Nogueira, J.M., Ciric, A. and Glamoclija, J., 2018. Insight into the biological properties and phytochemical composition of Ballota macrodonta Boiss. Et Balansa,−an endemic medicinal plant from Turkey. *Industrial Crops and Products*, 113, 422‑428.

Vallverdú-Queralt, A., Regueiro, J., Alvarenga, J. F. R., Martinez-Huelamo, M., Leal, L. N. and Lamuela-Raventos, R. M., 2015. Characterization of the phenolic and antioxidant profiles of selected culinary herbs and spices: caraway, turmeric, dill, marjoram, and nutmeg. *Food Science and Technology*, 35, 189-195.

Van Wyk, B. E., Oudtshoorn, B. V. and Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria, South Africa.

Venditti, A., Frezza, C., Guarcini, L., Maggi, F., Bianco, A. and Serafini, M., 2016. Reassessment of *Melittis melissophyllum* L. subsp. *melissophyllum* iridoidic fraction. *Natural Product Research*, 30 (2), 218-222.

Volpi, N. and Bergonzini, G., 2006. Analysis of flavonoids from propolis by on-line HPLC–electrospray mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, 42(3), 354-361.

Wagner, H, Bauer, R, Melchart, D, Xiao, P-G. and Staudinger, A., 2004. Chromatographic Fingerprint Analysis of Herbal Medicines: Thin-layer and High-Performance Liquid Chromatography of Chinese Drug. Germany: Springer Wien New York. Pp 903-921.

Wansi, S. L., Nyadjeu, P., Nguelefack, T. B., Fodouop, S. F., Donatien, A. A. and Kamanyi, A., 2012. In vivo antioxidant and vasodilating activities of *Gmelina arborea* (Verberaceae) leaves hexane extract. *Journal of complementary and Integrative Medicine*, 9(1).

Warra, A. A., 2019. Analyzing Physicochemical Properties of Wild Grapes (*Lannea Microcarpa*) Seed Oil. *Indonesian Journal of Computing, Engineering and Design (IJoCED)*, 1(1), 37-43.

Wright, R.J., Lee, K.S., Hyacinth, H.I., Hibbert, J.M., Reid, M.E., Wheatley, A.O. and Asemota, H.N., 2017. An investigation of the antioxidant capacity in extracts from Moringa oleifera plants grown in Jamaica. *Plants*, 6(4), 48.

Wu, H., Guo, J., Chen, S., Liu, X., Zhou, Y., Zhang, X. and Xu, X., 2013. Recent developments in qualitative and quantitative analysis of phytochemical constituents and their metabolites using liquid chromatography–mass spectrometry. *Journal of pharmaceutical and biomedical analysis*, 72, 267-291.

Wu, Y., Yin, Y., Li, Y., Guo, F. and Zhu, G., 2014. Secoiridoid/iridoid subtype bis-iridoids from *Pterocephalus hookeri*. *Magnetic Resonance in Chemistry*, 52(11), 734-738.

Yaouba, S., Koch, A., Guantai, E. M., Derese, S., Irungu, B., Heydenreich, M. and Yenesew, A., 2018. Alkenyl cyclohexanone derivatives from *Lannea rivae* and *Lannea schweinfurthii*. *Phytochemistry Letters*, 23, 141-148.

Yaouba, S., Koch, A., Guantai, E.M., Derese, S., Irungu, B., Heydenreich, M. and Yenesew, A., 2018. Alkenyl cyclohexanone derivatives from *Lannea rivae* and *Lannea schweinfurthii*. *Phytochemistry Letters*, 23, 141-148.

Yap, W. H. and Lim, Y. M., 2015. Mechanistic perspectives of maslinic acid in targeting inflammation. *Biochemistry research international*, 279356.

Yoshinari, K., Shimazaki, N., Sashida, Y. and Mimaki, Y., 1990. Flavanoexyloside and lignans from *Prunus jamasakura* bark. *Phytochemistry*, 29, 1675-1678.

Yun, X.J., Shu H.M., Chen, G.Y., Ji, M.H. and Ding, J.Y., 2014. Chemical constituents from barks of *Lannea coromandelica*. *Chinese herbal medicines*, 6(1), 65-9.

Zengin, G., Mahomoodally, M.F., Aktumsek, A., Ceylan, R., Uysal, S., Mocan, A., Yilmaz, M.A., Picot-Allain, C.M.N., Ciric, A. and Glamoclija, J., 2018. Functional constituents of six wild edible Silene species: a focus on their phytochemical profiles and bioactive properties. *Food Bioscience* 23, 75‑82.

Zhu, D., Li, B., Wang, C., Jiang, P., Tang, F. and Li, Y., 2023. Echinocystic acid induces the apoptosis and inhibits the migration and invasion of non-small cell lung cancer cells. *Medical Oncology*, 40(6), 182.

CHAPTER 5

The effect of acetone and water solvents on the appearance of metabolites in the organs of *Lannea discolor* and the evaluation of their *in vitro* antioxidant, and antibacterial activities

Abstract

Lannea discolor is a valuable medicinal plant widely used in the southern African region and due to its health-promoting properties. The phytochemicals present in the plant are responsible for these consequent health benefits it confers. Nevertheless, there is sound scientific research that supports the use of the plant, even though limitations exist because of the concern about the safety of the solvents used, and the bioavailability of the phytometabolites extracted from inorganic solvents over water. The present study compared the presence of metabolites through conventional phytochemical screening, Liquid chromatography mass spectrometry and the Folin-Ciocalteu and Aluminium chloride assays to estimate the Total phenolic (TPC) and Total flavonoid content (TFC) respectively. Furthermore, the *in vitro* antioxidant, and antibacterial activities were determined using 2,2’ -diphenyl-1-picrylhydrazyl radical (DPPH), respectively Phytochemical testing revealed the presence of hydrolysable tannins, alkaloids, triterpenoids, and flavonoids in both extracts. LC-MS led to the identification of 27 metabolites from the leaves, 10 from the stem and 20 from the roots from extracts of both solvents. Many of these compounds included phenolic acid derivatives (Gallic acid, P-Coumaric acid glucoside, Digalloyl glucose, 2-O-Caffeoyltartronic acid, Anacardoside) and flavonoids (Quercetin-3-glucuronide, Kaempferol 3-(2''-galloylglucoside), Kaempferol 3-(2''-galloylglucoside), Procyanidins and Epicatechin. TPC ranged between 121.99 - 243.90, 65.46 -176.29, and 11.31 -176.41 mg GAE/g for the leaves, stem, root water and acetone extract respectively. Furthermore, the TFC range between 149.26 -308.39, 31.07 -326.76, 189.71- 308.65 mg QE/g. Moreover, in the DPPH assay the IC_{50} value of the extract with the highest activity was 0.001541 µg/ml, relative to 2.5733 µg/ml of ascorbic acid. Most of the bacteria exhibited various levels of susceptibility to the extracts, with the acetone extracts having a remarkable MIC compared to the water extracts. These results allow the investigation of suitable non-toxic solvents for the investigation of unidentified metabolites. The derived chemical profile may facilitate the assessment of *Lannea discolor* quality for further pharmacological applications and may be used for validation of the species’ potential for its much-acclaimed traditional medicinal uses.

Keywords: flavonoids; phenolic acids; phytochemical screening; extract yield; acetone and water extracts; *Lannea discolor*.

5.1 Introduction

Traditional herbal remedies stand as one of the oldest solutions, exerting a significant impact on various alternative medicines over synthetic medicines whose ingredients are unknown. Due to their ease of access, affordability, ease of administration, and convenience, plants have gained favour on a global scale as a foundation of alternative medicine. Furthermore, traditional medicines could be a valuable option to conventional therapy in cases of negative side effects and microbial resistance (Mothibe and Sibanda, 2019). Medicinal plants can be prepared for experimentation or extracted and processed for direct use as herbal concoctions or tinctures. The latter is particularly true when working with user-friendly homemade extraction conditions, which may usually pose limitations if they were to be prepared at an industry scale (Abubakar and Haque, 2020). The idea of preparing a medicinal plant for experimental uses entails the timely and appropriate gathering of the plant, expert authentication, suitable drying, and grinding. When necessary, the bioactive ingredient is then extracted, fractionated, and isolated. It also includes figuring out how much and what kind of bioactive chemicals are present (Abubakar and Haque, 2020; Stéphane et al., 2021). Extraction mainly involves the separation of different groups of bioactive secondary metabolites from different vegetative parts of the plant, using an array of prepared solvents and techniques, and mostly depends on how the extracts are going to be used (Tiwari, 2015; Jha and Sit, 2022). One of the most important aspects that determine the turnaround of phytochemicals from plants during extraction and their subsequent health benefits is the solvent. In turn, the plant organ being extracted, the solubility of the bioactive components, polarity, and miscibility of the solvent may all influence the appearance of specific metabolites (Altemimi et al., 2017; Nawaz etal., 2020; Stéphane et al., 2021). In general, non-polar solvents such as chloroform and hexane and are used to extract non-polar metabolites, while polar solvents like water and ethanol are used to extract compounds that are inclined towards the higher polarity index (Singh et al., 2023; Takibayeva et al., 2023). Several attributes lead to the selection of a suitable extraction solvent, such as the solvents' ability to solubilise the required active ingredients, and its level of hazardousness. It is also recommended that the solvent of extraction should be feasible and be thermally stable (Stéphane et al., 2021; Zabot et al., 2021; Prasad et al., 2022).

Traditional healers have always used aqueous plant extracts for the treatment and management of different ailments. In traditional and alternative medicine, water is considered the most beneficial solvent as it is extremely polar, inexpensive, harmless, and dissolves a variety of compounds (Stéphane et al., 2021). Contrarily, its disadvantages include the induction of the breakdown of compounds, permitting the growth of germs and molds, and that, it may require a lot of heat to make a crude concentrate out of it (Das et al., 2010; Tiwari et al., 2011).

Many kinds of water products have been offered commercially, suggesting some efficacy beyond our scientific knowledge, even now at which various advanced scientific and technological research have been highly promoted. Since water is the safest and is efficient in extracting polar molecules which have been said to exhibit more antioxidant properties (Oboh et al., 2008), it may be a preferred solvent for extraction in the food industry, while in the case of less polar compounds, greater efficiency could be achieved by using organic solvents or binary solvents (Lim et al., 2019; Awad et al., 2021). Furthermore, the organic solvents used might be significantly removed at the end of extraction by rotary evaporation, however, there can be residual solvents remaining, raising a concern for quality and safety (Plaskova and Mlcek, 2023).

In scientific research, acetone has been the preferred solvent of extraction, due to its ability to solubilise both metabolites across all polarities, that impede the growth of pathogenic microorganisms (Eloff, 1998; Eloff et al., 2017; Borges et al., 2020). However, for normal traditional treatment, acetone plant extracts may not be suitable for human consumption due to several critical reasons. Acetone, commonly used as a solvent in industrial settings, may be present in plant extracts as a residue from the extraction process (Abubakar and Haque, 2020). Additionally, the drying of these extracts may not eliminate the potential risks associated with acetone, as it can concentrate any impurities or contaminants present in the original extract. Moreover, the concentration of acetone itself can become unpredictable during the drying process, leading to variations in the final product's composition. Furthermore, toxicology reports pointed out that long term exposure to acetone levels above 500 ppm by either oral or inhalation exposure in any way posed harm such as, respiratory, ocular, renal, and neurological effects (Luttrell and LaGrow, 2014; Garcia, 2000; Liu et al., 2021).

Traditionally, *Lannea discolor* has been macerated or decocted in water for use as a traditional medicine in several southern African countries (Maroyi, 2018). Previous studies have used organic solvents such as alcohols for the extraction of highly bioactive compounds from plants for further biological analysis (Chakuma et al., 2015; Kabongo-Kayoka et al., 2016; Mwamatope et al., 2020). Although the extracts have been expansively studied in these studies, no previous studies have compared solvent polarities and the influence they exert on residue yield or the appearance of the metabolites in *L. discolor*. In the present study, leaves, stem, and roots of *L. discolor* were selected to assess the impact of the extraction solvents (water and acetone) on the appearance of metabolites. An integrated strategy combined with untargeted LC-MS was established for comprehensively characterising the metabolites and simultaneously determining the antioxidant and antibacterial, potentials of leaves, stem and root extracts from both water, and acetone solvents. The results laid the theoretical basis for the potential utilization of *L. discolor* extracts in traditional medicine and pharmaceutical industries.

5.2 Materials and Methods

5.2.1 Plant Material

Leaves, stems and roots of *Lannea discolor* were collected at Vuwani in November 2018. The collected plant materials were dried over six weeks at room temperature, ground into powder using a laboratory electrical mill and thereafter stored in airtight containers for further use.

5.2.2 Extraction Process

Two commonly used solvents were used for the extraction of the organs of *Lannea discolor*, which were water and acetone (the polarity indexes are 10.2, and 5.1, respectively). Six different extracts of the dried material (1 kg) were prepared using a ratio of 1:10 (plant part powder to solvent). The extracts were prepared by maceration for 9 days with frequent shaking. The plant extracts were filtered using filter paper (Whatman 1), left out to dry and the exudates were stored in containers at 4 °C.

5.2.3 Qualitative phytochemical screening

The qualitative phytochemical screening of the extracts was performed to identify the main classes of compounds (phenols, glycosides, steroids, and terpenoids, tannins, saponins, flavonoids, alkaloids,) present in the extracts following standard protocols as described by (Tiwari et al., 2011; Dubale et al., 2023).

5.2.4 LC-MS analysis of water and acetone crude extracts of *Lannea discolor*

The solution made of 0.02 g of crude acetone and water extracts of *L. discolor* was dissolved in 2 mL of LC-MS grade methanol, centrifuged at 5000 xg for 20 minutes and filtered into a conical bottom glass insert (0.2 ml), fitted into a 2 ml vial. Mass spectrometry was used to monitor analyte elutions in negative ionization using conditions previously described by Madala and Kabanda in 2021. The m/z ranges of 100 – 1000 were used to identify and calculate corresponding molecular formulae in comparsion with those found in previous literature reports, and by searching the Knapsack Family (www.knapsackfamily.com), ChemSpider (www.chemspider.com), and Pubchem (www.pubchem.ncbi.nlm.nih.gov) databases.

5.2.5 Evaluation of total phenolic contents

The Folin–Ciocalteu reagent assay was conducted in triplicate to determine the phenolic content of each extract, using gallic acid as a standard (Herald et al., 2011; Akwu et al., 2021). An aliquot of 150 µl and 120 µl of 0.7 M Na_2CO_3 was added to 30 µl of each extract (240 µg/ml) from the different plant parts. The mixtures were allowed to incubate for approximately 30 minutes at ambient temperature. The results were expressed. A SpectraMax M3 spectrophotometer was used to determine the absorbance at 765 nm with and results were denoted as milligrams of gallic acid equivalents (GAE) per gram of dry weight using the following formula.

$$C_{tp} = C * \frac{V}{m}$$

Where, C_{tp} = Total phenolic content (mg/g) in GAE (gallic acid) equivalent

C is the concentration of gallic acid calculated from the standard curve in mg/ml

V is thevolume of *L. discolor* water/acetone extracts in ml

m is the mass of extracts used, in grams

5.2.6 Evaluation of total flavonoid contents

The TFC of *L. discolor* extracts was determined using the aluminium chloride colorimetric method described by Olajuyigbe and Afolayan in 2011. An aliquot of 100 µl of each extract was pipetted into wells of a 96-well plate. 100 µl of 1% aluminium chloride solution was added to each well. Quercetin was used to generate a standard curve. The loaded plate was incubated at room temperature for 60 minutes, after which absorbance was measured at 420 nm using a microplate reader. TFC was calculated based on the quercetin standard curve and expressed as quercetin equivalents (mg QE/g) of dry mass. The tests were performed in triplicates.

5.2.7 Minimum inhibitory concentration assay

The *L. discolor* extracts were individually tested against a panel of microorganisms, including *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 35923), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 700603) and *Escherichia coli* (ATCC 15213). For the minimum inhibitory concentration (MIC) of the extracts, a slightly modified resazurin microtiter-plate assay was used as reported by (Hussain et al., 2011). An aliquot of 100 µl of water and acetone extracts and Gentamicin and negative control (DMSO) was added to the first row of wells. After which, 50 µl of nutritional broth was added to each well followed by two-fold dilutions. An aliquot of 10 µl of Resazurin indicator solution was pipetted into each well followed by an aliquot of 10 µl of bacterial suspension. The plates were wrapped in parafilm to avoid dehydration and incubated at 34 °C overnight. A color shift from purple, to pink, then colourless, indicated the growth of the microorganism. As such the MIC value was determined by considering the lowest concentration at which a color change was observed.

5.3 Results and Discussion

5.3.1 Qualitative preliminary phytochemical screening

For this study, acetone and water are contrasted, thus, providing an inference that all tested compounds are extractable by both solvents. During preliminary tests, the presence of similar secondary metabolites was observed in the leaves, stem and bark and summarised in Table 5.1. The phytochemical tests employed indicated the presence of hydrolysable tannins, alkaloids triterpenoids, and flavonoids, which have all been suggested to infer antimicrobial, antioxidant, antiviral, anti-inflammatory, and anticancer activity (Mhlongo et al., 2018; Sabu et al., 2019; Cojandaraj et al., 2020; Ombouma et al., 2022).

Coumarins, carbohydrates, fats and saponins were absent in both acetone and water. Cardiac glycosides tested positive in all extracts indicating the ability of both the solvents to extract their class of compounds. However, the tests performed were basic and preliminary in nature and therefore required the validation of other instrumental analyses.

Table 5.1. Qualitative phytochemical screening of acetone and water extracts of the leaves, stem bark and root of *Lannea discolor*.

	Acetone extracts			Water extracts		
	Leaf	**Stem**	**Root**	**Leaf**	**Stem**	**Root**
TANNINS • **Ferric Chloride**	++	++	++	++	++	++
ALKALOIDS • **Dragendorff's**	++	++	++	++	++	++
• **Wagner's**	++	++	++	++	++	++
TRITERPENOIDS • **Salkowski's**	++	++	++	++	++	++
FLAVANOIDS • **Conc. Sulphuric acid**	++	++	++	++	++	++
• **Ammonia Test**	++	++	++	++	++	++
COUMARINS	-	-	-	-	-	-
SAPONINS • **Foam Test**	+	-	-	+	-	-
CARDIAC GLYCOSIDES • **Keller-Killiani Test**	++	++	++	++	++	++
CARBOHYDRATES • **Fehling's Test**	++	++	-	- ++	- ++	-
FATS & OILS	-	-	-	-	-	-

Intense present = ++; presence not pronounced; absent = –

5.3.2 LC-MS profiling of the metabolites in acetone and water extracts of *Lannea discolor*

Nowadays, LC-MS techniques are extensively used methods of analysis in the field of phytochemistry. They have been effectively used in the search and identification of novel bioactive secondary metabolites. An untargeted metabolomics approach was used to determine the phytochemical profile of water and acetone extracts of *L. discolor*. The molecular diversity in its vegetative organs, and the evaluation of the metabolite content were determined using high-resolution mass spectrometry. This was complemented by assessing the similarity of the MS data by comparing it with the spectral libraries and identifying potential candidates using specialised platforms such as Knapsack in negative ionisation modes. Tables 5.2 – 5.7 show the list of compounds tentatively identified for the first time in *L. discolor*, along with their retention times (RT), detected accurate mass (m/z) in negative (-) ionisation mode the molecular formula of each phytochemical, and the literature references used in the characterisation process. While Appendix Figure B1 – B6 shows chromatograms acquired after separation of compounds. Based on the library matches, phenolic acids and flavonoids were the major metabolites in all the extracts run on the LC-MS, information which corroborates with the data reported for most species in *Lannea* (Maroyi, 2019). Regarding the different plant parts, no considerable variations were observed in metabolite content.

L. discolor leaf water and acetone extracts have a total of 27 compounds in a 53 min elusion, and one of the first eluted and tentatively identified compounds was Hexahydroxydiphenic acid, a gallic acid derivative with a mass of 337.02 *m/z*, which is already reported by Suntar et al. (2019). Other phenolic acids such as Gallic acid, 2-O-Caffeoyltartronic acid, P-Coumaric acid glucoside, and Digalloyl glucose, were also present in the acetone leaf extracts and identified with the masses of 169.01 *m/z*, 281.03 *m/z*, 325.09 *m/z*, 483.08 *m/z* respectively and have been previously reported in other plants from Anacardiaceae (Picerno et al., 2006; Adebiyi et al., 2019; Rodrigues et al., 2021; Selemani et al., 2021). Dalmaisione A (587.14 *m/z*) previously identified in *Polygala dalmaisiana* (Kobayashi et al., 2002) was also identified for the first time in *L. discolor*. LC-MS also confirmed the presence of important previously reported flavonoids Quercetin 3-galacturonide, Quercetin 3-(2"-galloylgalactoside), Kaempferol 3-(2" galloylglucoside), in both water and acetone leaf extracts which were identified with masses of 615.10 m/z, 477.07 *m/z* and 599.10 *m/z* respectively, which were previously reported by (Abu-Reidah et al., 2015; Mansour et al., 2017; Shoko, 2018; Abu-Lafi et al., 2020). Other flavonoids in the water leaf extract included Nevadensin 7-rutinoside (651.19 *m/z*) identified in *Trema orientalis* Linn. (Das et al., 2023), and Catechin-3-o-rhamnoside (435.13 *m/z*) isolated previously from *Lannea kerstingii* (Stanislaus et al., 2021). In the leaf acetone extract, Icariside D1 was identified with the mass 415.16 *m/z* previously reported in *Punica granatum* (Wang et al., 2004; Basheera et al., 2021). Bistortaside, (511.11 *m/z*), previously identified in *Melastoma malabathricum* Linn. (Hosni et al., 2023), and Trigalloyl glucose (635.09 *m/z*) identified in *Pleiogynium timorense* (Sayed et al., 2010) were some of the tannins identified in leaf acetone extract. The acetone leaf extract is also considered promising sources of other phenolic compounds such as a Gallic acid derivative with *m/z* 469.06 (Bvenura and Kambizi, 2022), Protocatechuic acid with *m/z* 153.02 (Queiroz et al., 2011) and Brevifolincarboxylic acid with *m/z* 291.01 (Lee et al., 2021). From both the water and acetone extracts, 1 sesquiterpene previously identified in *Pulicaria canariensis* was given the assignment Pulicanol, with a *m/z* of 311.19 (Triana et al., 2005).

For the stem water extracts, the first compound to be identified was monogalloyl glucose (331.07 *m/z*) a hydrolysable tannin previously reported by Rodriques et al. (2021). The molecular ions at 153.02 *m/z* and 447.15 *m/z* were produced from Protocatechuic acid and Anacardoside, respectively which were previously documented (Umadevi et al.,1988; Lv et al., 2020; Queiroz et al., 2021). Three commonly known flavonoids were majorly found in the acetone stem extract and were identified as Catechin 3-O-beta-D-glucopyranoside (451.12 *m/z*), Epicatechin (289.07 *m/z*) and Procyanidin C1 at *m/z* 865.20 (Nocchi et al., 2017; Sobeh et al., 2018; Siqueira et al., 2023). Whereas the compound Myricetin 3-O-glucuronide (493.06 *m/z*), previously reported in *Rhus coriaria* L was found only in the stem water extract (Regazzoni et al., 2013). Phlorigidoside B, a phenolic acid, was also identified in the stem acetone extract at m/z 463.15 (Barrientos et al., 2020).

At the beginning of the analysis of the root water extract, a very strong signal of a compound with *m/z* 207.07 was identified as Ferulic acid, which has been previously reported in *Rhus semialata* by Ouyang et al., (2007). The ion at m/z 521 identified in both the water and acetone root extract was assigned to cyclolariciresinol hexoside, a lignan derivative identified previously in *Cissus rotundifolia* (Forssk.) Vahl. (Said et al., 2018). Tianshic acid, identified previously in *Mangifera indica*, was assigned to the *m/z* 329.23 as reported by Wilkinson et al., (2011). Flavonoids found abundantly in the acetone root extract included Procyanidin A (575.12 *m/z*), Procyanidin B (739.19 *m/z*), Epicatechin-3-O-hexoside isomer (451.12 *m/z*), three isomers with an ion at *m/z* 577.13 that were tentatively identified as Procyanidin B4 and two isomers with *m/z* 289.07 assigned to Epicatechin (Sobeh et al., 2018). Another flavonoid found in *Pistacia atlantica* subsp. *Mutica*, a plant from the family Anacardiaceae, had an m/z 357.06 and was identified as Luteolin 7-lactate (Rezaie et al., 2016). A phenolic compound with *m/z* 463.15 was identified as Phlorigidoside B (463.15 *m/z*) was also observed to be present in the root acetone extract as was observed in the stem acetone extract (Barrientos et al., 2020). Both the extractants used to carry out extraction of the leaf, stem and root brought out results that confirmed the presence of compounds with a comprehensive range of properties including antibacterial, anti-inflammatory, and antioxidant properties (Hubert et al., 2015; Mbaoji and Nweze, 2020; Akoro et al., 2022). The proposed method in this study also indicated that the acetone root extracts had more phytochemical compounds appearing on the profile, compared to the water extract profile. This may be due to polarity, and that polar compounds such as acetone have a superior extraction power in contrast to water when they are used to extract certain compounds (Borges et al., 2020).

Nevertheless, as determined by the LC-MS data, there are some compositional differences as well as similarities between the samples depending on the solvent. The data obtained revealed that the acetone extract contains large amounts of metabolites, meanwhile, the profiles obtained from the water extracts showed low signals of the presence of compounds. This could be because the active ingredients in the organic solvent are more soluble in it than in water, improving the acetone extracts' effectiveness (Plaskova and Mlcek, 2023). Water is said to extract mainly tannins, anthocyanin, terpenoids, and saponins, while acetone extracts mainly flavonoids (Rasul, 2018), however, this study observed a mirage of compound classes in both the water and acetone extracts, even though they differ in abundance. Flavonoids such as Procyanidin, Quercetin, Kaempferol Epicatechin, and Myricetin are found to be higher than all other compounds which may be responsible for elevated responses to various ailments and infections that are traditionally treated with *L. discolor* and other plants from *Lannea* (Picerno et al., 2006; Thomas-Oates et al., 2007; Sobeh et al., 2018; Mbaoji and Nweze, 2020).

Table 5.2. Tentative identification of secondary metabolites in water extracts from leaves of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Tentatively identified compounds	Class	References
1.66	337.02	$C_{14}H_{10}O_{10}$	Hexahydroxydiphenic acid gallic acid derivative	phenolic acid	*Rhus chinensis* Mill Suntar et al., 2019
2.13	169.01	$C_7H_6O_5$	Gallic acid	phenolic acid	Picerno et al., 2006
2.74	281.03	$C_{12}H_{10}O_8$	2-O-Caffeoyltartronic acid Caftaric acid	phenolic	Adebiyi et al., 2019
4.05	313.09	$C_{14}H_{18}O_8$	Vanilloside	phenolic glycoside	Ruellia patula; Samy et al., 2011
8.23	651.19	$C_{30}H_{36}O_{16}$	Nevadensin 7-rutinoside	flavonoid	Das et al., 2023
8.26	325.09	$C_{15}H_{18}O_8$	P-Coumaric acid glucoside	phenolic acid	Selemani et al., 2021 in Xeroderris stuhlmannii
9.52	483.08	$C_{20}H_{20}O_{14}$	Digalloyl glucose	phenolic acid	Rodrigues et al., 2021 *Spondias purpure*a
11.39	473.04	$C_{21}H_{14}O_{13}$	Trigallic acid	polyphenolic	Pistacia atlantica subsp. Benamar et al., 2018
12.06	369.05	$C_{15}H_{14}O_{11}$	2-O-Caffeoylhydroxycitric acid	phenolic	Corthout et al., 1992
15.21	469.00	$C_{21}H_{10}O_{13}$	Valoneic acid dilactone	hydrolysable tannin	Ghareeb et al., 2019 eucalyptus globulus
15.53	637.01	$C_{28}H_{14}O_{18}$	Gallagic acid polyphenolic	polyphenolic	
16.78	631.09	$C_{28}H_{24}O_{17}$	Myricetin 3-(2"-galloylgalactoside)	flavonoid	Mansour et al., 2017 Myrtus nivellei
20.39	615.10	$C_{28}H_{24}O_{16}$	Quercetin 3-(2"-galloylgalactoside)	flavonoid	Mansour et al., 2017 Myrtus nivellei
21.51	477.07	$C_{21}H_{18}O_{13}$	Quercetin 3-galacturonide	Flavonol glycoside	Shoko., 2018
21.95	435.13	$C_{21}H_{24}O_{10}$	catechin-3-o-rhamnoside	flavonoid	Stanislaus et al., 2021 Lannea kerstingii
23.82	599.10	C28H24O15	Kaempferol 3-(2" galloylglucoside)	flavonoid	Abu-Reidah et al., 2015 Rhus coriaria L. Abu-Lafi et al., 2020 Pistacia palaestina
26.05	587.14	$C_{28}H_{28}O_{14}$	Dalmaisione A	Phenolic glycoside	Kobayashi et al., 2002
41.39	311.19	$C_{17}H_{28}O_5$	Pulicanol	sesquiterpene	Triana et al., 2005

Table 5.3. Tentative identification of secondary metabolites in acetone extracts from leaves of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Compound	Class	References
2.13	169.01	$C_7H_60_5$	Gallic acid	phenolic acid	Picerno et al., 2006
3.20	469.06	$C_{19}H_{18}O_{14}$	Gallic acid derivative	phenolic acid	Bvenura and Kambizi, 2022
3.77	153.02	$C_7H_6O_4$	Protocatechuic acid	phenolic acid	Umadevi et al., 1988; Queiroz et al., 2011
6.88	325.09	$C_{15}H_{18}O_8$	P-Coumaric acid glucoside	phenolic acid	Selemani et al., 2021 *Xeroderris stuhlmannii*
8.14	325.09	$C_{15}H_{18}O_8$	P-Coumaric acid glucoside	phenolic acid	Selemani et al., 2021 in *Xeroderris stuhlmannii*

10.14	291.01	$C_{13}H_8O_8$	Brevifolincarboxylic acid	phenolic	Lee at al., 2021
10.63	483.08	$C_{20}H_{20}O_{14}$	Digalloyl glucose	phenolic acid	Rodrigues et al., 2021 *Spondias purpurea*
10.82	511.11	$C_{22}H_{24}O_{14}$	Bistortaside	tannin	Hosni et al., 2023
13.53	635.09	$C_{27}H_{24}O_{18}$	Trigalloyl glucose	tannin	Al Sayed et al 2010
13.53	415.16	$C_{19}H_{28}O_{10}$	Icariside D1	flavonoid	Basheera et al; Wang et al., 2004
17.30	477.10	$C_{22}H_{22}O_{12}$	0-Methoxy myricetin 3-O-α-L-rhamnopyranoside	flavonoid	Picerno et al., 2006 *Lannea microcarpa*
17.88	493.06	$C_{21}H_{18}O_{14}$	Myricetin 3-glucuronide	flavonoid	Regazzoni et al., 2013 *Rhus coriaria* L
18.55	477.10	$C_{22}H_{22}O_{12}$	0-Methoxy myricetin 3-O-α-L-rhamnopyranoside	flavonoid	Picerno et al., 2006 *Lannea microcarpa*
20.38	615.10	$C_{28}H_{24}O_{16}$	Quercetin 3-(2"-galloylgalactoside)	flavonoid	Mansour et al., 2017 *Myrtus nivellei*
21.50	477.07	$C_{21}H_{18}O_{13}$	Quercetin-3- glucoronide	flavonoid	Shoko ., 2018
22.59	599.10	$C_{28}H_{24}O_{15}$	Kaempferol 3-(2"-galloylglucoside)	flavonoid	Abu-Reidah et al., 2015 *Rhus coriaria* L. Abu-Lafi et al., 2020 *Pistacia palaestina*
23.78	599.10	$C_{28}H_{24}O_{15}$	Kaempferol 3-(2" galloylglucoside)	flavonoid	Abu-Reidah et al., 2015 *Rhus coriaria* L. Abu-Lafi et al., 2020 *Pistacia palaestina*
25.30	615.10	$C_{28}H_{24}O_{16}$	Quercetin 3-(2"-galloylgalactoside)	flavonoid	Mansour et al., 2017 *Myrtus nivellei*
25.97	615.10	$C_{28}H_{24}O_{16}$	Quercetin 3-(2"-galloylgalactoside)	flavonoid	Mansour et al., 2017 *Myrtus nivellei*
41.25	311.19	$C_{17}H_{28}O_5$	Pulicanol	sesquiterpene	Triana et al., 2005

Table 5.4. Tentative identification of secondary metabolites in water extracts from the stem of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Compound	Class	References
1.24	331.07	$C_{13}H_{16}O_{10}$	monogalloyl glucose	tannin	Rodrigues et al., 2021
3.78	153.02	$C_7H_6O_4$	Protocatechuic acid	phenolic acid	Umadevi et al., 1988; Queiroz et al., 2011
5.32	447.15	$C_{19}H_{28}O_{12}$	Anacardoside	phenolic acid	Lv et al., 2020
18.02	493.06	$C_{21}H_{18}O_{14}$	Myricetin 3-O-glucuronide	flavonoid	Regazzoni et al., 2013 *Rhus coriaria* L

Table 5.5. Tentative identification of secondary metabolites in acetone extracts from the stem of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Compound	Class	References
3.79	153.02	$C_7H_6O_4$	Protocatechuic acid	phenolic acid	Umadevi et al., 1988; Queiroz et al., 2011
5.30	477.15	$C_{19}H_{28}O_{12}$	Anacardoside	phenolic acid	Lv et al., 2020
5.99	463.15	$C_{19}H_{28}O_{13}$	Phlorigidoside B	phenolic acid	Barrientos et al, 2020
6.74	497.13	$C_{22}H_{26}O_{13}$	10-O-Protocatechuyl-catalpol	Phenolic acid	Castro et al., 2020 *Myracrodruon urundeuva*
6.77	451.12	$C_{21}H_{24}O_{11}$	Catechin 3-O-beta-D-glucopyranoside	flavonoid	Siqueira et al 2023; *Rhamnidium elaeocarpum*
11.37	289.07	$C_{15}H_{14}O_6$	Epicatechin	flavonoid	Sobeh et al., 2018 *Lannea stuhlmannii*
12.63	865.20	$C_{45}H_{38}O_{18}$	Procyanidin C1	flavonoid	Nocchi et al., 2017 *Schinus terebinthifolia*
19.75	491.19	$C_{25}H_{32}O_{10}$	epi-12-Palmatoside G	glycoside	

Table 5.6. Tentative identification of secondary metabolites in water extracts from the root of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Compound	Class	References
9.14	207.07	$C_{11}H_{12}O_4$	Ferulic acid methyl ester	Hydroxycinnamic acid	Ouyang et al., 2007 *Rhus semialata*
18.40	551.21	$C_{27}H_{36}O_{12}$	Nudiposide	aromatic glucosides	Sadhu et al., 2007 *Saraca asoca*
19.18	521.20	$C_{26}H_{34}O_{11}$	cyclolariciresinol hexoside	lignan	Said et al., 2018
45.45	329.23	$C_{18}H_{34O5}$	Tianshic acid	Fatty acid	Wilkinson et al., 2011 *Mangifera indica*

Table 5.7. Tentative identification of secondary metabolites in acetone extracts from the root of *L. discolor* by LC-MS.

RT (min)	M/Z	Molecular formula	Compound	Class	References
2.10	169.01	$C_7H_6O_5$	Gallic acid	phenolic acid	Picerno et al., 2006
3.76	153.02	$C_7H_6O_4$	Protocatechuic acid	Phenolic acid	Umadevi et al., 1988; Queiroz et al., 2011
5.26	447.15	$C_{19}H_{28}O_{12}$	Anacardoside	phenolic acid	Lv et al., 2020
5.54	739.19	$C_{36}H_{36}O_{17}$	Procyanidin B	Flavonoid	Siqueira et al., 2023 *Rhamnidium elaeocarpum*

5.96	463.15	$C_{19}H_{28}O_{13}$	Phlorigidoside B	phenolic acid	Barrientos et al, 2020
6.48	577.13	$C_{30}H_{26}O_{12}$	Procyanidin B4	Flavonoid	Siqueira et al., 2023 *Rhamnidium elaeocarpum*
7.16	289.07	$C_{15}H_{14}O_6$	Epicatechin	Flavonoid	Sobeh et al., 2018 L. stuhlmannii
7.38	577.13	$C_{30}H_{26}O_{12}$	Procyanidin B4	Flavonoid	Siqueira et al., 2023 *Rhamnidium elaeocarpum*
9.85	577.13	$C_{30}H_{26}O_{12}$	Procyanidin B4	Flavonoid	Siqueira et al., 2023 *Rhamnidium elaeocarpum*
10.40	575.12	$C_{30}H_{24}O_{12}$	Procyanidin A	Flavonoid	Nocchi et al., 2017 *Schinus terebinthifolia*
11.37	357.06	$C_{18}H_{14}O_8$	Luteolin 7-lactate flavonoid	Flavonoid	*Pistacia atlantica* subsp. *Mutica* Rezaie et al., 2016
11.50	289.07	$C_{15}H_{14}O_6$	Epicatechin	Flavonoid	Sobeh et al., 2018 *Lannea stuhlmannii*
17.53	521.20	$C_{26}H_{34}O_{11}$	cyclolariciresinol hexoside	lignan	Said et al., 2018
19.26	521.20	$C_{26}H_{34}O_{11}$	cyclolariciresinol hexoside	lignan	Said et al., 2018
19.87	491.19	$C_{25}H_{32}O_{10}$	epi-12-Palmatoside G	glycoside	
21.23	451.12	$C_{21}H_{24}O_{11}$	Epicatechin-3-O-hexoside isomer	Flavonoid	Siqueira et al., 2023 *Rhamnidium elaeocarpum*
41.63	535.14	$C_{25}H_{27}O_{13}$	Malvidin 3-(6"-acetylglucoside)	phenolic	Bavaresco et al., 2016
46.58	285.21	$C_{16}H_{30}O_4$	1,16-Hexadecanedioic acid	Fatty acid	-

5.3.3 Quantitative phytochemical screening

5.3.3.1 Impact of Extraction Solvents on extraction yield, Total phenolic, flavonoid content, and antioxidant activity

The extraction yield of the metabolites from plant materials is known to be dependent on the ratio of solvent to raw material, which is an important factor. The results in Table 5.8 indicated that between the two solvents, the extraction yield was similar, and this may suggest that both solvents may be suitable for optimizing extraction yield from *L. discolor*. Among solvents tested, water resulted in a satisfactory extraction yield 10 % for the leaves, followed by acetone 6.9% for the stem, which can be attributed to their capability to dissolve polar along with non-polar molecules. Also, polarity exerts a substantial part in increasing the recovery of compounds (Truong et al., 2019). The lower variation in the yields from both solvents might be attributed to the solubility of the extractable components, depending on the different chemical composition of plant organs. Literature is scarce concerning yields or any data concerning *L. discolor* extracts.

A quantitative study was further conducted to detect the amount of the total flavonoids and total phenols in the extracts for direct comparison. Results acquired showed that extraction solvents had a positive

impact on the extraction yields of the TPC and TFC from plant parts of *L. discolor*. A consistent variation in the phenolic content in the experimental group between solvents used to extract and the plant parts extracted was observed and the observations are summarised in Table 5.9. The TPC from the acetone varied widely from 243.90± 0.053, 176.29±0.053, 176.41± 0.053 (mg GAE/g) for the leaves, stem, and roots respectively. In the water extracts, the TPC of the leaves was 121.99±0.128 mg GAE/g, but lower in the stem and root extracts at 65.46±0.128 and 11.31± 0.1287 mg GAE/g. The highest TPC was observed in the acetone extracts, while it appeared lower in the water extracts. The findings indicated that phenolics have a substantial role in the pharmacological properties of *L. discolor* and are easily extracted by acetone. The TFC values acquired from the quercetin standard are summarised in Table 5.9. The acetone extracts exhibited notably high flavonoid content (308.39 ±0.013, 326.76 ±0.013, 308.65± 0.013 mg QE/g), for the leaves, stem, and root respectively, followed by the water extracts at 149.26 ±0.104, 31.07 ±0.104, 189.71±0.104 mg QE/g respectively. Maximum flavonoids were found to be consisted mostly of acetone extracts compared to the water extracts. These findings further emphasize the consideration of polarity when choosing a solvent considering that a slightly non-polar extraction solvent maybe more appropriate in the extraction of phenolic compounds from *L. discolor*. Furthermore, acetone seems to be the most suitable solvent for maximum extraction of TPC and TFC compared to water, which is a highly polar solvent. The present observation is substantiated by similar studies that also found that acetone can significantly affect the extraction yields of both the TPC and TFC in plants which are notably higher than their counterparts (Lamien-Meda et al., 2008; Mwamatope et al., 2020). Some authors defended the use of 70% (v/v) acetone, having stated that organic solvents without added water may be inefficient for the extraction of phenols (Skotti et al., 2014). However, the contrary was perceived in this study. The TPC and TFC of plant extracts was positively correlated with total antioxidant capacity summarised in Table 5.10. The antioxidant activities of the plant extracts were analysed by DPPH assay in comparison to the synthetic ascorbic acid (IC_{50}= 2.57 µg/mL). The IC_{50} of the acetone extracts (IC_{50}= 1.76 µg/mL) was lower than that of the standard, and the water extracts, denoting the influence of the extraction power of acetone over water. Other studies demonstrated that numerous phenolic and flavonoids contribute significantly to the total antioxidant activity acquired from plants, which is consistent with the findings of this study (Kumar and Jain, 2015). The variances can be attributed to the solvents' varying polarity, which extracts various phytochemicals across all polarities from the sample in a selective manner. This emphasizes the significance of exploring and identifying the solvents and extraction techniques that maybe bring out optimal results for each sample type.

Table 5.8. Extraction yield of *L. discolor* extracts.

Extract	100 % Water			100% Acetone		
	leaves	stem	root	leaves	stem	root
Color and nature	Dark green and gummy	Reddish brown and powdery solid	Reddish brown and powdery solid	Dark green, solid, and pasty	Reddish brown and resinous	Reddish brown and resinous
Extraction yield (% w/w)	10%	4.8%	3.9%	3%	6.9%	1.1%

Table 5.9. Quantification of Total Polyphenol and Flavonoid Contents from acetone and water extracts of *L. discolor*.

Samples (extracts)		Total Phenolic content (mg GAE/g extract)	Total flavonoid content (mg QE/g extract)
Acetone	leaves	243.90± 0.053	308.39 ±0.013
	stem	176.29±0.053	326.76 ±0.013
	root	176.41± 0.053	308.65±0.013
Water	leaves	121.99±0.128	149.26 ±0.104
	stem	65.46±0.128	31.07 ±0.104
	root	11.31± 0.128	189.71±0.104

All values were expressed as the mean of triplicates ± standard deviation (SD).

Table 5.10. Antioxidant activity of methanolic, acetone and water extracts of *Lannea discolor* using 2, 2-diphenyl1-pycrilhydrazil hydrate.

Sample		**IC_{50} ± SD µg/ml (DPPH)**
Acetone	leaves	1.761557 ± 0.030
	stem	0.453794 ± 0.020
	root	0.044672 ± 0.015
Water	leaves	0.001541 ± 0.007
	stem	29.10649 ± 0.059
	root	97.16748 ± 0.059
Standard		2.573369 ± 0.045

All values were expressed as the mean of triplicates ± standard deviation (SD).

5.3.4 Antibacterial activities of water and acetone extracts of *Lannea discolor*

Table 5.11 presents the minimum concentrations of the extract at which the highest antibacterial activity on the test organisms was recorded. The concentration of 0.78 mg/ml, followed by 1.56 mg/ml, 6.25 mg/ml and 12.5 mg/ml is the minimum at which the growth of *B. subtilis, P. aeruginosa, K. pneumoniae and E. coli* were inhibited when tested against acetone. The water extracts contributed moderately to the activity against *S. aureus* (50 mg/ml), *B subtilis* (25 mg/ml), *E. coli* (50 mg/ml), *P. aeruginosa* (0.78 mg/ml), and *K. pneumoniae* (6.25 mg/ml). The results of the study clearly demonstrated that *L. discolor* acetone extracts had a marked influence on the viability of *B. subtilis* and *P. aeruginosa* strain. In comparing the antibacterial activities of the acetone and water extracts, the activity varied fairly between the different plant parts. Generally, a difference in activity was observed between the acetone and water extracts, while for both solvents, it was also demonstrated that extracts did not have an appreciable influence on the inhibition of the growth of *S. aureus*. The resulting biological activity of the plant

extracts are greatly influenced by the overall properties of the extracting solvent due to the occurrence of different highly active compounds with a variation of properties and polarities that may or may not render them soluble in each solvent (Lefebvre et al., 2020). Eloff (1998) reported that acetone is the most suitable extractant for the screening of antimicrobial components in plants, validating the significant activity of the acetone extracts against the tested microorganisms (Kotze and Eloff, 2002). Thus, the two solvents employed in the study were chosen based on polarity differences as well as safety considerations. Moreover, from a toxicological point of view, water is safer than acetone which potentiate the acetonitrile poisoning, and other organic solvents which have been said to cause neurotoxicity (Joshi and Adhikari, 2019). No substantial activity was observed in the water extracts for all the plant parts, this may be because active compounds may be more soluble and more concentrated in the acetone extracts (Feng et al., 2019). Although water extracts had the ability to inhibit some bacteria as summarised, acetone had a higher ability to prevent the growth of all the organisms tested.

Table 5.11. MIC values in mg/ml of *Lannea discolor* after incubation against different bacterial microorganisms.

	MIC values (mg/ml)							
	Acetone			Water			Positive control	DMSO (10 %)
Organisms	leaf	stem	root	leaf	stem	root	Gentamicin	
S. aureus	25	25	25	50	50	50	0.39	ND
B. subtilis	0.78	0.78	0.78	12.5	25	25	0.39	ND
P. aeruginosa	1.56	0.78	1.56	12.5	12.5	25	0.39	ND
E. coli	12.5	12.5	12.5	50	ND	ND	0.39	ND
K. pneumoniae	6.25	6.25	6.25	25	25	25	0.39	ND

MIC = Minimum inhibitory concentration (mg/ml). Gentamicin was the positive control for bacteria.

5.3.5 Conclusion

In the current study, the LC-MS analysis led to the tentative identification of 57 compounds in the water and acetone extracts of *L. discolor*, based on the determination of the precise mass and molecular formulas obtained. Among the tentatively identified compounds, flavonoids and phenols were the major constituents. Moreover, acetone extracts demonstrated considerably high appearance of metabolites, significant TPC, TFC, as well as significant antioxidant and antibacterial activity compared to the water extracts. This study suggests that the extraction solvent can affect the phytochemical profile and the antioxidant activity of plant extracts. In contrast to the mainstream solvents currently used in modern herbal manufactures especially, pure-water-extracted materials should be reconsidered and could be reemerged for future studies and for the manufacture of traditional medicines. These are promising results that water extraction could be used to obtain a better yield of phytochemicals. However, it would also be worthwhile to optimize water extraction for development of an environmentally friendly procedure that brings about more phytochemicals that may infer more biological activity.

5.4 References

Abubakar, A. R., and Haque, M. 2020. Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes. *Journal of pharmacy & bioallied sciences*, *12*(1), 1.

Akoro, S. M., Omotayo, M. A., Ogundare, O. C., Akpovwovwo, S. A., and Bello, G. P. 2022. Phytochemical Analysis, Antioxidant and Cytotoxic Activity of *Lannea egregia* Engl. & K. Krause Stem Bark Extracts. *Pharmaceutical Sciences and Research*, *9*(3), 5.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, *6*(4), 42.

Awad, A.M., Kumar, P., Ismail-Fitry, M.R., Jusoh, S., Ab Aziz, M.F., Sazili, A.Q. 2021. Green extraction of bioactive compounds from plant biomass and their application in meat as natural antioxidant. *Antioxidants*. 10:1465. doi: 10.3390/antiox10091465.

Barrientos, R., Fernández-Galleguillos, C., Pastene, E., Simirgiotis, M., Romero-Parra, J., Ahmed, S., and Echeverría, J. 2020. Metabolomic analysis, fast isolation of phenolic compounds, and evaluation of biological activities of the bark from Weinmannia trichosperma Cav.(Cunoniaceae). *Frontiers in Pharmacology*, *11*, 780.

Basheera, S., Sivanandan, S., and Kamalan, B. C. 2021. Anti-tuberculosis activity in *Punica granatum*: In silico validation and identification of lead molecules. *Indian Journal of Pharmaceutical Sciences*, *83*(2), 316-330.

Bavaresco, L., De Rosso, M., Gardiman, M., Morreale, G., and Flamini, R. 2016. Polyphenol metabolomics of twenty Italian red grape varieties. In *BIO Web of Conferences* (Vol. 7, p. 01022). EDP Sciences.

Borges, A., José, H., Homem, V., and Simões, M. 2020. Comparison of Techniques and Solvents on the Antimicrobial and Antioxidant Potential of Extracts from *Acacia dealbata* and *Olea europaea*. *Antibiotics*, *9*(2), 48.

Chakuma, N., Chipurura, B., Muchuweti, M., Chitindingu, K., Bhebhe, M., and Chagonda, L. 2015. Total phenolic content, free radical scavenging, and antioxidant potential of *Lannea discolor* (Sond.) Engl bark and root extracts. Journal of Biologically Active Products from Nature, 5(1), 71-77.

Cojandaraj, L. and Milton, M. J., 2020. Phytochemical screening, GC-MS analysis, antioxidant activity and in vitro anticancer activity of leaf extract of *Semecarpus Anacardium*. Linn (Anacardiaceae). *Journal of Advanced Scientific Research*, 11(02), 181-186.

Das, K., Tiwari, R. K. S., and Shrivastava, D. K. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *Journal of medicinal plants research*, *4*(2), 104-111.

Das, R., Agrawal, S., Kumar, P., Singh, A. K., Shukla, P. K., Bhattacharya, I., and Tripathi, A. K. 2023. Network pharmacology of apigeniflavan: a novel bioactive compound of *Trema orientalis* Linn. in the treatment of pancreatic cancer through bioinformatics approaches. *3 Biotech*, *13*(5), 160.

Drosou, C., Kyriakopoulou, K., Bimpilas, A., Tsimogiannis, D., and Krokida, M. 2015. A comparative study on different extraction techniques to recover red grape pomace polyphenols from vinification byproducts. *Industrial Crops and Products*, *75*, 141-149.

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., and Suleman, S. 2023. Phytochemical screening and antimicrobial activity evaluation of selected medicinal plants in Ethiopia. Journal of Experimental Pharmacology, 51-62.

Eloff, J. N. 1998. Which Extractant Should Be Used for the Screening and Isolation of Antimicrobial Components from Plants. *J. Ethnopharmacol* 60, 1–8. doi:10.1016/s0378-8741(97)00123-2

Eloff, J. N., Angeh, I. E., and McGaw, L. J. 2017. Solvent-solvent fractionation can increase the antifungal activity of a *Melianthus comosus* (Melianthaceae) acetone leaf extract to yield a potentially useful commercial antifungal product. *Industrial Crops and Products*, *110*, 103-112.

Garcia, H. D. 2000. B1 ACETONE. *Spacecraft Maximum Allowable Concentrations for Selected Airborne Contaminants: Volume 4*, *4*, 17. National Academy Press; Washington D.C, USA.

Hosni, S., Gani, S. S. A., Orsat, V., Hassan, M., and Abdullah, S. 2023. Ultrasound-Assisted Extraction of Antioxidants from *Melastoma malabathricum* Linn.: Modeling and Optimization Using Box–Behnken Design. *Molecules*, *28*(2), 487.

Hubert, B. J., Adama, H., Moussa, C., Ahmed, C. Y., Martin, K., and Germaine, N. O. 2015. Anti-inflammatory activities of fruit and leaves extract of *Lannea microcarpa* england K. Kraus (Anacardiaceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*, 177-82.

Hussain, A.I., Anwar, F., Nigam, P.S., Sarker, S.D., Moore, J.E., Rao, J.R. and Mazumdar, A., 2011. Antibacterial activity of some Lamiaceae essential oils using resazurin as an indicator of cell growth. *LWT-Food Science and Technology*, 44(4), 1199-1206.

Jha, A. K., and Sit, N. 2022. Extraction of bioactive compounds from plant materials using combination of various novel methods: A review. *Trends in Food Science & Technology*, 119, 579-591.

Joshi, D. R., and Adhikari, N. 2019. An overview on common organic solvents and their toxicity. *Journal of Pharmaceutical Research International*, *28*(3), 1-18.

Kabongo-Kayoka, P. N., Eloff, J. N., Obi, C. L., and McGaw, L. J. 2016. Antimycobacterial activity and low cytotoxicity of leaf extracts of some African Anacardiaceae tree species. Phytotherapy Research, 30(12), 2001-2011.

Kai Dührkop, Louis-Félix Nothias, Markus Fleischauer, Raphael Reher, Marcus Ludwig, Martin A. Hoffmann, Daniel Petras, William H. Gerwick, Juho Rousu, Pieter C. Dorrestein and Sebastian Böcker Systematic classification of unknown metabolites using high-resolution fragmentation mass spectra Nature Biotechnology, 2020. https://doi.org/10.1038/s41587-020-0740-8

Kobayashi, S., Miyase, T., and Noguchi, H. 2002. Polyphenolic Glycosides and Oligosaccharide Multiesters from the Roots of *Polygala dalmaisiana*. *Journal of natural products*, *65*(3), 319-328.

Kumar, T., and Jain, V. 2015. Appraisal of total phenol, flavonoid contents, and antioxidant potential of folkloric *Lannea coromandelica* using in vitro and in vivo assays. *Scientifica*, 203679.

Lee, B. Z., Kim, C. S., Jeong, S. K., Lee, S., Lee, I. S., and Hong, K. 2021. Anti-inflammatory isocoumarins from the bark of *Fraxinus chinensis* subsp. rhynchophylla. *Natural Product Research*, 35(22), 4380-4387.

Lefebvre, T., Destandau, E., and Lesellier, E. 2021. Selective extraction of bioactive compounds from plants using recent extraction techniques: A review. *Journal of Chromatography A*, 1635, 461770.

Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Taboada, E. B., and Lacks, D. J. 2019. Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol–water binary solvent systems. *Journal of food science and technology*, 56, 2536-2544.

Liu, D., Pervaiz, E., Adimi, S., Thomas, T., Qu, F., Huang, C., Wang, R., Jiang, H., and Yang, M. 2021. Theoretical study on W-Co3O4 (111) surface: Acetone adsorption and sensing mechanism. *Applied Surface Science*, 566, 150642.

Luttrell, W. E., and LaGrow, A. L. 2014. Acetone. *Journal of Chemical Health & Safety*, *21*(3), 29-31.

Madala, N. E., and Kabanda, M. M. 2021. LC-MS based validation and DFT investigation on the antioxidant properties of clovamide:• OH and• OOH scavenging and Cu (II) chelation mechanisms. *Journal of Molecular Structure*, 1236, 130349.

Maroyi, A. 2019. Review of ethnomedicinal, phytochemical and pharmacological properties of *Lannea schweinfurthii* (Engl.) Engl. *Molecules*, 24(4), 732.

Mbaoji, F. N., and Nweze, J. A. 2020. Antioxidant and hepatoprotective potentials of active fractions of *Lannea barteri* Oliv. (Anarcadiaceae) in rats. *Heliyon*, 6(6).

Mhlongo, N. Y., Naidu, K. S. B., Himakar, R. K., Cheriti, A. and Govender, P., 2018. Phytochemical screening, antioxidant, and antimicrobial efficacy of *Protorhus longifolia* (Bernh. Ex C. krauss) Engl. (Anacardiaceae) seed extracts. *Current Trends in Biotechnology and Pharmacy*, 12(2), 128-138.

Mothibe, M. E., and Sibanda, M. 2019. African traditional medicine: South African perspective. *Traditional and Complementary Medicine*, *2019*, 1-27.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., and Nyirenda, C. 2020. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*, 9, e00481.

Mwamatope, B., Tembo, D., Chikowe, I., Kampira, E., and Nyirenda, C. 2020. Total phenolic contents and antioxidant activity of *Senna singueana*, *Melia azedarach*, *Moringa oleifera* and *Lannea discolor* herbal plants. *Scientific African*, 9, e00481.

Nawaz, H., Shad, M. A., Rehman, N., Andaleeb, H., and Ullah, N. 2020. Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56, e17129.

Oboh, G., Raddatz, H., and Henle, T. 2008. Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. *Journal of the Science of Food and Agriculture*, *88*(14), 2486-2492.

Ombouma, J. G., Mebale, A. J. A., Abessolo, D. D. M., Mve, O. A. and Mboma, R., 2022. Phytochemical screening, total polyphenols and flavonoids content and antiradical activity of methanolic extract of *Lannea welwitschii* (Hiern) Engl. (Anacardiaceae) from Gabon. *International Journal of Biological and Chemical Sciences*, 16(1), 308-314.

Ouyang, M. A., Wein, Y. S., Su, R. K., and Kuo, Y. H. 2007. Rhusemialins A—C, new cyclolignan esters from the roots of *Rhus javanica* var. roxburghiana. *Chemical and pharmaceutical bulletin*, *55*(5), 804-807.

Picerno, P., Mencherini, T., Loggia, R. D., Meloni, M., Sanogo, R., and Aquino, R. P. 2006. An extract of *Lannea microcarpa*: composition, activity, and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. *Journal of pharmacy and pharmacology*, *58*(7), 981-988.

Plaskova, A., and Mlcek, J. 2023. New insights of the application of water or ethanol-water plant extract rich in active compounds in food. *Frontiers in Nutrition*, *10*, 1118761.

Prasad, W., Wani, A. D., Khamrui, K., Hussain, S. A., and Khetra, Y. 2022. Green solvents, potential alternatives for petroleum-based products in food processing industries. *Cleaner Chemical Engineering*, 100052.

Rasul, M. G. 2018. Conventional extraction methods use in medicinal plants, their advantages, and disadvantages. *International Journal of Basic Sciences and Applied Computing*, 2, 10-14.

Sabu, V., Peter, J., Sasidharan Nair, A. I. B., Krishnan, S., Sathyaseelan Suja, L. P., Helen, A. and Radhakrishna Pillai, G., 2019). Combinatorial action of triterpenoid, flavonoid, and alkaloid on inflammation. *Natural Product Communications*, 14(8), 1-9.

Sayed, E. A., Martiskainen, O., Sinkkonen, J., Pihlaja, K., Ayoub, N., Singab, A. E. N., and El-Azizi, M. (2010). Chemical composition and bioactivity of *Pleiogynium timorense* (Anacardiaceae). *Natural Product Communications*, *5*(4), 1934578X1000500410.

Singh, S.K., Shrivastava, S., Mishra, A.K., Kumar, D., Pandey, V.K., Srivastava, P., Pradhan, B., Behera, B.C., Bahuguna, A. and Baek, K.H., 2023. Friedelin: Structure, Biosynthesis, Extraction, and Its Potential Health Impact. *Molecules*, *28*(23), 7760.

Skotti, E., Anastasaki, E., Kanellou, G., Polissiou, M., and Tarantilis, P. A. 2014. Total phenolic content, antioxidant activity and toxicity of aqueous extracts from selected Greek medicinal and aromatic plants. *Industrial Crops and Products*, *53*, 46-54.

Sobeh, M., Mahmoud, M. F., Hasan, R. A., Abdelfattah, M. A., Sabry, O. M., Ghareeb, M. A., and Wink, M. 2018. Tannin-rich extracts from *Lannea stuhlmannii* and *Lannea humilis* (Anacardiaceae) exhibit hepatoprotective activities in vivo via enhancement of the anti-apoptotic protein Bcl-2. *Scientific reports*, *8*(1), 9343.

Stéphane, F.F.Y., Jules, B.K.J., Batiha, G.E.-S., Ali, I., and Bruno, L.N. 2021. Extraction of Bioactive Compounds from Medicinal Plants and Herbs. In *Medicinal Plants from Nature*; IntechOpen: London, UK, 2021. DOI: 10.5772/intechopen.98602.

Takibayeva, A. T., Zhumabayeva, G. K., Bakibaev, A. A., Demets, O. V., Lyapunova, M. V., Mamaeva, E. A., and Ibrayev, M. K. 2023. Methods of Analysis and Identification of Betulin and Its Derivatives. *Molecules*, *28*(16), 5946.

Thomas-Oates, E. B., Reubsaet, L., Diallo, D., and Paulsen, B. S. 2007. Cell protective antioxidants from the root bark of *Lannea velutina* A. Rich., a Malian medicinal plant. *Journal of Medicinal Plants Research*, *1*(4), 066-079.

Tiwari, P., Kumar, B., Kaur, M., Kaur, G., and Kaur, H. 2011. Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., and Nguyen, H. C. 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. *Journal of food quality*, 2019, 1–9.

Wang, R. F., Xie, W. D., Zhang, Xing, D. M., Ding, Y., Wang, W., and Du, L. J. 2004. Bioactive Compounds from the Seeds of *Punica granatum* (Pomegranate). *Journal of Natural Products*, *67*(12), 2096-2098.

Zabot, G. L., Viganó, J., and Silva, E. K. 2021. Low-frequency ultrasound coupled with high-pressure technologies: Impact of hybridized techniques on the recovery of phytochemical compounds. *Molecules*, *26*(17), 5117.

CHAPTER 6

Green Synthesis of Gold and Copper Nanoparticles by *Lannea discolor:* Characterization and Antibacterial Activity

Abstract

Green synthesis using plant extracts has emerged as an eco-friendly, clean, and viable alternative to chemical and physical approaches. Herein, the leaf, stem, and root extracts of *Lannea discolor* were utilised as a reducing and stabilising agent in synthesizing gold (AuNPs) and copper (CuNPs) nanoparticles. The formation of AuNPs and CuNPs, confirmed by their color change, was characterised by UV-Vis spectroscopy (UV-Vis), scanning electron microscopy analysis, and energy-dispersive X-ray (SEM-EDX), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). Furthermore, a minimum inhibitory concentration (MIC) antibacterial assay was carried out. Gold nanoflowers (AuNFs), NPs, and CuNPs peaked at wavelengths of 316, 544, and 564 nm, respectively. TEM showed unexpected nanoflowers (30-97 nm) in the leaf extracts and spherical NPs (10–33 nm; 9.3–37.5 nm) from stem and root extracts, while spherical CuNPs (20–104 nm) were observed from all the extracts. EDX confirmed the presence of metal salts, and FTIR revealed stable capping agents. AuNPs and NFs from *L. discolor* extracts showed appreciable antibacterial activity against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603), and *Bacillus subtilis* (ATCC 6633) when compared to the plant extracts. At the same time, none was observed from the CuNPs. These AuNPs and CuNPs are particularly appealing in various biomedical and conductivity manufacturing applications due to their shapes, sizes, and economical and environmentally friendly production. To our knowledge, this is the first study of the synthesis of gold and copper nanoparticles from *L. discolor*.

Keywords: gold nanoflowers; plant extracts; nanosynthesis; transmission electron microscopy (TEM)

6.1 Introduction

Nanotechnology, a globally active research discipline, is rapidly progressing, with nanoparticles being a topic of interest since the 1970s (Malike et al., 2023). Nanoparticles (NPs) are microscopic particles with at least one dimension less than 100 nm. They can be categorized into different types according to morphology, size, physicochemical properties, and the type of precursor from which they are synthesized (Khan et al., 2019). Metal nanoparticles (NPs) like gold and copper are created from metal precursors using synthetic or chemical methods, which may use environmentally unfriendly reducing agents (Szczyglewska et al., 2023). This has resulted in scientists turning to safer synthesis methods by employing biological reductants such as bacterial, fungal, and plant material to curb any possible negative effects from nanosynthesis (Singh et al., 2018).

Gold nanoparticles (AuNPs) are used in various fields, such as gene therapy, protein delivery, cancer diagnosis, photodermal and photodynamic therapy, the delivery of antitumor agents, and DNA detection and catalysis (Egorova and Ananikov, 2016). Ancient Indian healers used them for asthma and arthritis. Later, the Romans used them for cathedral glassware decoration. Recently, they have functioned as photocatalytic air purifiers, gaining significant therapeutic applications (Louis and Pluchery, 2012). AuNPs are compatible with living tissue, producing no toxic or immunological response. They have been used as nanocarriers for anti-inflammatory drugs due to their small sizes, improved stability, and adsorption efficiencies (Chiang et al., 2021; Leary, 2022). Plant extracts have gained attention as reducing agents for AuNP synthesis due to their low toxicity, eco-friendliness, and simplicity of production (Singh et al., 2018; Kulkarni et al., 2023). Various plants, such as *Anacardium occidentale* (Sheny et al., 2011), *Spondias dulcis* (Pechyen et al., 2022), and *Pistacia chinensis* (Alhumaydhi, 2022), have been reported to be effective in reducing gold ions into differently shaped and sized nanoparticles. Nanotechnology has enabled the development of modern techniques for nanoscale copper generation over the past decade (Crisan et al., 2021).

Copper nanoparticles (CuNPs) are gaining attention due to their ease of availability and economic feasibility, unlike some noble metals that are resistant to oxidation and easy to work with but may be costly to acquire, such as silver, gold, and platinum. They are widely utilised in cancer imaging due to their efficient light-to-heat transformation under near-infrared laser irradiation (Crisan et al., 2021). Other uses include enhancing heat transfer liquids, photonic devices, sensors, and electrochemical devices (Athanassiou et al., 2006; Zhou et al., 2019). CuNPs, with their unique properties, have gained significant applications in various industries such as cosmetology, agriculture, food, textiles, and construction (Martínez-Vieyra et al., 2020; Wu et al., 2020). In several studies, CuNPs providing higher environmental mobility were synthesized using various plant extracts, including *Cissus vitiginea* (Wu et al., 2020), *Zingiber officinalis* and *Curcuma longa* (Varghese et al., 2020), *Brassica oleracea* (Sundaram et al., 2020), and *Hyptis suaveolens* (L.) (Shubhashree et al., 2022).

Lannea discolor from the family Anacardiaceae is a deciduous tree that usually grows up to 15 m on rocky slopes or sandy soil. The leaves are discolorous, having a green-coloured adaxial surface and a gray, dense trichome layer on the abaxial surface. Its traditional uses include treatment for diarrhea, stomach complaints, and an array of infections (Maroyi, 2018). The plant's pharmacological activities are purported to result from various secondary metabolites such as phenolic flavonoids, alkaloids, and tannins (Mwamatope et al., 2021). CuNPs are a stable substitute for gold, so they may be a low-cost replacement for unattainable precious metals. Conclusively, in this study, all NPs were characterised by UV-Vis spectrometry, FTIR spectroscopy, SEM-EDX, and TEM to investigate the optical, morphology, and elemental composition and the antibacterial activities of nanoparticles, respectively.

6.2 Materials and Methods

6.2.1. Collection of plant material

Lannea discolor material was collected from Vuwani (Vhembe district) (23°09′42.9″ S 30°25″22.6″ E) in November 2018. The plant species were identified by U.R. (Unarine Rambau) with the aid of the literature, and online herbariums (https://plants.jstor.org/compilation; https://powo.science.kew.org) (Accessed on 5 November 2018) and voucher specimens (UR 01) were prepared and deposited at the University of Venda Herbarium (UVH) in South Africa. *Lannea discolor* material reserved for experiments was air-dried, ground with a pestle and mortar, and stored for further use.

6.2.2 Extraction of plant material

The air-dried material was ground into a fine powder using a grinding mill (NETZSCH, Selb, Germany). The extracts were obtained by maceration for three 3-day periods at a ratio of 1:10 in 3 solvents; mainly, methanol, acetone, and water. They were filtered using filter paper (Whatman No. 1), evaporated to dryness, and stored at 4 °C for subsequent tests.

6.2.3 Synthesis of Nanoparticles

AuNPs and CuNPs were synthesized using *Lannea discolor* (10mg/mL) extracts. After dissolving 250 mg of gold precursor in 50 mL of distilled water, a stock solution of gold (III) chloride hydrate ($HAuCl_4 \cdot aq$) with a concentration of 14 mM. (Millipore, Merck) was prepared. After, the stock solution was diluted to a concentration of 1 mM $HAuCl_4$. The addition of 10 mL of *L. discolor* extract to a mixture containing 40 mL of 1 mM $HAuCl_4$ solution was incubated at 25 °C and 80 °C for an hour, then in the dark for twenty-four hours. The hue of the solution changed, indicating AuNP formation. The mixture was centrifuged for ten minutes at 10,062 xg after being washed twice with distilled water. AuNP pellets were gathered, air-dried, and kept at 4 °C.

To synthesize CuNPs, 1 mM copper (II) sulphate ($CuSO_4$) was made in distilled water, after which 10 mL of *L. discolor* extracts was added to 40 mL of copper sulphate solution (1 mM) and incubated at 25

°C and 80 °C for one hour. The color of the mixtures changed, indicating the formation of CuNPs. After twenty-four hours, the nanoparticles were rinsed twice with distillation water and centrifuged at 12,000 rpm for 15 min. CuNP pellets were dried at 60 °C overnight and then stored at 4 °C for future use.

6.2.4 Characterisation

6.2.4.1 Physicochemical Characterisation of AuNPs and CuNPs

UV-Vis spectroscopy analysis recorded the absorbance of AuNPs and CuNPs in 96-well plates containing 160 µL of NPs using a SpectraMax M3 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) at wavelengths of 200–800 nm and a scan speed of 200 nm/min.

6.2.4.2 Scanning Electron Microscopy Analysis and Energy-Dispersive X-ray Microanalysis

The AuNPs and CuNPs were sonicated for 20 min, after which about 40 µl of each sample was mounted on a glass coverslip attached to a brass stub with adhesive carbon tape and dried under a mercury lamp for about 60 min. The NPs were made conductive with a layer of gold (ca. 25 mm) using an automated Quorum (Q15OR ES) Module sputter coater (vacuum of 0.1 Torr for 2.5 min) and examined at varying magnifications, using a Zeiss Ultra-Plus FEG-SEM at an acceleration voltage of 5 kV. The images were captured with NIS-D image software (AzTec analysis software, V. 1.2, Oxford Instruments, High Wycombe, UK). At the same time, elemental analysis was performed with an energy-dispersive X-ray spectrometer (EDX), coupled to an Astronomical Thermal Emission Camera (Aztec) version 1.2 at 20 kV.

6.2.4.3 Transmission Electron Microscopy Analysis and Measurements of Zeta Potential

Carbon-coated 200-mesh copper grids were used to scoop a portion of the AuNPs and CuNPs and dried under a mercury lamp for about 30 min before analysis. The morphology and size of the nanoparticles were determined using a JEOL JEM-2100 TEM at an acceleration voltage of 200 kV. The number and the sizes of the nanoparticles were measured and analyzed using Image J software (https://imagej.net/).

Zeta analysis was used to measure the surface charge of the NPs using a Zetasizer Nano-Zs90 (Malvern International Ltd., MPT-Z, Malvern, London, UK). For each sample, the mean diameter ± SD for ten runs was calculated by applying a multimodal animation.

6.2.4.4. Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The ATR-IR FTIR (Alpha 1; Bruker, Germany, Europe) was used to describe the chemically possible compound interactions. The samples analysed at a 4000–400 cm^{-1} scan range. The spectra were interpreted by comparing the spectral data with references from identifying functional groups in the literature (Coates, 2000).

6.2.5 Antibacterial Assay

The antibacterial activity of the NPs, gentamicin, crude extracts, $HAuCl_4$ and $CuSO_4$ solutions was studied against *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC 700603) and *Bacillus subtilis* (ATCC 6633) using a minimum inhibitory concentration assay (MIC). A 96-well microtiter plate (Tarsons, Kolkata, India) was prepared by transferring 160 µl of Mueller–Hinton broth to the first row and 100 µl to the second row, under a laminar airflow chamber. A volume of 40 µl of NPs, extract, and gentamicin was added to the first row of the plate, followed by 10 µl of bacterial inoculum. After this, serial dilutions with concentration 7.8 – 1000 µg/ ml were performed, 100 µl of bacteria was added, and the plates were placed for twenty-four hours at 37 °C for incubation. Upon removal, 10 µl of Resazurin solution, as per Madikizela et al. (2013), was added to each well, followed by a further 30 min incubation to determine the MIC value by the observed color change (Madikizela et al., 2013).

6.3 Results and Discussion

6.3.1 Visual and UV-Vis Spectroscopic Analysis

This study was a simple and sustainable method for synthesizing AuNPs and CuNPs by mixing $AuCl_4$ and $CuSO_4$ solutions with the methanol, acetone, and water extracts of *Lannea discolor*. Following the incubation of $AuCl_4$ and $CuSO_4$ solutions with extracts, and solutions went from dark yellow to grayish for the leaves (Figure 6.1A, C), and intense violet for the stems and roots was observed (Figure 6.1B, D). When exposed to $CuSO_4$, the extracts changed from their usual color to a more intensified burnt orange for the leaves (Figure 6.1E, G) and a brown to a darker brown for the stems and roots (Figure 6.1H). The color changes observed suggest the formation of nanoparticles. This was observed in all extracts from all solvents.

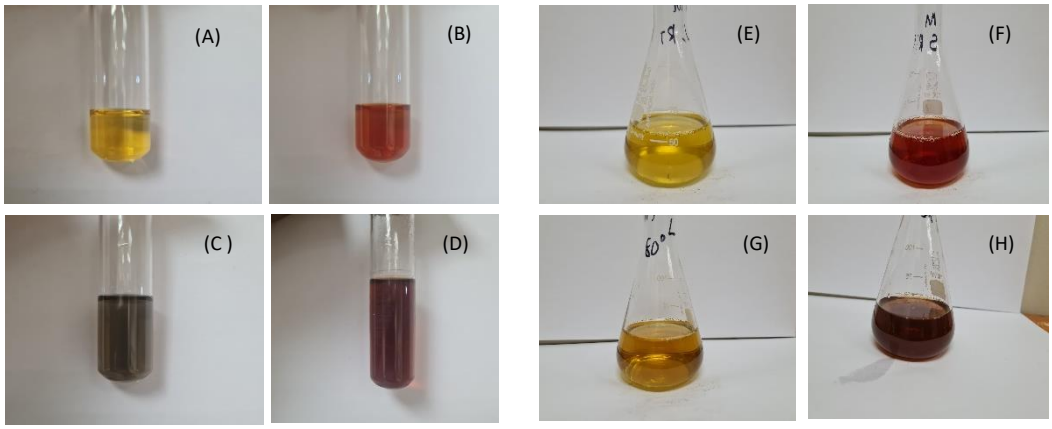


Figure 6.1. Visual representation of *Lannea discolor* extracts before and after NP synthesis. (A) Leaf, (B) stem and root before synthesis (C) AuNPs from leaves, and (D) AuNPs from stem and root bark

after synthesis. (E, F) are solutions before CuNP synthesis. CuNPs from leaf (G) and stem and root (H) after synthesis.

UV-Vis representative absorption spectra for the AuNPs and CuNPs are shown in Figure 6.2A and B. The absorption peak for the AuNPs from stem and root extracts had a maximal absorption at 544 nm, while the leaf extracts' NF solutions peaked at 316 nm. Consistent peaks were observed for the AuNPs, and NFs acquired from methanol, acetone, and water extracts. Similar results were observed in other studies (Liu et al., 2019; Abdallah et al., 2022). As much as the UV-Vis characteristic wavelength of AuNPs has been used to validate the synthesis of NPs, certain peaks may also be attributed to the size and shape of the nanoparticles. The AuNP solution's color change to gray is due to Ostwald ripening, a well-understood phenomenon involving the redeposition of smaller nanoparticles to larger ones to form popcorn, flower, or starlike shapes (Liebig et al., 2018). Correspondingly, the pointed peaks (at both 25 °C and 80 °C) observed in the AuNPs obtained from the leaf extracts (Figure 6.2A) suggest a different morphology in contrast to the AuNPs obtained from the stem and root extracts. This can be observed in the UV-Vis peaks of the rest of the samples in this study (Appendix Figure C1). Studies suggest that spherical AuNPs appear red but turn blue or gray when they form NFs (Gao et al., 2022; Mthembu et al., 2023). The CuNPs display an absorption peak at 556–564 nm for leaves and 540–546 nm for stems and roots. As Figure 6.2B shows, the increase in the temperature did not significantly reduce or increase the adsorption and synthesis of CuNPs. It is reported in the literature that the surface plasmon resonance band of CuNPs provides absorption from 500 to 600 nm (Das et al., 2022). Therefore, the UV-Vis peak acquired in this region after synthesis confirms the formation of CuNPs.

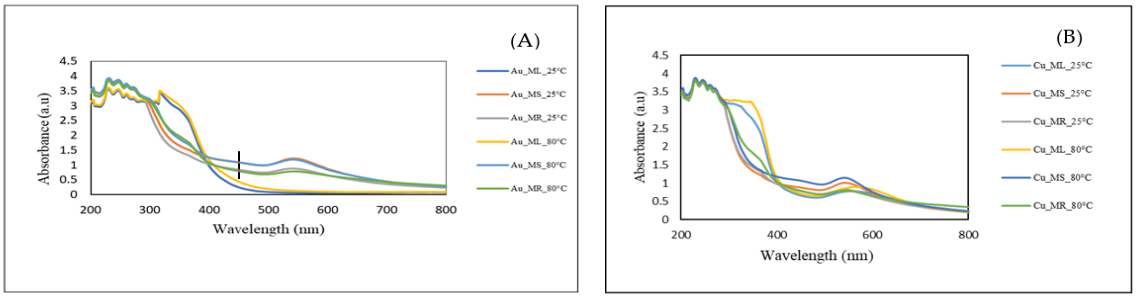


Figure 6.2. Representative UV-Vis spectra of SPR bands representing *Lannea discolor* (A) AuNPs/NFs and (B) CuNPs from methanol extracts. Sample keys: ML = Methanol leaf; MS= Methanol stem; MR = methanol root.

6.3.2 The Morphology of the Synthesized Nanoparticles Using Scanning Electron Microscopy and Energy-Dispersive X-ray Analysis

The SEM analysis in Figure 6.3A shows aggregated AuNPs that were present from all leaf extracts, thus confirming the influence of the leaf extracts from all extracts on the morphology observed. The fluorescence emission of the AuNPs synthesized using stem methanol extract is observed in Figure 6.3B inset. Under light irradiation, AuNPs emit fluorescence, a characteristic suggested to confer phototherapy potential and show the possible optical properties of the AuNPs (Kim et al., 2018).

The CuNPs from the leaf, stem, and root extracts were spherical (Figures 6.4A and Appendix Figure C3) and aggregated, resulting from static tension (Peng et al., 2019). Furthermore, unexpected sintering is observed in Figure 6.4B after exposure to heat. Sintering involves joining nanoparticles into a solid mass due to temperatures less than 250 °C, pressure, and leftover alcohols during evaporation (Babalola et al., 2023). As such, during both synthesis and drying, the CuNPs undergo sintering, and the well-connected features are observed in Figure 6.4B. Sintering techniques have been standardised to produce chemically synthesized CuNPs for copper inks used in conductivity (Hong et al., 2022; Babalola et al., 2023). Yet, no work has been reported employing a sintering temperature as low as 60 °C for CuNPs from *Lannea discolor* extracts. The literature reports that the optical and electronic properties of NPs depend on morphology (Sarwax et al., 2021; Hong et al., 2022), granting CuNPs potential in these applications.

The EDX measurements unambiguously confirmed the presence of metallic Au in all samples measured. The EDX profile shows a strong Au signal, along with C, O, Al, K, and Ca peaks from the biomolecules on the surface of the AuNPs (Figure 6.3C, D). These elements may also be present due to the elemental composition of the extracts, from which the AuNPs were not completely purified (Keskin et al., 2021). The grid on which the samples were examined caused Cu's presence. Thus, all measurement techniques confirmed the presence of AuNPs and NFs. Likewise, the EDX profile of the CuNPs shows strong elemental signals of copper, confirming the presence of CuNPs, as shown in Figure 6.4C, D. The weight percentages of different elements in the CuNPs were below 10%. The remaining Wt % was carbon and oxygen present in organic molecules, which act as capping molecules surrounding the nanoparticles, as well as the tape used to mount the CuNPs (Ismail et al., 2019).

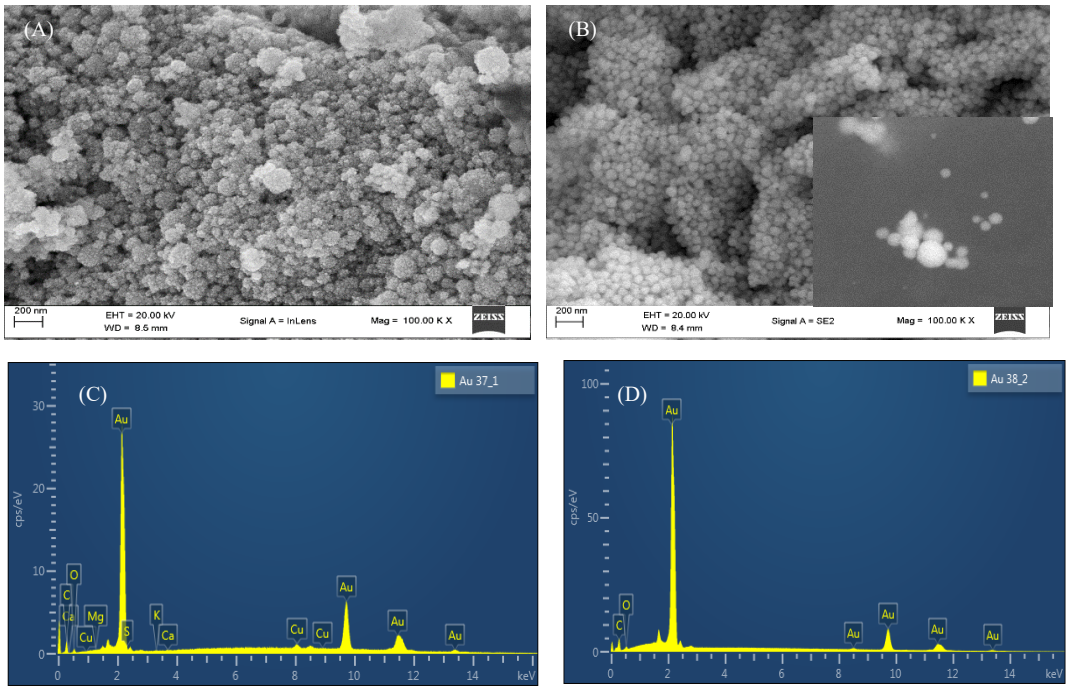


Figure 6.3. Representative SEM images of AuNFs from methanol leaf extracts. (A, B) AuNPs from methanol stem extract of *L. discolor* and (C, D) EDX spectra. Inset of (B) is image of fluorescence emission from AuNPs. Insets of (C, D) contain graphs of the abundance of Au to validate the formation of AuNFs and NPs.

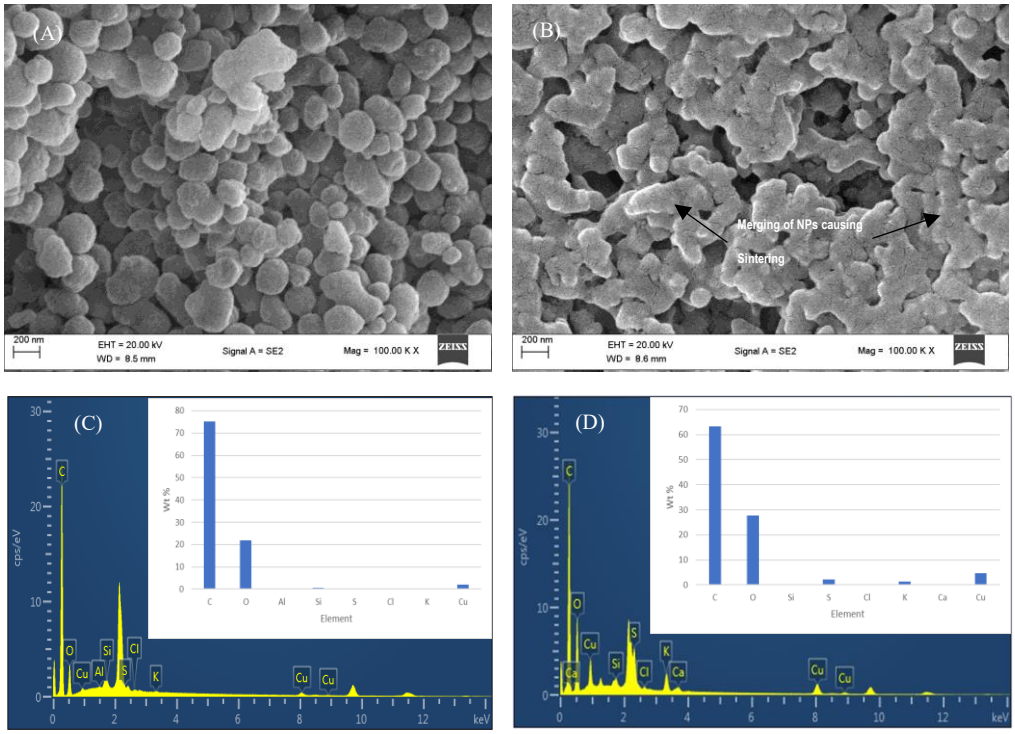


Figure 6.4. Representative SEM images and EDX spectra of CuNPs from the leaf methanol extracts of *Lannea discolor.* (A) Nanoparticles synthesized at 25 °C; (B) arrows showing sintering of CuNPs from leaf methanol extract. (C, D) show EDX spectra and abundance of Cu.

6.3.3 Transmission Electron Microscopy, Particle Size, and Zeta Potential Analysis

The TEM images show NPs from leaf methanol, acetone, and water extracts with protruding petal-like features, which are confirmed to be NFs (Sajanlal et al., 2011). As observed in Figure 6.5A, the particles are spherical dots with an average diameter of 10 nm. An in-depth morphological analysis suggests that the formation of AuNFs occurs by the initial formation of NPs, followed by rapid anisotropic growth, then assembly into a flower shape by secondary reduction at their interface (Sajanlal et al., 2011). The rapid color change from yellow to gray without intermediate colours support this theory of their progression (Sajanlal et al., 2011). Thus, we proposed the formation mechanism to be aggregation-based growth, as illustrated in Figure 6.6. Their three-dimensional orientation gives AuNFs better suspension stability and a greater surface area than spherical AuNPs (Ji et al., 2015; Jakhmola et al., 2019). Also, a significant enhancement of the local electrostatic fields and "petals" is advantageous in catalytic reactions, drug delivery, and nuclear medicine (Ji et al., 2015). The size of the NFs from *L. discolor* varies from 30 to 97 nm, with an average size of 67 nm. Borah et al. reported similar sizes using *Syzygium cumini* (Borah et al., 2018). Onmaz et al. (2022) reported NFs of sizes <100 nm synthesized using *Helichrysum italicum*. Other studies suggest that tannin-based polymers enhance reduction (Deshmukh and Kim, 2021), explaining the unexpected formation of NFs from *L. discolor*. NFs can potentially be used to develop biosensors, such that, through surface modification, a color change can confirm their interactions with biomolecules of interest (Deshmukh and Kim, 2021). A familiar example of NFs used in sensing is the home pregnancy test that detects pregnancy (β-HCG hormone) and confirms its presence by a change in the colour of the test solution (Pluchery et al., 2013). In other studies, the gray suspension of AuNFs was obtained by chemical synthesis, where trisodium citrate and water-soluble polymer were added to $HAuCl_4$ (Tanaka et al., 2006; Merza et al., 2011). Moving to a greener synthesis of NFs, using *L. discolor* leaves may be less tedious and more environmentally friendly. Under the given conditions**,** spherical AuNPs were formed from stem and root methanol, acetone, and water extracts ranging from 10 to 33 and 9.3 to 37.5 nm, respectively (Figure 6.5B), with smaller sizes (Figure 6.5D) than the NFs obtained in the leaf samples. Shabestariana et al. acquired spherical AuNPs with an average size of 20.83 from *Rhus coriaria* L. (sumac), confirming that significant molecules in the extracts act as ligands that control NP growth (Shabestarian et al.,2016; Kariuki et al., 2017). Similarly, Donga et al. (2020), reported even smaller-sized NPs from *Mangifera indica* ranging from 12.33 to 24.05 nm. Comparably, Pechyen et al. (2022) reported similar-sized spherical AuNPs from *Spondias dulcis*. Figure 6.5B insets show triangular and hexagonal NPs, which were acquired from acetone stem and root extracts and have been reported by Song et al. (2009) to be caused by low concentrations of extracts. However, the effect on the size and shape of AuNPs in the

present study may be in response to the plant part and solvent used in extraction, indicating the uniqueness of the phytochemicals in the extracts and their specific requirements for higher temperatures to initiate the reduction process and produce other morphologies (Amjad et al., 2021).

Figure 6.7A shows the TEM images of spherical CuNPs from all plant parts and their respective solvents, with particle sizes of 20–104 nm (Figure 6.7B). In Figure 6.7A and the inset, NPs are similarly sintered, as observed in Section 6.3.2. It is suggested that the large sizes of the CuNPs observed in the present study may be due to the concentration and ability of the biomolecules in the extracts to reduce the volume of the $CuSO_4$. Similarly, in a study by Amjad et al. (2021) copper nanoparticle size increased with smaller concentrations of the precursor salt.

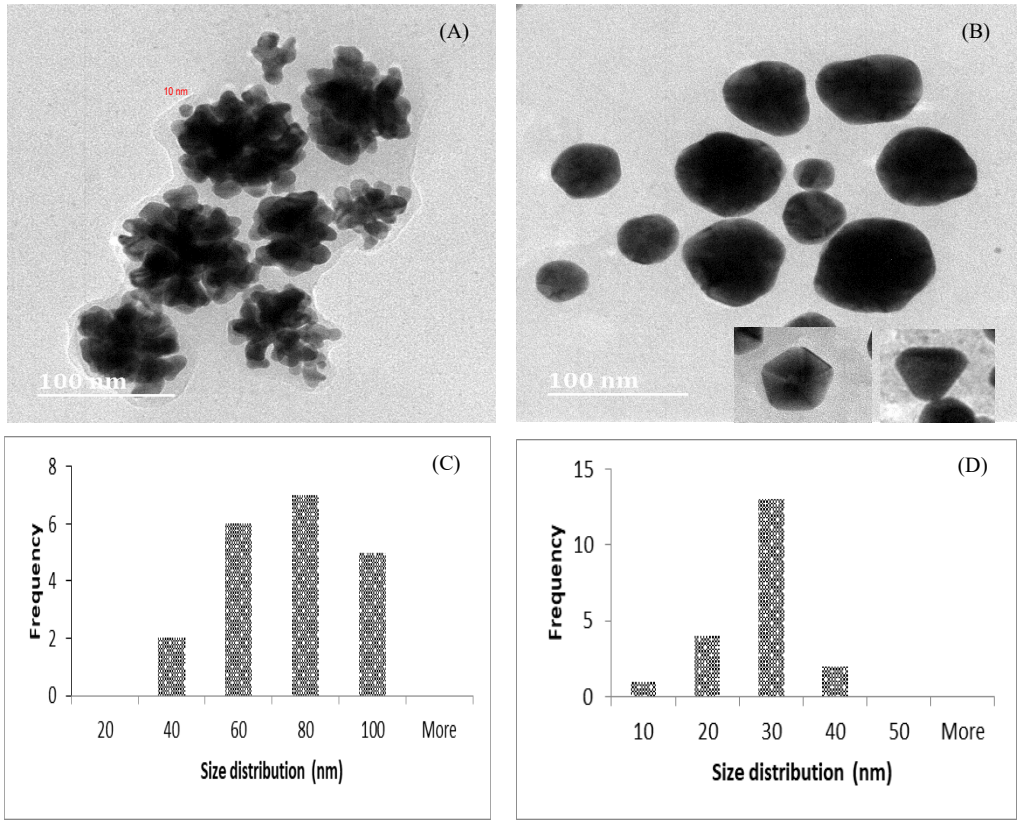


Figure 6.5. Representative TEM images of the AuNFs (A) from methanol leaf extracts and AuNPs (B) from *L. discolor* methanol stem extract. Insets in B show triangular and hexagonal NPs from stem and root acetone extracts. Histograms show the size distribution of the AuNFs (C) and spherical AuNPs (D).

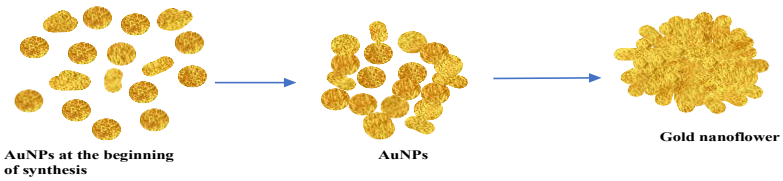


Figure 6.6. Schematic diagram showing proposed growth mechanism of AuNFs from *Lannea discolor* leaves.

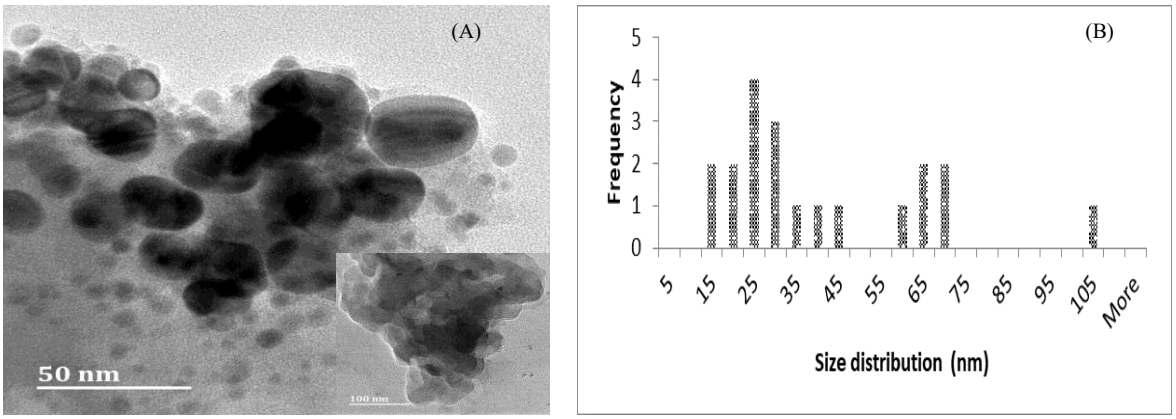


Figure 6.7. Representative micrograph of (A) CuNPs from stem methanol extract and (B) size distribution. The inset shows a TEM image of sintering in CuNPs exposed to high temperatures.

AuNFs and NPs are soluble in water, suggesting high biofluid solubility and potential as injectables (Chopra et al., 2022). Their stability may improve the corresponding adsorption of these NPs in the body, which is measured as zeta potential. In addition, the absolute values of zeta potentials for AuNFs were −14.6, −8.3, −10.8, and −6.39 mV, indicating their stability due to the larger electrostatic repulsion (Patra et al., 2018; Khan et al., 2019). CuNPs had a stability range of −43.3 to −13.4 mV. A zeta potential greater than 30 mV or less than −30 mV indicates stable dispersions of NPs in solutions (Chopra et al., 2022).

6.3.4 FTIR Analysis

FTIR measurements of the NPs were conducted to identify the major functional groups of phytochemicals in *L. discolor* extracts and the AuNFs, NPs, and CuNPs. Strong bands were observed in the methanol, acetone, and water extracts, with peaks corresponding to the O-H stretching of alcohols

and carboxylic acids. Similar bands in the extracts were observed to represent alkanes, alkynes, aldehydes, allenes, thiocyanates, and isothiocyanates (Appendix Figure C5 - C7). The typical FTIR spectra of AuNPs and CuNPs synthesized in the presence of *L. discolor* methanol stem and root extracts, respectively, are shown in Figure 6.8. Bands at 2780, 2873,2816, 3015, 3255, 3272, 3299, and 3313 cm^{-1} are due to N–H stretching of both amines and amides and/or to O–H stretching of alcohols, phenols, or carboxylic acids. The aromatic stretches (C-H) observed at peaks 1894, 1935, 1938, 1954, 1973, and 1994 cm^{-1} in the AuNPs and CuNPs may be among the main capping agents. The binding characteristics of amino groups in the samples could cause the CuNPs to aggregate, which resulted in sintering (Gawande et al., 2016; Sizochenko et al., 2021).

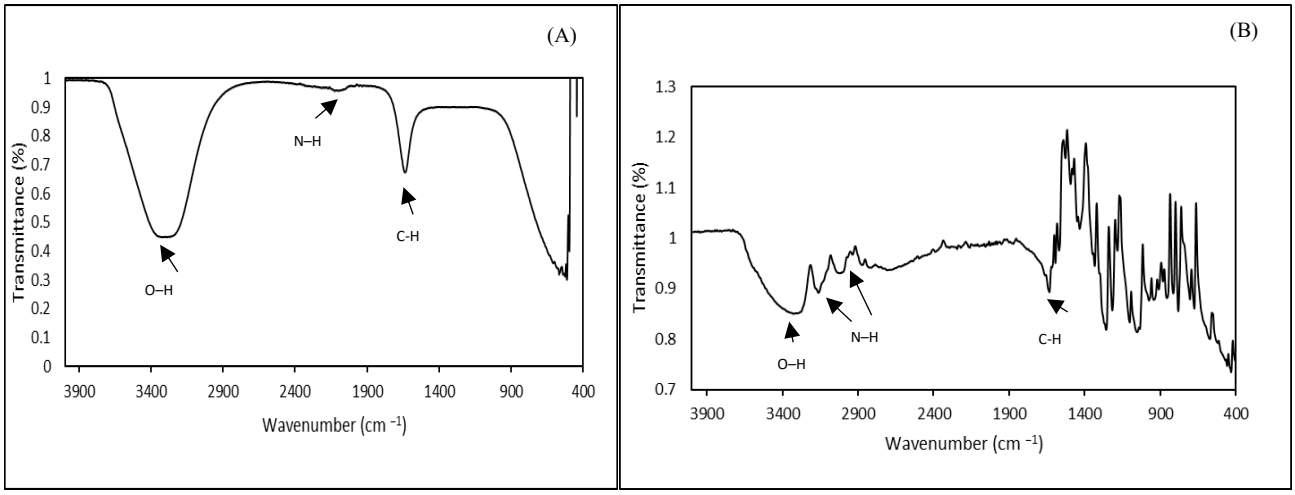


Figure 6.8. FTIR spectrum of AuNPs from methanol stem extract (A) and CuNPs from methanol root extract (B) from *Lannea discolor*.

6.3.5. The Antibacterial Activity of AuNPs and CuNPs

In addition to the previously mentioned applications, NPs may be a potent antibacterial agent. Although extensive research from the literature has been devoted to proving the antibacterial activity of other metal-based NPs (Murthy et al., 2020), the focus on the antibacterial activity of AuNPs/NFs and CuNPs is limited in *Lannea*; as such, the present study contributes hugely. MIC was employed to estimate the inhibition AuNPs/NFs and CuNPs may have on microorganisms. The MICs for the AuNPs and NFs are summarized in Table 6.1. Interestingly, the MIC values of the NPs were similar for the bacterial strains. *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), and *Bacillus subtilis* (ATCC 6633) were the most susceptible to AuNFs from all extracts (leaf, stem, and root) and solvents (methanol, acetone, and water) synthesized at both 25 °C and 80 °C and showed the lowest viability compared to *Klebsiella pneumoniae* (ATCC 700603) and *Escherichia coli* (ATCC 25922). The authors attributed the activity of the AuNFs to the high aspect ratio of the elongated petal-like surfaces, which induce localized tension and increase reactive oxygen species, thus causing bacterial membrane rupture

(Yu et al., 2020). Additionally, the enhancement of *L. discolor* extracts by NPs improves the ability to disrupt the bacterial cell membrane (Yu et a., 2020). The MICs from the NPs from stem and root methanol, acetone, and water extracts were similar and exhibited good antibacterial activity against all the bacteria due to their similar sizes. Contrastingly, all the crude extracts displayed antibacterial activity at only 1000 µg/mL, explaining the significance of nanoparticle enhancement. On the other hand, the controls for the crude extracts, which are the solvents used for extraction, showed no activity.

Table 6.1. Antibacterial activity of AuNPs and NFs containing *Lannea discolor* extracts. Values of minimum inhibitory concentrations (MIC).

	AuNFs and NPs (µg/mL)																					
	37	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**Extracts (µg/mL)**	**solvents**	**G**	**$HAuCl_4$ (1 mM)**
P. aeruginosa	250	125	62.5	62.5	62.5	62.5	62.5	62.5	62.5	125	62.5	7.81	500	500	500	500	1000	1000	1000	0	7.8	500
K. pneumoniae	250	125	125	250	250	250	250	62,5	125	15.62	15.62	1000	1000	0	0	7.81	62.5	0	1000	0	7.8	500
E. coli	250	125	1000	500	125	62.5	15.62	15.62	125	125	125	500	500	500	500	31.25	31.25	0	1000	0	7.8	500
B. subtilis	250	31.25	31.25	31.25	31.25	31.25	31.25	31.25	15.62	62.5	62.5	62.5	62.5	62.5	15.62	15.62	7.81	0	1000	0	7.8	1000
S. aureus	62.5	62.5	0	62.5	62.5	0	15.62	62.5	125	250	250	500	500	250	500	250	250	0	1000	0	7.8	1000

Sample keys: 37 = Au_ML_25 °C; 38 = Au_MS_25 °C; 39 = Au_MR_25 °C; 40 = Au_ML_80 °C; 41 = Au_MS_80 °C; 42 = Au_MR_80 °C; 43 = Au_AL_25 °C; 44 = Au_AS_25 °C; 45 = Au_AR_25 °C; 46 = Au_AL_80 °C; 47 = Au_AS_80 °C; 48 = Au_AR_80 °C; 49 = Au_WL_25 °C; 50 = Au_WS_25 °C; 51 = Au_WR_25 °C; 52 = Au_WL_80 °C; 53 = Au_WS_80 °C; 54 = Au_WR_80 °C; G = gentamicin; $HAuCl_4$ = gold(III) chloride hydrate.

Reports show that the antibacterial activity of AuNPs is associated with their surface modifications, such as the shape and the molecules involved in capping the NPs during synthesis (Yu et al., 2020). Some bacterial strains, like *S. aureus,* are considered dreadful to humans; hence, introducing AuNPs as antibacterial agents is fundamental. Morphological interactions depend on surface orientations to enhance antibacterial activity (Yougbare et al., 2019). NPs with edges rupture the membrane of bacteria and disrupt them. Many experiments on nanoscale surfaces have observed the morphological interactions of AuNPs/NFs with bacteria, confirming the significance of surface orientation for activity (Yougbare et al., 2019; Mousavi et al., 2020). In the present study, the high activity can be attributed to the large surface area and disruptive ability of AuNPs/NFs. Studies have also ascribed the high activity of gold NPs against Gram (−) bacteria to the thin peptidoglycan membrane, and that lower activity is usually observed in Gram (+) bacteria with a peptidoglycan membrane with a thickness that is 50% higher (Okkeh et al., 2021). However, this is not the case in this study, as activity varies equitably within the Gram (−) and Gram (+) bacteria. Similarly, several other studies have observed impressive antibacterial activity from AuNPs in both Gram (−) and Gram (+) bacteria (Umadevi et al., 2011; Zheng et al., 2017; Rovati et al., 2019). The total MIC for different extracts resembling that of $AuCl_4$ confirms that the activity of phytochemicals improves when incorporated into different nanoformulations. The outcome demonstrates that the AuNPs/NFs synthesized from *L. discolor* can inhibit the growth of the tested bacteria even at low concentrations. The CuNPs in this work showed no activity against the tested bacteria, ideally indicated by a change in colour after adding Resazurin. This is attributed to the sintered NPs that could not interact with the bacterial cells as they are more of a solid mass instead of polydispersed. Other causes may include the size, shape, specific surface area, and surface curvature of the nanoparticles (Montes de Oca-Vásquez et al., 2020). However, CuNPs in other studies have shown

comparable activity in contrast to their counterparts (Qamar et al., 2020). A higher concentration of precursor salts and a longer incubation period may produce CuNPs with better antibacterial activity. All these data reported here support using the *L. discolor* extracts as an alternative to toxic chemical reductants in producing AuNPs, NFs, and CuNPs that can inhibit the growth of different bacteria.

6.4 Conclusion

In the current study, the straightforward synthesis of AuNPs, NFs, and CuNPs was reported for the first time, employing *Lannea discolor* leaves, stem, and root extracts in a more cost-effective, green technique. The ability of the functional groups in the phytochemicals of the organs of *L. discolor* to function as a reducing and capping agent is linked to the formation of AuNFs and NPs as well as spherical CuNPs. TEM micrographs provided evidence toward the morphology of AuNPs with several shapes, including flowers, from methanol, acetone, and water leaf extracts, while spherical, triangular, and hexagonal AuNPs were observed in the stems and root of all solvents from which extracts were acquired. Due to their merged surface area, the sintered CuNPs from the leaf, stem, and root extracts can potentially find a unique position in many technological applications, such as sensors, flexible devices, and conductivity. Despite the noticeable bacterial activity of AuNPs and NFs, more study is required into their intricate cytotoxicity profiles. Critical parameters for synthesizing the AuNFs and NPs in this study include the concentration and ratio of gold salts, leaf, stem and root extracts, time, and temperature. The techniques used in this study present several significant advantages, such as biocompatibility, multifunctionality, and economic viability. On the other hand, there may be a need to optimise techniques to produce AuNFs and sintered CuNPs from *L. discolor,* which could be exploited to develop novel extract-formulated nanoparticles with prospective applications in different fields.

6.5 References

Abdallah, B.M. and Ali, E.M., 2022. Therapeutic Potential of Green Synthesized Gold Nanoparticles Using Extract of *Leptadenia hastata* against Invasive Pulmonary Aspergillosis. *Journal of Fungi,* 8 (5), 442.

Alhumaydhi, F.A., 2022. Green synthesis of gold nanoparticles using extract of *Pistacia chinensis* and their *in vitro* and *in vivo* biological activities. *Journal of Nanomaterials*, 1, 1-11.

Amjad, R., Mubeen, B., Ali, S.S., Imam, S.S., Alshehri, S., Ghoneim, M.M., Alzarea, S.I., Rasool, R., Ullah, I. and Nadeem, M.S., 2021. Green Synthesis and Characterization of Copper Nanoparticles Using *Fortunella margarita* Leaves. *Polymers*, 13(24), 4364.

Athanassiou, E.K., Grass, R.N. and Stark, W.J., 2006. Large-scale production of carbon-coated copper nanoparticles for sensor applications. *Nanotechnology*, 17(6), 1668.

Babalola, B.J., Ayodele, O.O. and Olubambi, P.A., 2023. Sintering of nanocrystalline materials: Sintering parameters. *Heliyon*, *9*, e14070.

Borah, D., Hazarika, M., Tailor, P., Silva, A.R., Chetia, B., Singaravelu, G. and Das, P., 2018. Starch-templated biosynthesis of gold nanoflowers for in vitro antimicrobial and anticancer activities. *Applied Nanoscience*, 8, 241-253.

Chiang, M.C., Nicol, C.J., Lin, C.H., Chen, S.J., Yen, C. and Huang, R.N., 2021. Nanogold induces anti-inflammation against oxidative stress induced in human neural stem cells exposed to amyloid-beta peptide. *Neurochemistry international*, *145*, 104992.

Chopra, H., Bibi, S., Singh, I., Hasan, M.M., Khan, M.S., Yousafi, Q., Baig, A.A., Rahman, M.M., Islam. and Emran, T.B., 2022. Green metallic nanoparticles: Biosynthesis to applications. *Frontiers in Bioengineering and Biotechnology*, 10, 548.

Coates, J., 2000. Interpretation of infrared spectra, a practical approach. In *Encyclopedia of Analytical Chemistry*, Meyers, R.A., Ed., John Wiley & Sons, Ltd.: Hoboken, NJ, USA, p. 10815.

Crisan, M.C., Teodora, M. and Lucian, M., 2021. Copper nanoparticles: Synthesis and characterization, physiology, toxicity, and antimicrobial applications. *Applied Sciences*, *12*(1), 141.

Das, P.E., Abu-Yousef, I.A., Majdalawieh, A.F., Narasimhan, S. and Poltronieri, P., 2022. Green synthesis of encapsulated copper nanoparticles using a hydroalcoholic extract of *Moringa oleifera* leaves and assessment of their antioxidant and antimicrobial activities. *Molecules*, 25 (3), 555.

Deshmukh, A.R. and Kim, B.S., 2021. Flower-like biogenic gold nanostructures for improved catalytic reduction of 4-nitrophenol. *Journal of Environmental Chemical Engineering,* 9(6), 106707.

Donga, S., Bhadu, G.R. and Chanda, S., 2020. Antimicrobial, antioxidant, and anticancer activities of gold nanoparticles green synthesized using *Mangifera indica* seed aqueous extract. *Artificial Cells, Nanomedicine, and Biotechnology*, 48(1), 1315-1325.

Egorova, K.S. and Ananikov, V.P., 2016. Which metals are green for catalysis? Comparison of the toxicities of Ni, Cu, Fe, Pd, Pt, Rh, and Au salts. *Angewandte Chemie International Edition,* 55(40), 12150-12162.

Gao, L., Mei, S., Ma, H. and Chen, X., 2022. Ultrasound-assisted green synthesis of gold nanoparticles using citrus peel extract and their enhanced anti-inflammatory activity. *Ultrasonics Sonochemistry,* 83, 105940.

Gawande, M.B., Goswami, A., Felpin, F.X., Asefa, T., Huang, X., Silva, R., Zou, X., Zboril, R. and Varma, R.S., 2016. Cu and Cu-based nanoparticles: Synthesis and applications in catalysis. *Chemical reviews,* 116(6), 3722-3811.

Hong, G.B., Wang, J.F., Chuang, K.J., Cheng, H.Y., Chang, K.C. and Ma, C.M., 2022. Preparing copper nanoparticles and flexible copper conductive sheets. *Nanomaterials*, 12(3), 360.

Ismail, M., Gul, S., Khan, M.I., Khan, M.A., Asiri, A.M. and Khan, S.B., 2019. Green synthesis of zerovalent copper nanoparticles for efficient reduction of toxic azo dyes congo red and methyl orange. *Green processing and synthesis,* 8(1), 135-143.

Jakhmola, A., Vecchione, R., Gentile, F., Profeta, M., Manikas, A.C., Battista, E. and Netti, P.A., 2019. Experimental and theoretical study of biodirected green synthesis of gold nanoflowers. *Materials Today Chemistry,* 14, 100203.

Ji, Y., Ren, M., Li, Y., Huang, Z., Shu, M., Yang, H., Xiong, Y. and Xu, Y., 2015. Detection of aflatoxin B1 with immunochromatographic test strips: Enhanced signal sensitivity using gold nanoflowers. *Talanta,* 142, 206-212.

Kariuki, V.M., Hoffmeier, J.C., Yazgan, I. and Sadik, O.A. 2017. Seedless synthesis and SERS characterization of multi-branched gold nanoflowers using water-soluble polymers. *Nanoscale,* 9(24), 8330-8340.

Keskin, C., Atalar, M.N. Firat Baran, M. and Baran, A., 2021. Environmentally friendly rapid synthesis of gold nanoparticles from *Artemisia absinthium* plant extract and application of antimicrobial activities. *Journal of the Institute of Science and Technology*, 11(1), 365-375.

Khan, I., Saeed, K. and Khan, I., 2019. Nanoparticles: Properties, applications, and toxicities. *Arab. J. Chem. Arabian journal of chemistry,* 12(7), 908-931.

Kim, H.S. and Lee, D.Y., 2018. Near-infrared-responsive cancer photothermal and photodynamic therapy using gold nanoparticles. *Polymers,* 10(9), 961.

Kulkarni, D., Sherkar, R., Shirsathe, C., Sonwane, R., Varpe, N., Shelke, S., More, M.P., Pardeshi, S.R., Dhaneshwar, G. and Junnuthula, V., 2023. Biofabrication of nanoparticles: Sources, synthesis, and biomedical applications. *Frontiers in Bioengineering and Biotechnology,* 11, 1159193.

Leary, J., 2002. Attaching Biomolecules to Nanoparticles, Cambridge University Press: Cambridge, UK, pp. 103–121.

Liebig, F., Henning, R., Sarhan, R.M., Prietzel, C., Bargheer, M. and Koetz, J., 2018. A new route to gold nanoflowers. *Nanotechnology,* 29(18), 185603.

Liu, K., He, Z., Curtin, J.F., Byrne, H.J. and Tian, F., 2019. A novel, rapid, seedless, in situ synthesis method of shape and size controllable gold nanoparticles using phosphates. *Scientific reports*, 9(1), 7421.

Louis, C. and Pluchery, O., 2012. Gold Nanoparticles in the Past: Before the Nanotechnology Era Gold Nanoparticles for Physics, Chemistry, and Biology, World Scientific Publishing: Singapore, pp. 1–28.

Madikizela, B., Ndhlala, AR., Finnie, J.F. and Staden, J.V., 2013. In vitro antimicrobial activity of extracts from plants used traditionally in South Africa to treat tuberculosis and related symptoms. *Evidence-Based Complementary and Alternative Medicine*, *1*, 840719.

Malike, S., Muhammad, K. and Waheed, Y., 2023. Nanotechnology: A revolution in modern industry. *Molecules,* 28(2), 661.

Maroyi, A., 2018. *Lannea discolor* its botany, ethnomedicinal uses, phytochemistry, and pharmacological properties. *Asian Journal of Pharmaceutical and Clinical Research,* 11(10), 49.

Martínez-Vieyra, C., Olguin, M.T., Gutiérrez-Segura, E. and López-Tellez, G., 2020. Comparison of Ag, Cu and Zn nanoparticles obtained using *Aloe vera* extract and gamma ionizing radiation. *Journal of applied research and technology,* 18(5), 289-314.

Merza, K.S., Al-Attabi, H.D., Abbas, Z.M. and Yusr, H., 2011. A Comparative Study on Methods for Preparation of Gold Nanoparticles. *Green and Sustainable Chemistry,* 2(1), 26-28.

Montes de Oca-Vásquez, G., Solano-Campos, F., Vega-Baudrit, J.R., López-Mondéjar, R., Odriozola, I., Vera, A., Moreno, J.L. and Bastida, F., 2020. Environmentally relevant concentrations of silver nanoparticles diminish soil microbial biomass but do not alter enzyme activities or microbial diversity. *Journal of hazardous materials,* 391, 122224.

Mousavi, S.M., Zarei, M., Hashemi, S.A., Ramakrishna, S., Chiang, W.H., Lai, C.W. and Gholami, A., 2020. Gold nanostars-diagnosis, bioimaging and biomedical applications. *Drug metabolism reviews,* 52(2), 299-318.

Mthembu, S.B., Akintayo, D.C., Moodley, B. and Gumbi, B.P., 2023. Development of gold plasmonic nanoparticles for detection of polydiallyldimethylammonium chloride at Umgeni water treatment plants: An optimised study and case application. *Heliyon*, *9*, e17136.

Murthy, H.C., Desalegn, T., Kassa, M., Abebe, B. and Assefa, T., 2020. Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) JF Gmel leaf extract: Antimicrobial properties. *Journal of nanomaterials*, *1*, 3924081.

Mwamatope, B., Tembo, D., Kampira, E., Maliwichi-Nyirenda, C. and Ndolo, V., 2021. Seasonal variation of phytochemicals in four selected medicinal plants. *Pharmacognosy Research,* 13(4), 218.

Okkeh, M., Bloise, N., Restivo, E., De Vita, L., Pallavicini, P. and Visai, L., 2021. Gold nanoparticles: Can they be the next magic bullet for multidrug-resistant bacteria? *Nanomaterials,* 11(2), 312.

Onmaz, N.E., Demirezen Yilmaz, D., Imre, K., Morar, A., Gungor, C., Yilmaz, S., Gundog, D.A., Dishan, A., Herman, V. and Gungor, G., 2022. Green synthesis of gold nanoflowers using *Rosmarinus officinalis* and *Helichrysum italicum* extracts: Comparative studies of their antimicrobial and antibiofilm activities. *Antibiotics,* 11(11), 1466.

Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V., Rodriguez-Torres, M.D., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K. and Sharma, S., 2018.Nano based drug delivery systems: Recent developments and future prospects. *Journal of nanobiotechnology,* 16(1), 1-33.

Pechyen, C., Ponsanti, K., Tangnorawich, B. and Ngernyuang, N., 2022. Biogenic synthesis of gold nanoparticles mediated by *Spondias dulcis* (Anacardiaceae) peel extract and its cytotoxic activity in human breast cancer cell. *Toxicology Reports*, 9, 1092-1098.

Peng, C., Tong, H., Yuan, P., Sun, L., Jiang, L. and Shi, J., 2019. Aggregation, Sedimentation, and Dissolution of Copper Oxide Nanoparticles: Influence of Low-Molecular-Weight Organic Acids from Root Exudates. *Nanomaterials*, 9(6), 841.

Pluchery, O., Remita, H. and Schaming, D., 2013. Demonstrative experiments about gold nanoparticles and nanofilms: An introduction to nanoscience. *Gold Bulletin,* 46, 319-327.

Qamar, H., Rehman, S., Chauhan, D.K., Tiwari, A.K. and Upmanyu, V., 2020. Green Synthesis, Characterization and Antimicrobial Activity of Copper Oxide Nanomaterial Derived from *Momordica charantia*. *International journal of nanomedicine*, 15, 2541-2553.

Rovati, D., Albini, B., Galinetto, P., Grisoli, P., Bassi, B., Pallavicini, P., Dacarro, G. and Taglietti, A., 2019. High Stability Thiol-Coated Gold Nanostars Monolayers with Photo-Thermal Antibacterial Activity and Wettability Control. *Nanomaterials*, 9(9), 1288.

Sahu, M.K., Yadav, R. and Tiwari, S.P., 2023. Recent advances in nanotechnology. *International Journal of Nanomaterials, Nanotechnology and Nanomedicine*, *9*(1), 015-023.

Sajanlal, P.R., Sreeprasad, T.S., Samal, A.K. and Pradeep, T., 2011. Anisotropic nanomaterials: Structure, growth, assembly, and functions. *Nano reviews,* 2(1), 5883.

Sarwax, N., Choi, S.H., Dastgeer, G., Humayoun, U.B., Kumar, M., Nawaz, A., Jeong, D.I., Zaidi, S.F. and Yoon, D.H., 2021. Synthesis of citrate-capped copper nanoparticles: A low temperature sintering approach for the fabrication of oxidation stable flexible conductive film. *Applied Surface Science,* 542, 148609.

Shabestarian, H., Homayouni-Tabrizi, M., Soltani, M., Namvar, F., Azizi, S., Mohamad, R. and Shabestarian, H., 2016. Green synthesis of gold nanoparticles using *sumac* aqueous extract and their antioxidant activity. *Materials Research,* 20, 264-270.

Sheny, D.S., Joseph, M. and Daizy, P., 2011. Phytosynthesis of Au, Ag and Au-Ag bimetallic nanoparticles using aqueous extract and dried leaf of *Anacardium Occidentale. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* 79(1), 254-262.

Shubhashree, K.R., Reddy, R., Gangula, A.K., Nagananda, G.S., Badiya, P.K., Ramamurthy, S.S. and Reddy, N., 2022. Green synthesis of copper nanoparticles using aqueous extracts from *Hyptis suaveolens* (L). *Materials Chemistry and Physics,* 280, 125795.

Singh, J., Dutta, T., Kim, K.H., Rawat, M., Samddar, P. and Kumar, P., 2018. Green synthesis of metals and their oxide nanoparticles: Applications for environmental remediation. *Journal of nanobiotechnology*, 16(1), 1-24.

Sizochenko, N., Mikolajczyk, A., Syzochenko, M., Puzyn, T. and Leszczynski, J., 2021. Zeta potentials (ζ) of metal oxide nanoparticles: A meta-analysis of experimental data and a predictive neural network modeling. *NanoImpact, 22,* 100317.

Song, J.Y., Jang, H.K. and Kim, B.S., 2009. Biological synthesis of gold nanoparticles using *Magnolia kobus* and *Diopyros kaki* leaf extracts. *Process Biochemistry*, 44(10), 1133-1138.

Sundaram, C.S., Kumar, J.S., Kumar, S.S., Ramesh, P.L.N., Zin, T. and Rao, U.M. 2020. Antibacterial and anticancer potential of *Brassica oleracea var acephala* using biosynthesised copper nanoparticles. *Medical Journal of Malaysia, 75*(6), 677-684.

Szczyglewska, P., Feliczak-Guzik, A. and Nowak, I., 2023. Nanotechnology–General Aspects: A Chemical Reduction Approach to the Synthesis of Nanoparticles. *Molecules,* 28(13), 4932.

Tanaka, R., Yuhi, T., Nagatani, N., Endo, T., Kerman, K., Takamura, Y. and Tamiya, E., 2006. A novel enhancement assay for immunochromatographic test strips using gold nanoparticles. *Analytical and bioanalytical chemistry*, 385, 1414-1420.

Umadevi, M., Rani, T., Balakrishnan, T. and Ramanibai, R., 2011. Antimicrobial activity of silver nanoparticles under an ultrasonic field. *International Journal of Pharmaceutical Sciences and Nanotechnology, 4, 1491-1496.*

Varghese, B., Kurian, M., Krishna, S. and Athira, T.S., 2020. Biochemical synthesis of copper nanoparticles using *Zingiber officinalis* and *Curcuma longa*: Characterization and antibacterial activity study. *Materials Today: Proceedings,* 25, 302-306.

Wu, S., Rajeshkumar, S., Madasamy, M. and Mahendran, V., 2020. Green synthesis of copper nanoparticles using *Cissus vitiginea* and its antioxidant and antibacterial activity against urinary tract infection pathogens. *Artificial Cells, Nanomedicine, and Biotechnology*, 48(1), 1153-1158.

Yougbare, S., Chang, T.K., Tan, S.H., Kuo, J.C., Hsu, P.H., Su, C.Y. and Kuo, T.R., 2019. Antimicrobial gold nanoclusters: Recent developments and future perspectives. *International journal of molecular sciences,* 20(12), 2924.

Yu, Z., Li, Q., Wang, J., Yu, Y., Wang, Y., Zhou, Q. and Li, P., 2020. Reactive oxygen species-related nanoparticle toxicity in the biomedical field. *Nanoscale research letters,* 15(1), 115.

Zheng, K., Setyawati, M.I., Leong, D.T. and Xie, J., 2017. Antimicrobial Gold Nanoclusters. *ACS Nano,* 11(7), 6904-6910.

Zhou, Q., Zhou, M., Li, Q., Wang, R., Fu, Y. and Jiao, T., 2019. Facile biosynthesis and grown mechanism of gold nanoparticles in *Pueraria lobata* extract. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 567, 69-75.

CHAPTER 7

Plant mediated synthesis of silver nanoparticles by *Lannea discolor*: Antibacterial activities and cytotoxic effects on Caco-2 cell line

Abstract

The global research community has consistently focused on medicinal plants given the multitude of benefits they can provide. The synthesis of useful nanoparticles from plants has also gained traction for the application in different fields. This study outlines a method for producing bioactive nanoparticles from the vegetative organs of *Lannea discolor* and silver nitrate. The formation of nanoparticles from *L. discolor* was validated and characterised using innumerable analytical procedures. Ultraviolet-visible spectroscopy (UV-vis) was used to analyse the particles optically. Fourier-transform infrared spectroscopy (FTIR) provided information about the chemical composition and existing functional groups. Scanning electron microscopy (SEM) allowed for examination of particle morphology and size. Energy dispersive x-ray (EDX) spectroscopy supported elemental analysis and transmission electron microscopy (TEM) facilitated imaging at nanoscale resolution for structural characterization. They were then assessed for their antibacterial efficacy and cytotoxicity. After the reduction reactions of silver into nanoparticles, color change was confirmed, plasmon resonance peaked at 440 nm and the results of the zeta potential revealed a highly stable formulation, as evidenced by a competative negative zeta potential values in the range of -12.8 to -19.5 mV. Elemental mapping reveals particles consisting of silver, among the main elements. Highly aggregated and polydispersed spherical silver nanoparticles of 2 - 45 nm sizes were observed with electron microscopy. The biosynthesized AgNPs showed good antibacterial activity (ranging from 15 – 30 mm) and *in vitro* antioxidant activities (scavenging effects at 42.7 – 85.45%) increased as concentration increased. The cytotoxic properties of the nanoparticles were observed and found to exhibit high toxicity against Caco-2 colorectal adenocarcinoma cells. Cytotoxicity of the nanoparticles was greater compared to extracts of the *L. discolor* plant. This study represents the initial research on the biosynthesis of nanoparticles using *L. discolor* and silver and underscores the prospect for safe methods in fabricating nanoparticles that could prove useful in the battle against pathogenic infections, and other non-communicable diseases, as well as advancement of nanoparticles derived from traditional medicine.

Keywords: *Lannea discolor,* silver nanoparticles, biosynthesis, Antibacterial activity, cytotoxicity,

7.1 Introduction

Nanosynthesis is a revolutionary technique in nanotechnology which deals with the synthesis of matter of any kind at nanoscale (1 – 100 nm). Owing to their morphology, size and efficient biodistribution, nanoparticles have constantly been considered for applications in different fields (Anjum et al., 2021). As an emerging technological revolution, nanotechnology has gained its fronts in disciplines such as biology, chemistry, medicine, and the health sector (Malik et al., 2023). Conventionally, nanoparticles are synthesised by various methods, including physical and chemical processes some of which have been proven to be costly and toxic to the surrounding environment (Patra and Baek, 2015). Conventional methods for the fabrication of nanoparticles commonly employ chemical and physical procedures that utilize environmentally hazardous materials that may pose an array of biological risks once they interact with most biotic factors. In this regard, it is essential to come up with reliable, nontoxic, clean, eco-friendly protocols for nanosynthesis (Gopinath et al., 2010). In the pursuit of more feasible and environmentally friendly ways of nanosynthesis, scientists have turned to "green synthesis", a process by which reduction reactions are performed by the bioactive compounds in the plant or microorganism. These bioactive compounds have properties such as the ability reduce toxicity within the metal precursor, provide stability and cap or encapsulate nanoparticles to avoid overgrowth. (Adeyemi et al., 2022; Hiba and Thoppil, 2022). Various metals and their oxide forms have been employed as precursors in green synthesis routes to generate nanoparticles. However, commonly produced and studied nanoparticles usually contain silver (AgNPs) are the most common type of nanoparticles produced, due to intrinsic characteristics, exceptional chemical stability, and significant biological properties (Simon et al., 2022). Different extracts from a variety of plants have been widely used in green nanotechnology to research alternative methods for nanosynthesis, as plant extracts boost biocompatibility, accessibility, and simplicity and are eco-friendly (Adeyemi et al., 2022; Khan et al., 2022). Parameters such as the temperature environment, amount of time, and controlling the amounts of precursors introduced as well as any reducing agents employed represents a factor that has been validated to impact the outcome of a synthesis procedure (Shaikh et al., 2021).

Literature suggests and validates that *Lannea discolor* plant parts have an ample number of medicinal uses around the world (Mølgaard et al., 2001; Maroyi, 2013). However, previous studies have not demonstrated utilizing the plant for nanosynthesis. Furthermore, many authors have used many other plants for silver nanoparticle synthesis for use in many applications, owing to their several activities such as antibacterial, and apoptotic activities. Earlier studies have demonstrated the ability of *Lannea grandis* (Kumar et al., 2017), *Anacardium occidentale* (Adelere et al., 2020) and *Lannea coromandelica* (Wahab et al., 2023), to synthesise nanoparticles from silver that demonstrated high antibacterial activities against common pathogenic bacteria. In the face of fighting against cancer in the past few

years, studies aimed at the determination of the toxicity of nanoparticles towards human body systems, and different cancers have been on a rampant increase particularly those being developed with biological applications in mind (Ghorbani et al., 2018; Reis Luz et al., 2018). McShan et al. (2014), theorizes that changes to the nanoparticles' structure and properties during their interaction with external factors, contribute to observed toxic effects, which further establish the toxicity of the said nanoparticles against cancer cells. The safety of *L. discolor* has been determined in a study by Kabongo-Kayoka et al. (2016), where the leaf extracts revealed low cytotoxicity activities of *L. discolor* against Vero monkey kidney and RAW 264.7 cells serving as an indication of their safety. Furthermore, the extracts showed toxicity against hepatoma cells, showing some potential against hepatocellular carcinoma. However, there has not been work done on cytotoxicity activity against other cancer cells. The current research utilized the human colorectal adenocarcinoma cell line (Caco-2) to evaluate the potential anticancer activity of *L. discolor* extracts in comparison to the nanoparticles. The present study stands significant, as it deals with the first study on silver nanoparticles synthesis from *Lannea discolor* plant extracts and testing their efficiency as antibacterial and cytotoxic agents to establish new grounds for the treatment of infections and potentiate the plant for anticancer research. This would bring new advancements to nanomedicine in terms of enhancing the efficiency of drug administration.

7.2. Materials and Methods

7.2.1 Plant collection and extraction

Lannea discolor vegetative organs which were previously collected (Vuwani, 23°09′42.9″ S 30°25″22.6″ E) and air-dried *were* ground using a grinding mill (NETZSCH, Selb, Germany), and extracted using methanol, acetone and water at 3 day intervals at ratios of 1:10. The extracts and plant material were separated by filtering through a No.1 Whatman paper, dried into powder and stored in a cool dry place for further analysis.

7.2.2 Plant mediated Synthesis and collection of Silver Nanoparticles (AgNPs)

The biosynthesis of the nanoparticles was conducted using the standard means from Akwu et al., (2021) with slight revisions. Aliquots of 5 ml of plant extracts acquired from methanol, acetone, and water mixed into 50 ml of 1 mM silver nitrate (AgNO3) formulated in distilled water and stirred for 15 minutes to initiate the reduction process associated with nanoparticle formation. Reactions were carried out at 25°C and a stable concentration of 80 °C for 1 hour. The change in colour observed in the reaction mixtures prompted confirmation of nanoparticle synthesis, resulting the removal of the solutions from their respective reactions conditions. The solutions were then placed in a dark chamber for 24hrs to avoid continuous synthesis. 5 ml distilled water was substituted for the negative control and taken through the same reactions conditions as described for the extracts. All samples were synthesised in triplicates (n = 3) and tagged Ag_ML_/MS/MR (“Ag” for silver; “M” for methanol and “L” for leaf) depending on the plant part and the same was done for all the samples. Upon complete reduction, each the nanoparticle sample was pelletised by subjecting them to 4 cycles of centrifugation at 4000 rpm for 35 minutes at 4° C. Following centrifugation, the resulting pellets were washed down thrice distilled water to eliminate impurities. The pellets were dried in an oven at 50 ° C and kept at -4 ° C for further use.

7.2.3 UV-Visible Spectral Analysis

The synthesis of silver nanoparticles (AgNPs) was verified using ultraviolet-visible spectroscopy (UV-Vis) (SpectraMax 3). Samples containing AgNPs were added into the wells of a 96-well plate and their absorbance was quantified across a wavelength range of 200 to 800 nm with 1 nm spectral resolution. This allowed confirmation of AgNP formation through characteristic surface plasmon resonance absorption.

7.2.4 Zeta Potential of Nanoparticles

A 1 ml aliquot of the silver nanoparticles (AgNPs) was used to measure zeta potential via a Malvern Zetasizer Nano-ZS90 system produced by Malvern Inc. Triplicate measurements of the AgNPs were

conducted using a viscosity of 0.8872 mPa·s and a temperature of 25°C. Zetasizer software version 7.13 , which was purchased from the manufacturer, was used to analyze the data that was collected.

7.2.5 ATR-Fourier Transform Infrared Spectroscopy (FTIR)

An Alpha Platinum ATR-Fourier-transform infrared (ATR-FTIR) spectrophotometer (Bruker, Bryanston, South Africa) was used to analyse the AgNPs at parameters of 2 cm^{-1} resolution ranges of 4500 and 400 cm^{-1}. The purpose of this was to identify the functional groups interacting within the samples.

7.2.6 Scanning electron microscopy (SEM) analysis and energy dispersive x-ray (EDX) microanalysis

The silver nanoparticles were fixed on aluminum SEM stubs by means of double-sided carbon adhesive tape. They were then gold sputter coated using a Quorum K 150 RES sputter coater under a vacuum of 0.1 Torr for 2.5 minutes. The samples were then visualized at various magnifications utilizing a Zeiss Ultra Plus FEG scanning electron microscope with an operating voltage set to 5kV. Images were acquired and examined utilizing the NIS-D imaging software package in conjunction with the AzTec analytical platform, Version 1.2. Elemental composition analysis was conducted using an Oxford X-Max energy-dispersive X-ray spectroscopy detector attached to an Astronomical Thermal Emission Camera model 1.2, operated at 20 kV.

7.2.7 Transmission Electron Microscopy (TEM) Analysis

The previously sonicated AgNPs were suspended on 200 mesh carbon-coated formvar TEM grids, after which their morphology and size was observed and captured using a JEOL 2100 HRTEM (Japan) at a voltage of 200 kV.

7.2.8 Biological Evaluation of the plant synthesised nanoparticles

7.2.8.1 *In vitro* antioxidant DPPH scavenging activity

The radical scavenging activity based on 2,2'-diphenyl-1-picrylhydrazyl (DPPH) was determined using a revised version of the method described by Bhakya et al. (2016) with ascorbic acid as a standard control. In brief, 0.3 mM of DPPH was incorporated into 100 µl of all the test samples in a 96-well plate and left to stand for 30 minutes in dark conditions. At 517 nm, the silver nanoparticles' absorbance was measured against a blank control of DPPH without any test sample. The radical scavenging activity of the silver nanoparticles was calculated using the appropriate equation:

$$\text{Scavenging activity (\%)} = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} \times 100$$

where absorbance of control $Abs_{control}$ is the value measured for the mixture of DPPH + methanol

Abs $_{sample}$ is the absorbance measured for the mixture of DPPH + AgNPs (or ascorbic acid standard)

7.2.8.2 Antibacterial evaluation of AgNPs

7.2.8.2.1 Bacterial Strains and Antibacterial Activity Assay

The antimicrobial efficacy was assessed using five bacterial strains: *Escherichia coli* (ATCC® BAA-2469), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumoniae* (ATCC 700603), *and Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 43300).

An antibacterial assay was carried out using the technique of Madikizela et al. (2013), with some revisions. A loopful of bacteria from each cultured bacterium were incorporated into a nutritious broth (5 ml) (Merck, Germany) and allowed to grow overnight at 34 ± 1 °C. The solutions were further adulterated with sterilised nutrient broth and spread evenly onto Müller-Hinton agar plates and permitted to airdry at ambient temperature. Müller-Hinton agar plates containing 8 mm wells were topped with 100 µl of each AgNPs sample and incubated overnight at 34°C. The inhibition zones measured were considered as positive results. Silver nitrate ($AgN0_3$) was used as the negative control, while gentamicin, a positive control at a concentration of 10 µg/ml was employed. For the Alamar blue assay, the cultures (100 µl) were added to each well after serially diluting the silver nanoparticles (AgNPs). The agar plates were enclosed with parafilm and incubated overnight at 34 ° C. Subsequently, 40 µl resazurin was added to each well to assess growth inhibition. The results were observed after 30 min and the lowest concentration containing blue were considered as the minimum inhibitory concentration (MIC) values. The measurements were presented as mean ± standard deviation, and each of the tests were run in triplicates (n=3).

7.2.8.3 Cytotoxicity evaluation of AgNPs against human colorectal adenocarcinoma cells (Caco-2)

7.2.8.3.1 Sample preparation

The extracts and AgNPs were dissolved in dimethyl sulfoxide (DMSO) to generate stock solutions at a concentration of 100 mg/ml. The samples were exposed to sonication to ensure homogeneity and then stored at 4 ° C until required for further testing.

7.2.8.3.2 Cell line maintenance

The Caco-2 human colorectal adenocarcinoma cell line was obtained from Cellonex in South Africa. The Caco-2 cells were propagated in 10 cm culture dishes containing complete medium consisting of Dulbecco's Modified Eagle Medium with low glucose (DMEM-LG) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin. The cells were incubated at 37°C in humidity and carbon dioxide at 5%.

A 100 µl aliquot of Caco-2 cells were seeded into a 96-well plate at a density of 4,000 cells per well (100 microliter aliquots) and incubated overnight to allow for attachment. Three concentrations, 250, 500, and 1,000 µg/ml, of each sample were assessed. Treatments at double the final concentrations were added to the wells in 100 µl aliquots to achieve a total volume of 200 microliters per well. Incubation of the cells was for a period of 48 hours at 37 °C and 5% carbon dioxide. A 50 mM concentration of melphalan, using a 100 mM stock solution, served as a positive control. For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 100 µl of 0.5 mg/ml MTT in complete medium was added to each well containing cells, aspirated, and incubated for 3 hours. Subsequently, 100 µl of dimethyl sulfoxide was added to each well for solubilisation. A BioTek® PowerWave XS spectrophotometer ((Winooski, VT, USA) was then used to measure absorbance values at 540 nm.

7.3. Results and Discussion

7.3.1 Synthesis, UV-Vis, and Zeta Potential analysis of AgNPs

Nanoparticle solutions of *L. discolor* extracts and reduced silver nitrate were exposed to temperature and time factors for synthesis to take pace. After incubation at synthesis, a color change was observed in all the samples. The colour of the solutions varied from yellowish to burnt orange, and from light reddish brown to cloudy brown (Figure 7.1 A – D). The overall colour change of nanoparticle solutions observed in the present study was like in previous studies (Bodede et al., 2017; Kocazorbaz et al., 2021; Mukaratirwa-Muchanyereyi et al., 2022) and confirms as observed in the color changes from synthesis of silver nanoparticles from *Zanthoxylum capense*, *Quercus coccifera*, and *Erythrina abyssinica*, that the initial step to confirm nanosynthesis formation, is through the change in colour of the solutions (Chopra et al., 2022).

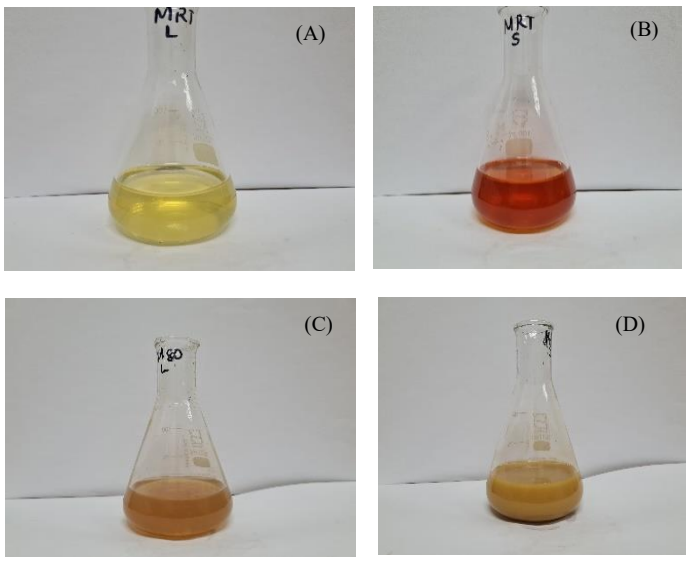


Figure 7.1. Representative images showing visual changes after synthesis of AgNPs using extracts of *Lannea discolor* leaves (A) and bark (B). Changes after synthesis observed in C and D.

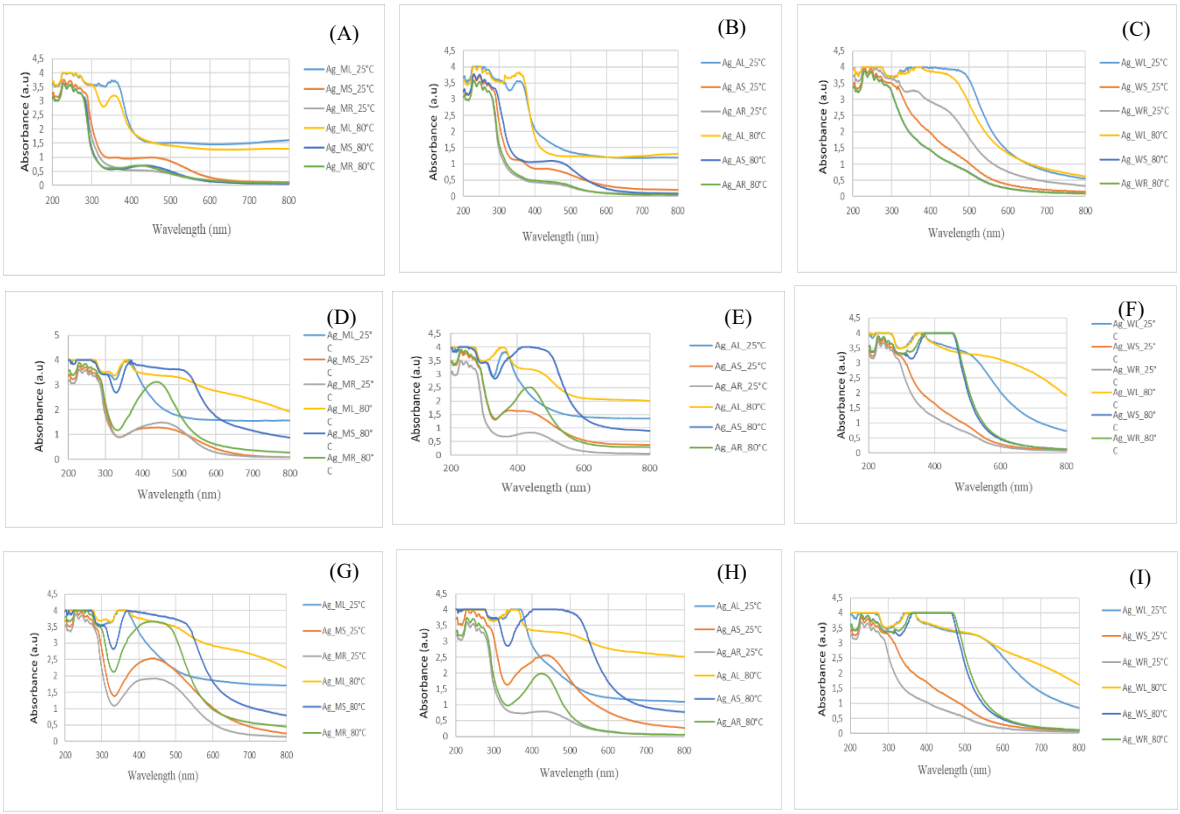


Figure 7.2. The ultraviolet-visible (UV-vis) spectroscopy results show the absorbance spectra recorded for various reactions indicating the formation of silver nanoparticles (AgNPs). In each reaction, a 1 mM aqueous silver nitrate (AgNO3) solution was reacted with different *Lannea discolor* extracts and incubated at temperatures of 25 °C and 80 °C. Each graph (A-I) represents AgNPs formation from methanol, acetone, and water extracts at varying times of synthesis (A – C) 0 minutes (D – F) 60 minutes (G – I) 24 hrs.

All metal nanoparticles have their own characteristic absorption pattern that is determined by UV-Vis spectroscopy as the reduction of Ag+ progresses, which is valuable in establishing the formation and stability of metal nanoparticles (Evanoff and Chumanov, 2005; Revathy et al., 2022). Presently, single maximum absorbance peaks were observed at 320 nm to 480 nm which indicates the excitation of the nanoparticles, referred to as surface plasmon resonance (Santhoshkumar et al., 2021). The spectra projected indicate the complete reduction of silver to nanoparticles at both 25 °C and 80°C by stem and root the extracts as denoted by some broad peaks (Figure 7.2), indicating possible formation of large nanoparticles as the reduction slows down due to the termination of the reaction (Donga and Chanda, 2021). The methanol extract demonstrated a narrower peak profile (Figure 7.2 A, D & G) and longer

wavelength absorption, which was almost identical to the spectral response for nanoparticles produced from acetone extracts (Figure 7.2 B, E & H).

UV-Vis analysis was similar to studies by Donga and Chanda, (2021), where in the characteristics peak of silver ranges from 380 to 580 nm. Nanoparticles with sizes of between 2 and 100 nm are said to characteristically peak between 200 – 800 nm, signifying (Safaepour et al., 2009; Ibrahim, 2015; Azis et al., 2022). Low wavelengths change evident in samples synthesised at both room and high temperatures, may suggest more presence of weak reducing bioactive compounds in the extracts, which would have been more concentrated had the synthesis occurred at temperatures beyond 80 °C clearly required higher temperatures and longer periods for the progression of reduction as suggested by El-Chaghaby and Ahmad (2011) and Mohanta et al. (2020). After 24 h of reaction time, no change is observed, as a confirmation that all the silver molecules in the solutions have already been reduced to AgNPs. On this basis, these conditions and time length were considered as optimum for forming AgNPs with the reducing agent of *Lannea discolor* aqueous bark extract. Both methanol and acetone have better extraction efficiency for phytochemicals which are more efficient in silver ion reduction similar to the study by Mukaratirwa-Muchanyereyi et al. (2022). The zeta potential was measured, and the value of zeta potential of the silver nanoparticle solution produced from the plant extracts was between − 19.5 mV and – 12 mV, suggesting long term stability of AgNPs. The acceptable stability values usually fall outside +30 mV and -30 mV, as such, AgNPs from *L. discolor* are confirmed to be highly stable (Eltarahony et al., 2016).

7.3.2 Surface Morphological analysis by SEM, and EDX Analysis

The properties of *L. discolor* AgNPs, which include surface morphology, overall shape and diameters were examined using SEM. The SEM micrographs of the nanoparticles in Figure 5.3 show few large particles as well as many spherical shaped silver nanoparticles, some of which are agglomerated together. Similarly, the spherical shaped silver nanoparticles were synthesized using *Odina wodier*, Roxb. (Arunkumar et al., 2013). The cause of the agglomeration could be due to the polarity and electrostatic attraction of the nanoparticles rising from the biological material as well as rising temperatures (Zare, 2016). It is noteworthy that in some areas, the nanoparticles were stacked together and formed an irregular structure (Figure 7.3C).

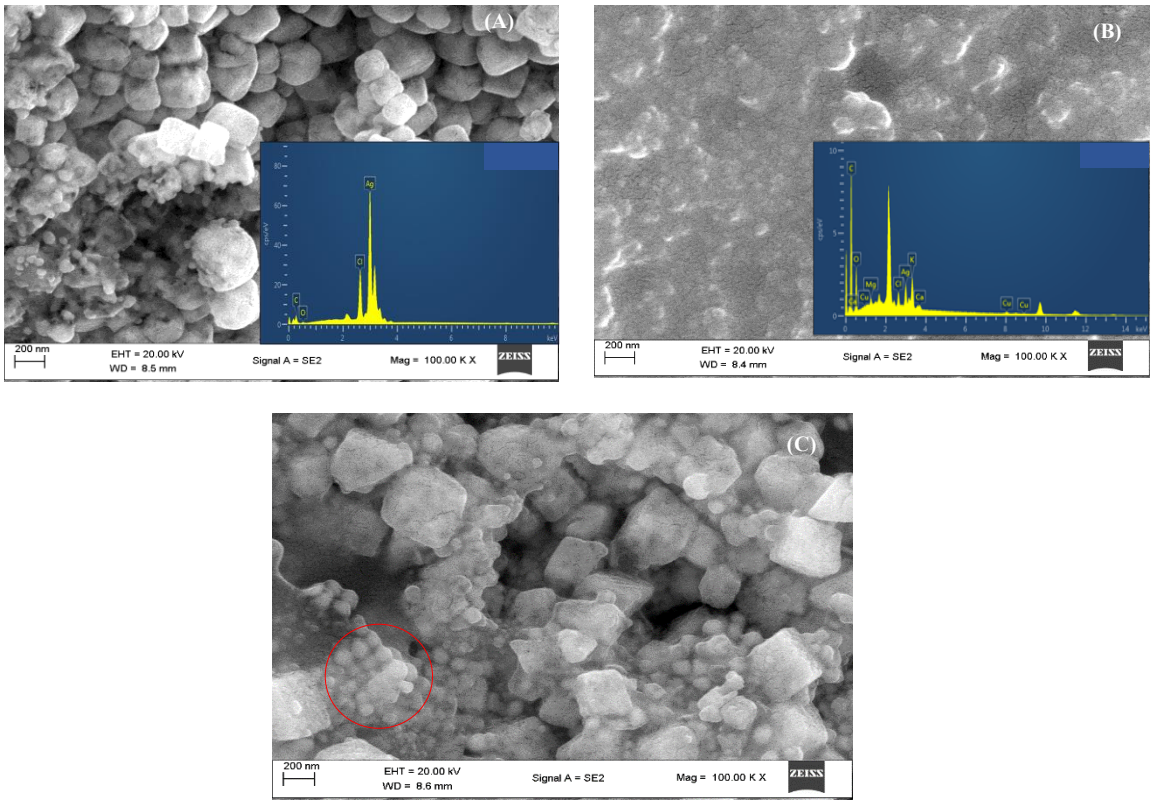


Figure 7.3. SEM images of AgNPs with their respective EDX spectra validating the presence of silver nanoparticles. Circle in (C) shows some spherical NPs. Insets in (A) and (B) showing signal peaks for the presence of elements.

EDX is a technique that determines the basic elements that are composed in substances across the field of nanotechnology. It is essential to validate the presence of desired elements in a sample after nanosynthesis to determine what influences certain properties (Scimeca et al., 2018). Presently, EDX validates the incidence of Ag in the AgNPs. (Figure 7.3 A and B insets) give a strong indication regarding the incidence of silver in the AgNPs for different samples. The EDX profile of silver nanoparticles showed a strong signal peak indicating an average of 79 wt %, 80 wt % from both the methanol and water extracts (high signal peaks in Figure 7.3A, and the weakest signals were seen in the acetone extracts with an average of 29 wt % (Figure 7.3B). All of the samples exhibited an optical absorption maximal peak at 3 keV, which aligns with the predicted absorption level for silver nanoparticles (Ali et al., 2023). The observed EDX suggested that *L. discolor* synthesised AgNPs have different amounts of silver as seen by the weight percentages. This further suggests maximum reduction of silver ions during synthesis. However, this is not always the case. In a study by Khiya et al. (2021), AgNPs from *Pistacia atlantica* leaves demonstrated a weight percentage of 60.2%, which is lower, compared the weights observed in the present study. Additional elements were present, some of which

are typically found in plant mediated nanoparticles. These were denoted by peaks of carbon (22.81%) and oxygen (16.03%) which are constituted in most metabolites (Shaik et al., 2018).

7.3.3 Microstructural studies by HRTEM and Size distribution

The morphology of the three differently shaped AgNPs is shown in the TEM micrographs in Figure 7.4, which include some short rod nanoparticles, triangular (Figure 7.4B) and spherical nanoparticles for all the extracts. The TEM micrographs depict mostly spherical nanoparticles (Figure 7.4A – D) were formed, ranging from approximately 2.85 to 46.6 nm in size (Figure 7.5C and F). Further examination of the TEM micrographs revealed that at both temperatures, AgNPs were similar in sizes, although AgNPs at 80 °C appeared insignificantly smaller (Figure 7.5 D and E) than the AgNPs synthesized at room temperature, giving a favourable nanoparticle size distribution.

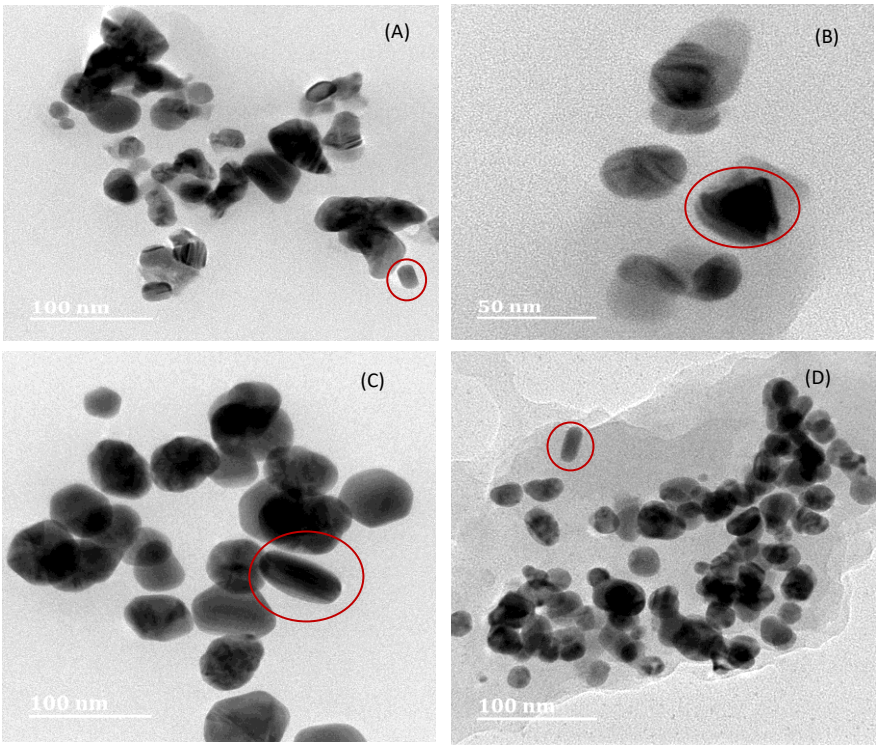


Figure 7.4. TEM silver nanoparticles with different shapes of differently shaped nanoparticles. (Circled in red) Short silver nanorods (A, C, D), spheres (A – D) and triangular shaped (B) particles.

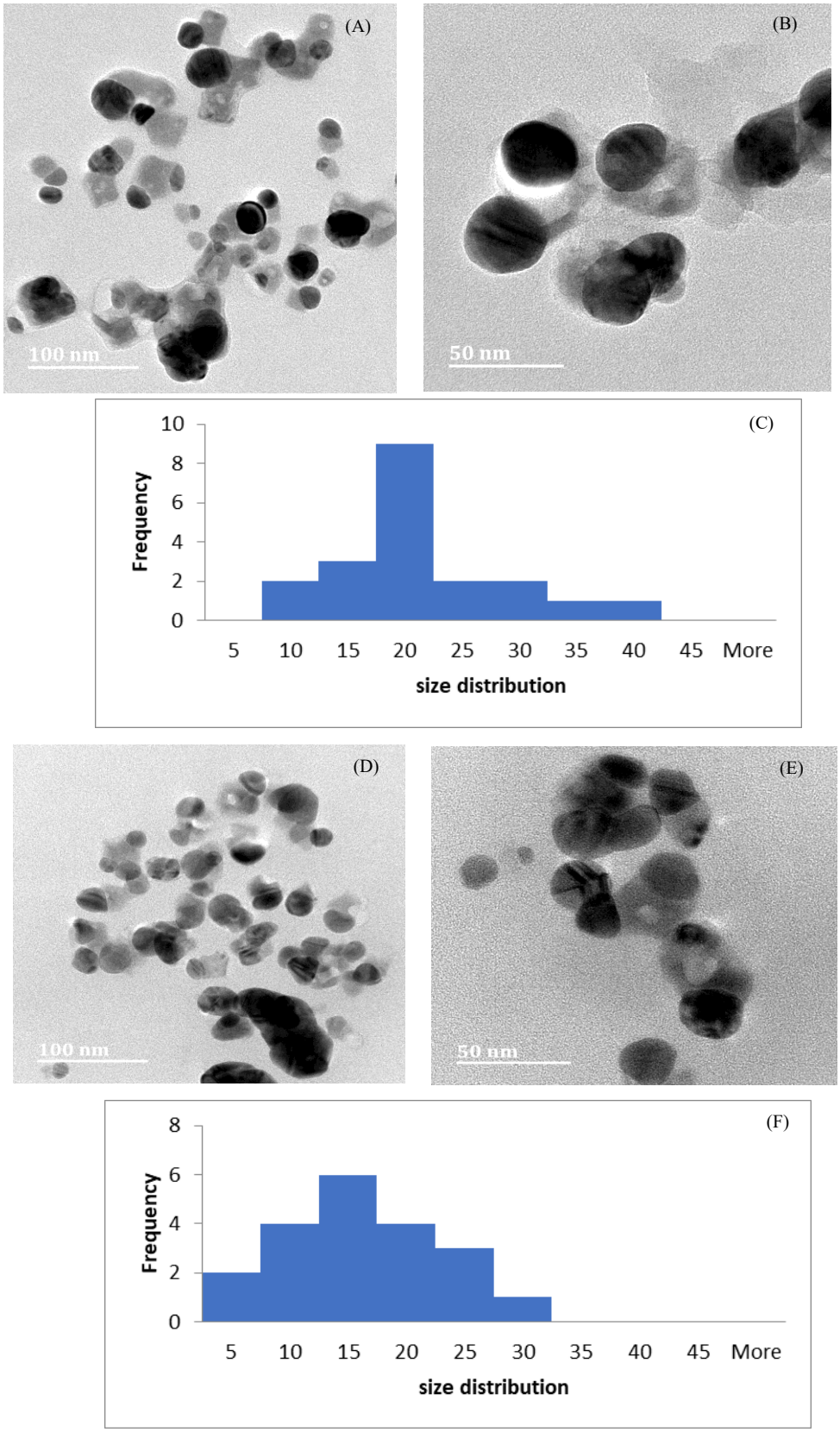


Figure 7.5. Representative TEM micrographs of AgNPs synthesized at 25 °C (A and B) and 80 °C (D and F) Shape and size at different magnification power. Notes: Ag nanoparticles are majorly spherical. AgNPs are dispersed homogeneously in solution and not agglomerated in some samples. The histograms (C and F) show silver nanoparticle size distribution of 2.85 to 46.6 nm.

Comparable results from AgNPs from *Anacardium occidentale* leaves were measured at 19 nm in a study by Sheny et al., (2011). Similar nanoparticle sizes were also obtained by Panwar et al., (2022) showing pseudo-spherical particles from *Mangifera indica* leaves with a diametere of 18.2 nm. In another study, synthesis of silver nitrate and *Mangifera indica* resulted in spherically shaped nanoparticles with a diameter of 22 nm (Rana et al., 2023). While in a similar study AgNPs synthesised from *Mangifera indica* had a size of 31.7 nm from the leaves (Sundeep et al., 2017). Synthesis of different solvent extracts at various concentrations determines disparities in morphology and size as well as other factors, like aggregation and dispersity. This also determines the types of applications they would be used in. Additionally, the properties of nanoparticles could also be determined by the plant part used in the synthesis, as well as the overall health of the plant. In biomedical applications nanoparticles with sizes less than 100 nm are more favourable for use as the benefits of their use highly depends on their sizes and the ability to manoeuvre into the cells (Hiba et al., 2022).

7.3.4 FTIR analysis

To determine the chemical makeup of AgNPs' surfaces, FT-IR analysis is performed as it provides a good picture of the biomolecules that were involved in synthesis as well as stabilization and reduction (Singh and Mijakovic, 2022). Recent studies claim that the many prevalent functional groups contributing to the production of AgNPs, have been discovered to be carbonyl (–CO), (–CO–NH2), and hydroxyl (–OH) (Dawadi et al., 2021). However, it was not the case for this present study. The presence of different phytochemicals is presumably indicated by the various molecular groups found in the different extracts and samples, confirming that the AgNPs are capped with different phytochemicals (Eid, 2022). As such, the FTIR examination has significant ramifications for the initial qualitative research. Strong absorbance bands in Figure 7.6 are observed in all samples in the region 4000 – 500 cm^{-1} and (after reduction) are approximately at 1618 - 3496 cm^{-1}. The strongest absorption bands consist of water (3290 – 3496 cm^{-1}) and others can be assigned to hydroxyl peaks (O-H stretching) at between 2109 - 3290 cm^{-1}, may be ascribed to carboxylic acids. O-H bonds indicate the presence of water, as verified in Nunes et al., (2019), these, perhaps constituted in proteins and carbohydrates within the samples. The bands at 1739.31 cm^{-1} indicate the existence of aldehyde groups C=O (de Souza et al., 2017). The absorption bands at 2891.37 and 2898.55 corresponds to C–H medium stretches of alkane groups (Donga et al., 2020). The bands at 2112.60, 2114.30, 2137.52, 2138.95 cm^{-1} denotes to C=C medium stretching of alkyne groups. Similar absorbance bands were observed by Ologunagba et al., (2017) and Donga et al., (2020). Alkene and enol carbon double bonds (C=C) with wave numbers around 1634.03 – 1646 cm^{-1} showed in a majority the spectrum of the samples, confirming a typical functional group in the aglycones of saponins as was observed in Akoto et al., (2020). Similar peak shifts were observed across the nanoparticle's samples synthesised from the samples that differed in terms of solvent used for extraction, plant part and temperature at which synthesis occurred. The absorption peak at 2095.75 and 2111.22 cm^{-1} is reported for isothiocyanate functional group (N=C=S),

suggesting interaction with, and capping of AgNPs by isothiocyanates, as in consistence with the observation by Wu et al., (2009).

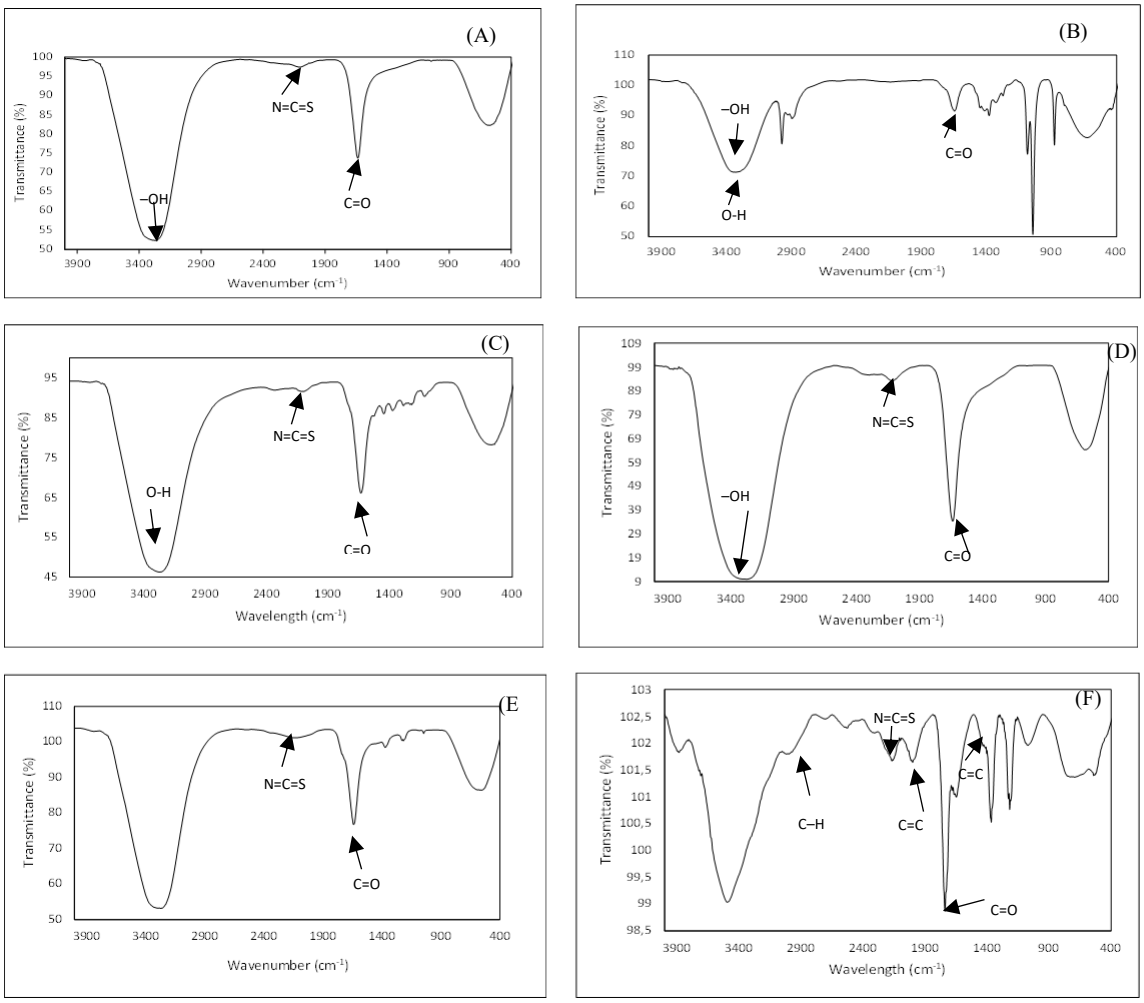
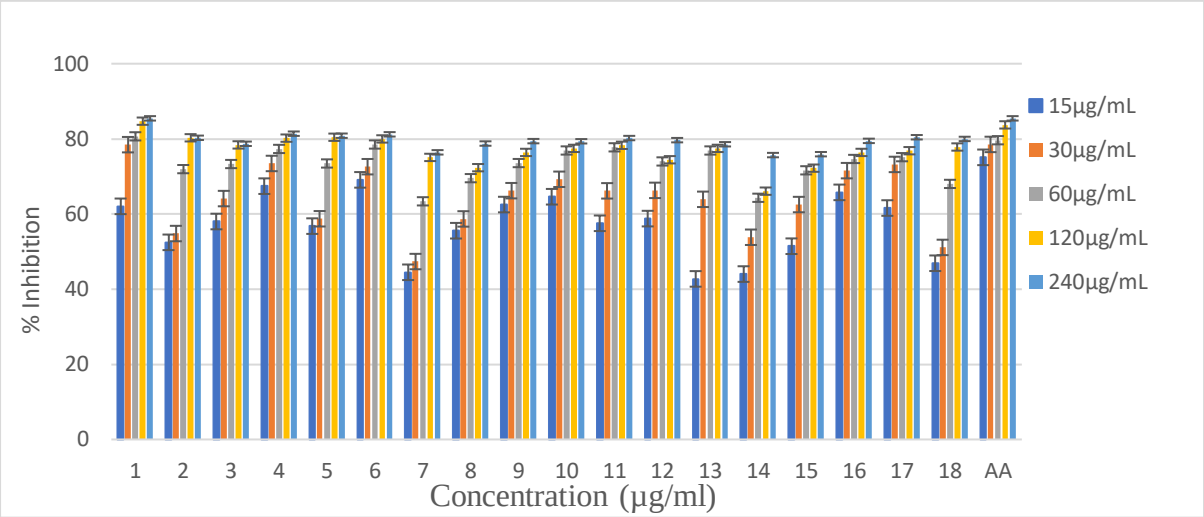


Figure 7.6. Representative Fourier transform infrared (FTIR) spectra are shown for silver nanoparticles (AgNPs) biosynthesized from various extracts of *Lannea discolor.* (A) leaf extract (25 °); (B) synthesis with stem bark (25 °C) (C) root bark (25 °); (D) leaf methanol extract (80 °C); (E) stem bark (80 °C) (F) root bark (80 °C).

7.3.5 Antioxidant and Antibacterial assay

The ability of AgNPs synthesised by *L. discolor* to scavenge free radicals was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. The NPs from various extracts and temperatures displayed dose-dependent action (Figure 7.7), and the DPPH scavenging effect peaked at 85.45% at 240 µg/ml of Ag-NPs after starting at 42.7 % at a concentration of 15 µg/ml. *Lannea discolor* AgNPs from methanol extracts in no order of significance, showed strong *in vitro* antioxidant potential against DPPH radicals which exhibited the inhibition percentages values ranging from above 50% to 85.45%. According to the percentage of inhibition values, it is observed that the anti-radical power increases

proportionately with concentration. Previous research has shown that biosynthesised AgNPs of different plants showed good antioxidant activity, suggesting that they may be highly effective in treating a various illness that are caused by intercellular stress, such as insulin resistance, cancer, and other neurological diseases (Khane et al., 2022; Erenler et al., 2023).



Sample keys: 1=Ag_ML_25°C; 2 = Ag_MS_25°C; 3 =Ag_MR_25°C; 4=Ag_ML_80°C; 5=Ag_MS_80°C; 6 = Ag_MR_80°C; 7 = Ag_AL_25°C; 8 = Ag_AS_25°C; 9 = Ag_AR_25°C; 10 = Ag_AL_80°C; 11 =Ag_AS_80°C; 12 = Ag_AR_80°C; 13 = Ag_WL_25°C; 14=Ag_WS_25°C; 15 = Ag_WR_25°C; 16 = Ag_WL_80°C; 17 = Ag_WS_80°C; 18 = Ag_WR_80°C

Figure 7.7. The figure shows the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity percentages of inhibition for the silver nanoparticles synthesised from the methanol, acetone, and water extracts of the leaves, stem, and root of *Lannea discolor* at room temperature and 80 °C.

The activity of the AgNPs against bacteria was assessed by well diffusion and Alamar blue MIC Assay. As summarised in Tables 7.1 – 7.3, it is distinct that AgNPs synthesised from *Lannea discolor* possess the ability to mitigate the growth of the tried microorganisms. A maximum activity was observed due to high zone of inhibitions in the AgNPs synthesised from methanol extracts for all the bacteria tested, with inhibition zones ranging from 15 – 26 mm. For the acetone and water extract synthesised nanoparticles, the inhibition zones ranged from 10 – 25 mm and 9 – 20 mm respectively. The positive control, Gentamicin had a good bioactivity against all the bacteria paralleled to its counterparts. Against the bacteria *P. aeruginosa, B. subtilis and S. aureus*, the AgNPs synthesised at 80° C and remained the most efficient with high activity just below that of the positive control. At room temperature, AgNPs demonstrated the lowest activity against *K. pneumoniae* and *E. coli* and, in contrast to their counterparts, yet can be considered as antimicrobial agents.

Table 7.1. Antibacterial activity of the synthesised nanoparticles from the methanol extracts of *Lannea discolor*.

	Zones of inhibition (mm)							
	AgNPs (Room temp)			**AgNPs 80 °C**			**Control**	
	ML	**MS**	**MR**	**ML**	**MS**	**MR**	**Gentamicin**	**AgNO3**
S. aureus	17	17	22	22	22	25	30	14
B. subtilis	15	20	20	21	23	25	30	13
P. aeruginosa	17	17	15	23	24	25	30	14
E. coli	15	21	21	20	25	26	30	14
K. pneumoniae	16	17	15	20	25	26	30	13

Table 7.2. Antibacterial activity of the synthesised nanoparticles from the acetone extracts of *Lannea discolor*.

	Zones of inhibition (mm)							
	AgNPs (Room temp)			**AgNPs 80 °C**			**Control**	
	AL	**AS**	**AR**	**AL**	**AS**	**AR**	**Gentamicin**	**AgNO3**
S. aureus	14	16	18	16	18	20	30	14
B. subtilis	10	13	15	17	20	24	30	13
P. aeruginosa	15	15	19	20	20	25	30	14
E. coli	12	14	15	16	20	20	30	14
K. pneumoniae	10	15	20	20	20	30	30	13

Table 7.3. Antibacterial activity of the synthesised nanoparticle from the water extracts of *Lannea discolor*.

	Zones of inhibition (mm)							
	AgNPs (Room temp)			**AgNPs 80 °C**			**Control**	
	WL	**WS**	**WR**	**WL**	**WS**	**WR**	**Gentamicin**	**AgNO3**
S. aureus	15	15	10	15	15	15	30	14
B. subtilis	10	15	17	17	17	20	30	13
P. aeruginosa	15	17	19	15	18	20	30	14
E. coli	12	10	10	15	12	18	30	14
K. pneumoniae	9	9	10	12	15	17	30	13

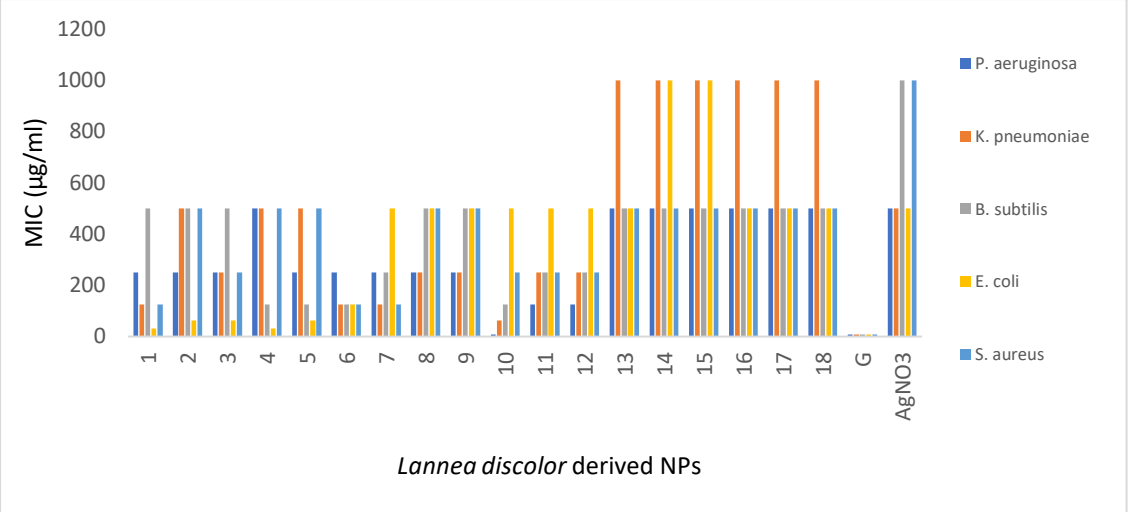
Sample keys (Table 5.1-5.3): ML (Methanol leaf extract); MS (Methanol stem extract); MR (Methanol root extract); AL (Acetone leaf extract); AS (Acetone stem extract); AR (Acetone root extract); WL (Water leaf extract); WS (Water stem extract); WR (Water root extract).; AgNO3 = silver nitrate.

The minimum inhibitory concentrations for the *L. discolor* synthesised AgNPs and extracts are expressed in Figure 5.8. Literature has suggested that the criteria with which significant effects of a plant extract/ drug can be considered, should be at 0.15 mg/ml (150 µg/ml) however, no study has suggested a criterion that can be used in nanoparticles-based assays. As such, a criterion recommended by Eloff, (2021) was considered for the interpretation and rating of the MIC values of the samples.

Additionally, the MIC activities of the present study were classified as: MIC ≤ 62.6 µg/ml: good, MIC ≥ 125 µg/ml: moderate, MIC ≥ 500 µg/ml: poor.

AgNPs synthesised using acetone and methanol extracts at room temperature and 80°C demonstrated the ability to impede the growth of *Bacillus subtilis* and *Pseudomonas aeruginosa* at concentrations of 125 µg/mL and 7.8 µg/ml. These findings indicate the AgNPs produced from acetone and methanol extracts under different temperature conditions have potential as effective antibacterial agents.

For all the bacteria the positive control had high activity at 7,8 µg/ml and $AgNO_3$ with a MIC of 250µg/ml of displayed a lower activity than most of the AgNPs. *E. coli* and $AgNO_3$ had moderate activities compared to all the AgNPs apart from water leaf and stem synthesised nanoparticles at 80 ºC (MIC of 1000 µg/ml). *S. aureus* growth was inhibited at MIC of 500 µg/ml with AgNPs synthesised from *the* water extract, whilst higher activities than this were obtained from the AgNPs from methanol and acetone extracts at 125 – 250 µg/ml. Furthermore, AgNPs from methanol and acetone extracts tested against *K. pneumoniae* showed higher activity in comparison with the AgNPs from water extracts that had and MIC of 1000 µg/ml. Previous research has documented analogous experiments demonstrating the antibacterial functionality of AgNPs synthesized in comparable investigations, lending credence to the antibacterial capacity of the AgNPs in the present study (Ghorbani et al., 2015; Lediga et al., 2018; Samuggam et al., 2021; Khan et al., 2022). Contradictory findings may be attributed to various physiochemical characteristics such as the morphology of the AgNPs, oxidation ratio and dispersibility will also depend on the phytochemical compounds used to cap the nanoparticles (Hassan et al., 2022). Significant observations include AgNPs displaying significant activity against *Pseudomonas aeruginosa* which has been said to be resistant to several antibiotics including streptomycin (Pang et al., 2019). *Pseudomonas aeruginosa* is usually isolated from the burn wounds, and the *Lannea*-AgNPs could be successfully applied to treat the multi-drug resistant bacteria in such medical conditions.



Sample ID: 1=Ag_ML_25°C; 2 = Ag_MS_25°C; 3 =Ag_MR_25°C; 4=Ag_ML_80°C; 5=Ag_MS_80°C; 6 = Ag_MR_80°C; 7 = Ag_AL_25°C; 8 = Ag_AS_25°C; 9 = Ag_AR_25°C; 10 = Ag_AL_80°C; 11 =Ag_AS_80°C; 12 = Ag_AR_80°C; 13 = Ag_WL_25°C; 14=Ag_WS_25°C; 15 = Ag_WR_25°C; 16 = Ag_WL_80°C; 17 = Ag_WS_80°C; 18 = Ag_WR_80°C; G+ Gentamicin; AgNO3 = silver nitrate.

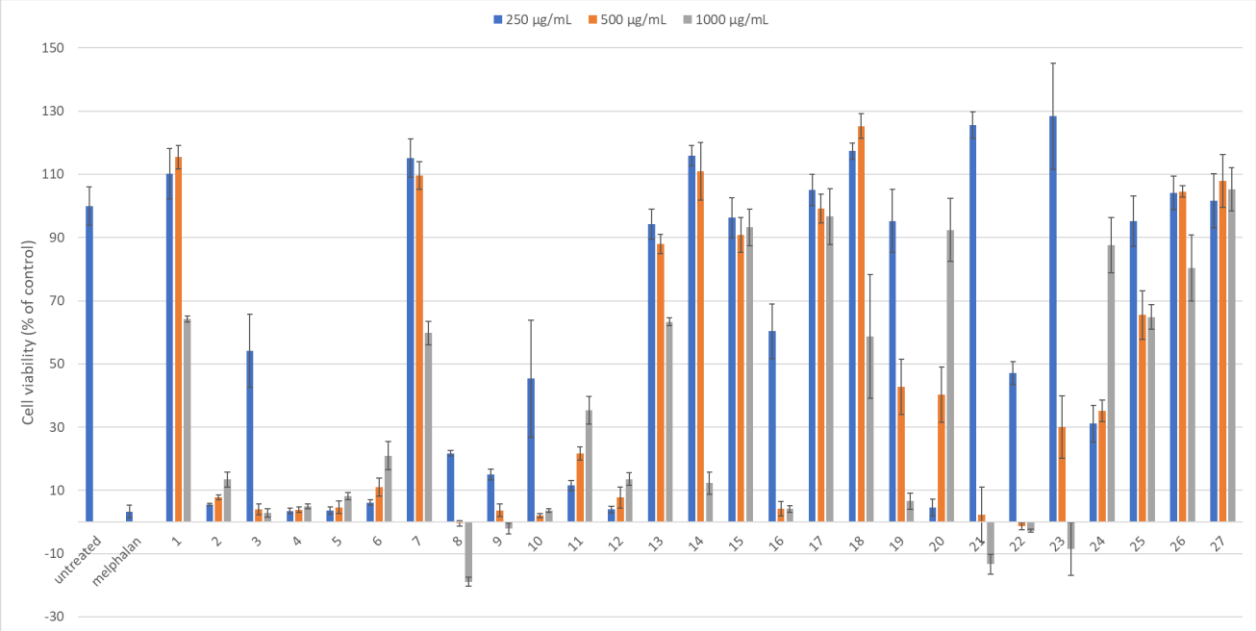
Figure 7.8. Results of minimum inhibitory concentration (MIC) of AgNPs particles and controls against bacteria.

7.3.6 Comparative Cytotoxicity Activity

The determination of cytotoxicity was performed by the MTT reduction colorimetric method (Sylvester, 2011; Vega-Avila and Pugsley, 2011). The efficiencies of the AgNPs and extracts of *L. discolor* were analysed in different concentrations against the Caco-2 cancer cell line. MTT is an accurate colorimetric method that is commonly used to determine cellular toxicity, as well as cell proliferation and viability. The samples were classified into three classes based on their cytotoxicity as follows: not cytotoxic (inhibition of cell growth by < 50%), moderately cytotoxic (inhibition of cell growth by 50% to 75%), and highly cytotoxic (inhibition of cell growth by > 75%).

In the triple concentration analysis (Figure 7.9), the methanol and water leaf and stem extracts showed cytotoxic effects when assayed against the cell cultures at higher concentrations of 500 and 1000 µg/ml, while the acetone leaf extract was cytotoxic at all concentrations. Considering that the leaves of *L. discolor* can be taken orally, being concurrently ingested that the gastrointestinal tract is the most exposed district to the action of dietary ingredients, it is possible to hypothesize a beneficiary effect of this extract, rich in phytocompounds, at the intestinal level. The methanol stem and acetone root extracts presented an inverse dose dependence cytotoxicity, whereas the water stem and root extracts presented no cytotoxic activity against the Caco-2 cells. The findings reported contradict those of research conducted by Varela-Rodriguez, et al., (2019) wherein, water extracts of *Rhus trilobata* decreased the proliferation of CaCo-2 cells. High cytotoxic values have also been observed in other plants of the same family *Rhus trilobata* (Híjar-Soto et al., 2014) and *Schinus polygamus* (Cav.) (El-Nashar et al 2021). In this respect, literature data has shown the ability of a large variety of classes of dietary phenols, (Zhang

et al., 2015; Youns and Hegazy, 2017; Kashyap et al., 2019) to affect glucose transport in Caco-2 cells, inhibiting cancer cell growth (Marbaniang et al., 2018; Müller and Kma, 2018; Schreck et al., 2019; Reckzeh et al., 2019). Therefore, in our case, the cytotoxic activity of *L. discolor* on the cells could be attributed to the reduced glucose internalization, mediated by the synergistic action of polyphenolic compounds.



Sample keys: 1=Ag_ML_25°C; 2 = Ag_MS_25°C; 3 =Ag_MR_25°C; 4=Ag_ML_80°C; 5=Ag_MS_80°C; 6 = Ag_MR_80°C; 7 = Ag_AL_25°C; 8 = Ag_AS_25°C; 9 = Ag_AR_25°C; 10 = Ag_AL_80°C; 11 =Ag_AS_80°C; 12 = Ag_AR_80°C; 13 = Ag_WL_25°C; 14=Ag_WS_25°C; 15 = Ag_WR_25°C; 16 = Ag_WL_80°C; 17 = Ag_WS_80°C; 18 = Ag_WR_80°C; 19 = ML (Methanol leaf extract); 20 = MS (Methanol stem extract); 21 = MR (Methanol root extract); 22 = AL (Acetone leaf extract); 23 = AS (Acetone stem extract); 24 = AR (Acetone root extract); 25 = WL (Water leaf extract); 26 = WS (Water stem extract); 27 = WR (Water root extract).

Figure 7.9. The graph depicts the results of an MTT assay demonstrating the cytotoxic effect of extracts from *L. discolor* and AgNPs on Caco-2 colorectal cancer cell viability.

Significant differences amongst the activities of the samples were observed, indicating the anticancer activity and potential of *Lannea discolor* –AgNPs on the tested cell line. The higher concentration (1000 µg/ml) of the nanoparticles AgNPs synthesised by methanol and acetone extracts at both temperatures (25 °C and 80° C) was found to be highly toxic against the Caco-2 cell line. For the same samples, all the concentrations (250 – 1000 µg/ml), showed toxicity against the cells, some more than others, confirming their high capacity of reducing the percentage of cell viability. The AgNPs from leaf and stem water extracts concentrations (500 – 1000 µg/ml) at room temperature significantly inhibited the cell growth by more than 50% and after 48h exposure time. The lowest concentration of silver AgNPs tested, 250 µg/ml, did not demonstrate a significance in cytotoxicity when compared to Melphelan.

However, there was an overall dose-dependent effect of AgNPs at all concentration on the Caco-2 cells, confirming a dose-dependent response relationship, and that, at higher concentrations of the samples, cytotoxicity may be induced.

This was also observed in *Pistacia lentiscus* Chios mastic, where the extract showed little to no toxic effects in Caco-2 cells treated with even lower concentrations (Zorzan et al., 2019). The cytotoxic properties of AgNPs are impacted by various factors including concentration, cell line tested, and particle size. Specifically, increasing Ag-NP concentration corresponds to higher cytotoxicity, while smaller Ag-NP sizes tend to result in greater cytotoxic potential compared to larger nanoparticles (Akter et a., 2018). Moreover, it has been reported that Caco-2 cellular nanoparticle uptake is higher for smaller particles less than 50 nm than that for larger particles (Banerjee et al., 2016) validating the effect of the small-sized particles synthesised by *L. discolor* extracts as well as the significance of nanoparticle size for cellular uptake, thus decreasing cell viability. Based on our findings, the *L. discolor* AgNPs manifested characteristics of cytotoxic nature against the intrinsically resistant human colorectal cancer cell line (Caco-2), depicting a promising candidature for biomedical application especially for cytotoxic activities.

7.3.7 Conclusion

The current investigation intended to assess the antioxidant, antibacterial and possible anticancer properties of silver nanoparticles biosynthesized from *L. discolor*. This represents the initial study to evaluate the aforementioned effects of silver nanoparticles derived from *L. discolor*.

Presently, it is observed that the benefits of plant-mediated synthesis are those of bioavailability of thhe phytochemicals that facilitate the reduction of silver ions. It is also wise to conclude that the need of specific solvents does not determine synthesis, even if it may offer a more favourable yield of bioactives that can efficiently reduce nanoparticles. The biosynthesized AgNPs from *L. discolor* may be a potential source of agents that have potent antioxidant and antibacterial activity, for the mitigation of different infections. *L. discolor* AgNPs exhibited effective cytotoxicity and anti-cancerous potential against the colorectal adenocarcinoma (Caco-2) cell line with high apoptosis inducing properties. Results presented in this research work presented that *L. discolor* mediated synthesised AgNPs with high cytotoxic potential against Caco-2 cells portrays potential for biomedical application and research especially for cancer work.

References

Adeyemi, J. O., Oriola, A. O., Onwudiwe, D. C. and Oyedeji, A. O., 2022. Plant extracts mediated metal-based nanoparticles: Synthesis and biological applications. Biomolecules. 12 (5), 627.

Akoto, C. O., Acheampong, A., Boakye, Y. D., Kokloku, B. K. and Kwarteng, G., 2020. In vitro anthelmintic, anti-inflammatory, antioxidant activities and FTIR analysis of *Sclerocarya birrea* root. *Journal of Pharmacognosy and Phytochemistry*, 9(2), 1389-1401.

Akter, M., Sikder, M. T., Rahman, M. M., Ullah, A. A., Hossain, K. F. B., Banik, S. and Kurasaki, M., 2018. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. *Journal of advanced research*, 9, 1-16.

Ali, I. A. M., Ahmed, A. B. and Al-Ahmed, H. I., 2023. Green synthesis and characterization of silver nanoparticles for reducing the damage to sperm parameters in diabetic compared to metformin. *Scientific Reports*, 13(1), 2256.

Anjum, S., Ishaque, S., Fatima, H., Farooq, W., Hano, C., Abbasi, B. H. and Anjum, I., 2021. Emerging applications of nanotechnology in healthcare systems: Grand challenges and perspectives. *Pharmaceuticals*, 14(8), 707.

Arunkumar, C., Astalakshmi A., Nima, P. and Ganesan, V., 2013. Plant mediated Synthesis of Silver nanoparticles using leaves of *Odina wodier* Roxb. *International Journal of Advanced Research*, 1, 265-272.

Azis, N., Wahab, A. W., Karim, A., La Nafie, N. and Febrianti, T., 2022. Synthesis of Silver Nanoparticles in an Eco-friendly Way using *Lannea coromandelica* Aqueous Bark Extract. *Jurnal Kimia Sains dan Aplikasi*, 25(6), 224-230.

Banerjee, A., Qi, J., Gogoi, R., Wong, J. and Mitragotri, S., 2016. Role of nanoparticle size, shape, and surface chemistry in oral drug delivery. *Journal of Controlled Release*, 238, 176-185.

Bhakya, S., Muthukrishnan, S., Sukumaran, M. and Muthukumar, M., 2016. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. *Applied Nanoscience*, 6, 755-766.

Bodede, O., Shaik, S., Govinden, R. and Moodley, R., 2017. Evaluating the bioreducing potential of the leaves, knobs, and roots of *Zanthoxylum capense* (small knobwood) for the synthesis of silver nanoparticles, applicable to in vitro fungal contamination control. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, 8(4), 045007.

Chopra, H., Bibi, S., Singh, I., Hasan, M. M., Khan, M. S., Yousafi, Q. and Cavalu, S., 2022. Green metallic nanoparticles: biosynthesis to applications. *Frontiers in Bioengineering and Biotechnology*, 10, 548.

Dawadi, S., Katuwal, S., Gupta, A., Lamichhane, U., Thapa, R., Jaisi, S. and Parajuli, N., 2021. Current research on silver nanoparticles: Synthesis, characterization, and applications. *Journal of nanomaterials*, 1-23.

de Souza, E. R. B., de Paula, J. A. M., Borges, L. L. and Caramori, S. S., 2017. Chemical Characterization and Bioprospecting of Cashew Tree Polysaccharide of Brazilian Cerrado *(Anacardium Othonianum* Rizz.*)*, Anacardiaceae. *Fronteiras: Journal of Social, Technological and Environmental Science*, 6, 230-46.

Donga, S. and Chanda, S., 2021. Facile green synthesis of silver nanoparticles using *Mangifera indica* seed aqueous extract and its antimicrobial, antioxidant, and cytotoxic potential (3-in-1 system). *Artificial Cells, Nanomedicine, and Biotechnology*, 49(1), 292-302.

Donga, S., Bhadu, G. R. and Chanda, S., 2020. Antimicrobial, antioxidant, and anticancer activities of gold nanoparticles green synthesized using *Mangifera indica* seed aqueous extract. *Artificial Cells, Nanomedicine, and Biotechnology*, 48(1), 1315-1325.

dos Reis Luz, L., Porto, D. D., Castro, C. B., Silva, M. F. S., de Godoy Alves Filho, E., Canuto, K. M. and Zocolo, G. J., 2018. Metabolomic profile of *Schinopsis brasiliensis* via UPLC-QTOF-MS for identification of biomarkers and evaluation of its cytotoxic potential. *Journal of Chromatography B*, 1099, 97-109.

Eid, M. M., 2022. Characterization of Nanoparticles by FTIR and FTIR-Microscopy. in: Mallakpour, H., Hussain, C.M. (Eds.), Handbook of Consumer Nanoproducts Singapore: Springer., Singapore, pp. 1-30.

El-Chaghaby G.A. and Ahmad, A.F., 2011. Biosynthesis of Silver Nanoparticles using *Pistacia lentiscus* Leaves Extract and Investigation of their Antimicrobial Effect. *Oriental journal of chemistry*, 27(3), 929-936.

El-Nashar, H. A., Mostafa, N. M., El-Badry, M. A., Eldahshan, O. A. and Singab, A. N. B., 2021. Chemical composition, antimicrobial and cytotoxic activities of essential oils from *Schinus polygamus* (Cav.) cabrera leaf and bark grown in Egypt. *Natural Product Research*, 35(23), 5369-5372.

Eloff, J. N. 2021. A proposal towards a rational classification of the antimicrobial activity of acetone tree leaf extracts in a search for new antimicrobials. *Planta Medica*, 87(10/11), 836-840.

Eltarahony, M., Zaki, S., Kheiralla, Z. and Abd-El-Haleem, D., 2016. Biogenic synthesis of Iron oxide nanoparticles via optimization of nitrate reductase Enzyme using statistical experimental design. *Journal of Advances in Biology & Biotechnology*, 5, 667–684.

Erenler, R., Chaoui, R., Yildiz, I., Genc, N., Gecer, E. N., Temiz, C. and Akkal, S., 2023. Biosynthesis, Characterisation, and Antioxidant Activity of Silver Nanoparticles using *Schinus molle* L. *Trends in Sciences*, 20(10), 6105-6105.

Evanoff Jr, D. D. and Chumanov, G., 2005. Synthesis and optical properties of silver nanoparticles and arrays. *ChemPhysChem*, 6(7), 1221-1231.

Ghorbani, P., Namvar, F., Homayouni-Tabrizi, M., Soltani, M., Karimi, E. and Yaghmaei, P., 2018. Apoptotic efficacy and antiproliferative potential of silver nanoparticles synthesised from aqueous extract of sumac (*Rhus coriaria* L.). *IET Nanobiotechnology*, 12(5), 600-603.

Ghorbani, P., Soltani, M., Homayouni-Tabrizi, M., Namvar, F., Azizi, S., Mohammad, R. and Boroumand Moghaddam, A., 2015. Sumac silver novel biodegradable nano composite for bio-medical application: Antibacterial activity. *Molecules*, 20, 12946-12958.

Gopinath P., Gogoi SK., Sanpui P., Paul A., Chattopadhyay A. and Ghosh SS., 2010. Signalling gene cascade in silver nanoparticle induced apoptosis. *Colloids and Surfaces B: Biointerfaces*, 77(2), 240-245.

Hassan, H., Sharma, P., Hasan, M. R., Singh, S., Thakur, D. and Narang, J., 2022. Gold nanomaterials–The golden approach from synthesis to applications. *Materials Science for Energy Technologies*, 3, 375-390.

Hiba, H. and Thoppil, J.E., 2022. Medicinal herbs as a panacea for biogenic silver nanoparticles. *Bulletin of the National Research Centre*, 46(1), 9.

Híjar-Soto, L. E., Gónzalez-Horta, C., Chávez-Flores, D., Torres-Muñoz, J. V. and Sánchez-Ramírez, B., 2014. Antineoplastic potential of bioactive fractions of *Rhus trilobata* on colon cancer cells CaCo-2. *Biotechnology Summit 2014. Santa María Huatulco, Oaxaca, Mexico, 8-10 October 2014*, 2-6.

Kabongo-Kayoka, P. N., Eloff, J. N., Obi, C. L. and McGaw, L. J., 2016. Antimycobacterial activity and low cytotoxicity of leaf extracts of some African Anacardiaceae tree species. *Phytotherapy Research*, 30(12), 2001-2011.

Kanchi, S. and Ahmed, S., 2018. Green metal nanoparticles: synthesis, characterization and their applications. John Wiley & Sons.

Kashyap, D., Garg, V. K., Tuli, H. S., Yerer, M. B., Sak, K., Sharma, A. K. and Sandhu, S. S., 2019. Fisetin and quercetin: promising flavonoids with chemopreventive potential. *Biomolecules*, 9(5), 174.

Kedi, P. B. E., Nanga, C. C., Gbambie, A. P., Deli, V., Meva, F. E. A., Mohamed, H. E. A. and Nko'o, M. H. J., 2020. Biosynthesis of silver nanoparticles from *Microsorum punctatum* (l.) copel fronds extract and an in-vitro anti-inflammation study. *Journal of Nanotechnology Research*, 2(2), 25-41.

Khan, F., Shariq, M., Asif, M., Siddiqui, M. A., Malan, P. and Ahmad, F., 2022. Green nanotechnology: plant-mediated nanoparticle synthesis and application. *Nanomaterials*, 12(4), 673.

Khan, W., Khan, N., Jamila, N., Masood, R., Minhaz, A., Amin, F. and Nishan, U., 2022. Antioxidant, antibacterial, and catalytic performance of biosynthesized silver nanoparticles of *Rhus javanica*, *Rumex hastatus*, and *Callistemon viminalis*. *Saudi Journal of Biological Sciences*, 29(2), 894-904.

Khiya, Z., Oualcadi, Y., Mourid, E. H., Tagnaout, I., Berrekhis, F., Zair, T. and El Hilali, F., 2021. Evaluation antioxidant and antibacterial activities of silver nanoparticles synthesized by aqueous extract of *Pistacia atlantica*. *Research on Chemical Intermediates*, 47(8), 3131-3144.

Kocazorbaz, E. K., Moulahoum, H., Tut, E., Sarac, A., Tok, K., Yalcin, H. T. and Zihnioglu, F., 2021. Kermes oak (*Quercus coccifera* L.) extract for a biogenic and eco-benign synthesis of silver nanoparticles with efficient biological activities. *Environmental Technology & Innovation*, 24, 102067.

Lade, B. D. and Shanware, A. S., 2020. Phytonanofabrication: methodology and factors affecting biosynthesis of nanoparticles. in: Tatyana Shabatina, T., Bochenkov, V. (Eds.) Smart nanosystems for biomedicine, optoelectronics, and catalysis. *IntechOpen*, 90918.

Lediga, M. E., Malatjie, T. S., Olivier, D. K., Ndinteh, D. T. and 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.

Madikizela, B., Aderogba, M. A. and Van Staden, J., 2013. Isolation and characterization of antimicrobial constituents of *Searsia chirindensis* L. (Anacardiaceae) leaf extracts. *Journal of ethnopharmacology*, 150(2), 609-613.

Malik, S., Muhammad, K. and Waheed, Y., 2023. Nanotechnology: A revolution in modern industry. *Molecules*, 28, 661.

Marbaniang, C. and Kma, L., 2018. Dysregulation of glucose metabolism by oncogenes and tumor suppressors in cancer cells. *Asian Pacific journal of cancer prevention: APJCP*, 19(9), 2377.

Maroyi, A., 2013. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. *Journal of ethnobiology and ethnomedicine*, 9(1), 1-18.

McShan, D., Ray, P. C. and Yu, H., 2014. Molecular toxicity mechanism of nanosilver. *Journal of food and drug analysis*, 22(1), 116-127.

Mohanta, Y. K., Biswas, K., Jena, S. K., Hashem, A., Abd'Allah, E. F. and Mohanta, T. K., 2020. Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. *Frontiers in Microbiology*, 11, 1143.

Mølgaard, P., Nielsen, S. B., Rasmussen, D. E., Drummond, R. B., Makaza, N. and Andreassen, J., 2001. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. *Journal of Ethnopharmacology*, 74(3), 257-264.

Mukaratirwa-Muchanyereyi, N., Gusha, C., Mujuru, M., Guyo, U. and Nyoni, S., 2022. Synthesis of silver nanoparticles using plant extracts from *Erythrina abyssinica* aerial parts and assessment of their anti-bacterial and antioxidant activities. *Results in Chemistry*, 4, 100402.

Müller, U., Stübl, F., Schwarzinger, B., Sandner, G., Iken, M., Himmelsbach, M. and Weghuber, J., 2018. In vitro and in vivo inhibition of intestinal glucose transport by guava (*Psidium guajava*) extracts. *Molecular nutrition & food research*, 62(11), 1701012.

Nunes, E. N., Rocha, A. P. T., de Araújo, G. T., de Paz, A. P., Rodrigues, L. M. D. S., Batista, R. S. D. A. and Melo, T. B. L., 2019. Characterization of yellow mombin biomass (*Spondias mombin* L.) for production of activated carbon. *Journal of Thermal Analysis and Calorimetry*, 135, 3281-3288.

Ologunagba, M. O., Jain, S., Thanki, K., Suresh, S., Furtado, S., Azubuike, C. P. and Silva, B. O., 2017. Extraction and Characterization of the Gum Exudate of *Anacardium occidentale* for its potential as an Excipient in Drug Delivery Systems. *Tropical Journal of Natural Product Research*, 1, 76-83.

Pang, Z., Raudonis, R., Glick, B. R., Lin, T. J. and Cheng, Z., 2019. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. *Biotechnology advances*, 37(1), 177-192.

Panwar, R. S., Pervaiz, N., Dhillon, G., Kumar, S., Sharma, N., Aggarwal, N. and Kumar, N., 2022. *Mangifera indica* leaf extract assisted biogenic silver nanoparticles potentiates photocatalytic activity and cytotoxicity. *Journal of Materials Science: Materials in Electronics*, 33, 16538-16549.

Patra, J. K. and Baek, K. H., 2015. Green nanobiotechnology: factors affecting synthesis and characterization techniques. *Journal of Nanomaterials*, 2014, 219-219.

Perez, C., 1990. Antibiotic assay by agar-well diffusion method. *Acta Biologiae Et Medicinae Experimentalis*, 15, 113-115.

Reckzeh, E. S., Karageorgis, G., Schwalfenberg, M., Ceballos, J., Nowacki, J., Stroet, M. C. and Waldmann, H., 2019. Inhibition of glucose transporters and glutaminase synergistically impairs tumor cell growth. *Cell chemical biology*, 26(9), 1214-1228.

Revathy, R., Joseph, J., Augustine, C., Sajini, T. and Mathew, B., 2022. Synthesis and catalytic applications of silver nanoparticles: a sustainable chemical approach using indigenous reducing and capping agents from *Hyptis capitata*. *Environmental Science: Advances*, *1*(4), 491-505.

Samuggam, S., Chinni, S. V., Mutusamy, P., Gopinath, S. C., Anbu, P., Venugopal, V. and Enugutti, B., 2021. Green synthesis and characterization of silver nanoparticles using *Spondias mombin* extract and their antimicrobial activity against biofilm-producing bacteria. *Molecules*, 26(9), 2681.

Santhoshkumar, R., Hima Parvathy, A. and Soniya, E. V., 2021. Phytosynthesis of silver nanoparticles from aqueous leaf extracts of *Piper colubrinum*: characterisation and catalytic activity. *Journal of Experimental Nanoscience*, 16(1), 294-308.

Schreck, K. and Melzig, M., 2018. Intestinal Saturated Long-Chain Fatty Acid, Glucose and Fructose Transporters and Their Inhibition by Natural Plant Extracts in Caco-2 Cells. *Molecules* 23, 2544.

Scimeca, M., Bischetti, S., Lamsira, H.K., Bonfiglio, R. and Bonanno, E., 2018. Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. *European journal of histochemistry: EJH*, 62(1), 2841.

Shaikh, W. A., Chakraborty, S., Owens, G. and Islam, R. U., 2021. A review of the phytochemical mediated synthesis of AgNP (silver nanoparticle): The wonder particle of the past decade. *Applied Nanoscience*, *11*(11), 2625-2660.

Sheny, D. S., Mathew, J. and Philip, D., 2011. Phytosynthesis of Au, Ag and Au–Ag bimetallic nanoparticles using aqueous extract and dried leaf of *Anacardium occidentale*. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 79(1), 254-262.

Simon, S., Sibuyi, N. R. S., Fadaka, A. O., Meyer, S., Josephs, J., Onani, M. O., Meyer, M. and Madiehe, A. M., 2022. Biomedical applications of plant extract-synthesized silver nanoparticles. *Biomedicines*, 10(11), 2792.

Singh, P. and Mijakovic, I., 2022. Strong antimicrobial activity of silver nanoparticles obtained by the green synthesis in *Viridibacillus* sp. extracts. *Frontiers in Microbiology*, *13*, 820048.

Sundeep, D., Vijaya Kumar, T., Rao, P. S., Ravikumar, R. V. S. S. N. and Gopala Krishna, A., 2017. Green synthesis and characterization of Ag nanoparticles from *Mangifera indica* leaves for dental restoration and antibacterial applications. *Progress in biomaterials*, *6*, 57-66.

Venugopal, K., Rather, H. A., Rajagopal, K., Shanthi, M. P., Sheriff, K., Illiyas, M. and Maaza, M., 2017. Synthesis of silver nanoparticles (Ag NPs) for anticancer activities (MCF 7 breast and A549 lung cell lines) of the crude extract of *Syzygium aromaticum*. *Journal of Photochemistry and Photobiology B: Biology*, 167, 282-289.

Wu, X., Zhou, Q. H. and Xu, K., 2009. Are isothiocyanates potential anti-cancer drugs? *Acta Pharmacologica Sinica*, 30(5), 501-512.

Youns, M. and Abdel Halim Hegazy, W., 2017. The natural flavonoid fisetin inhibits cellular proliferation of hepatic, colorectal, and pancreatic cancer cells through modulation of multiple signaling pathways. *PLoS One*, *12*(1), e0169335.

Zare, Y., 2016. Study of nanoparticles aggregation/agglomeration in polymer particulate nanocomposites by mechanical properties. *Composites Part A: Applied Science and Manufacturing*, 84, 158-164.

Zhang, X. A., Zhang, S., Yin, Q. and Zhang, J., 2015. Quercetin induces human colon cancer cells apoptosis by inhibiting the nuclear factor-kappa B Pathway. *Pharmacognosy magazine*, *11*(42), 404.

Zorzan, M., Collazuol, D., Ribaudo, G., Ongaro, A., Scaroni, C., Zagotto, G. and Pezzani, R., 2019. Biological effects and potential mechanisms of action of Pistacia lentiscus Chios mastic extract in Caco-2 cell model. *Journal of Functional Foods*, 54, 92-97.

CHAPTER 8

General conclusion and recommendations for future research

8.1 General summary and conclusion

This study explored the gap between traditional medicine and nanotechnology, and as such, looked at policies put in place by the south African scientific space as a 10-year strategic plan regarding nanotechnology research as well as establishment of its roots within society. As such, a base foundation for the integration of nanotechnology into societies through traditional medicine would be to review strategies initially placed for advancement of nanotechnology, and their progress within the country. So far, transparency and public awareness are being implemented through research outputs, which have had an increase of 508% by the year 2019. However, acceptability seems to be a hurdle as the safety and sustainability of nanoparticles needs to be addressed. As such concerns are still on the rise, the biggest question remains as to whether the 10-year plan has been accomplished, and if it has, then has nanotechnology broken barriers into fields such as traditional medicine or complementary medicine? Developing new methods for sustainable synthesis of nanoparticles is a stepping stone for new developments in nanotechnology. In search for greener alternatives to producing nanoparticles, this study investigated the use *Lannea discolor* for nanoparticle synthesis. *Lannea discolor* leaf, stem and roots extract were used as a potential reducing agent of copper sulphate, gold, and silver nitrate to produce green nanoparticles. The formation of nanoparticles was confirmed by the presence of surface plasmon resonance (SPR) absorption peaks in the visible light region of the spectrum. The green synthesis was optimized by changing the reaction conditions (temperature, solvent of extraction and plant part) and the study proved that size and shape of the synthesized nanoparticles can be determined by adjustment of reaction conditions. SEM and TEM analysis confirmed the morphological properties of the nanoparticles synthesised. Synthesis with copper and gold precursors resulted in differently shaped nanoparticles. Nanoflowers were observed from the reduction of gold by the leaf extracts of *Lannea discolor*, while the stems and roots produced small sized spherical nanoparticles as well as noticeable amounts of triangular and hexagonal shaped nanoparticles. Copper synthesised nanoparticles appeared to be spherical and large at all conditions, while exposure to heat resulted in sintering, a phenomenon that may be favourable for the manufacturing of conductive nanomaterials at an industrial level. The gold nanoparticles and nanoflowers showed antibacterial activity with MIC values lower than those of the copper nanoparticles. These results indicate that the nanoparticle sizes and shapes and phytochemicals capped on the nanoparticles play important part in the antibacterial activity. Moreover, due to the nature of nanoparticles produced by *L. discolor*, it is evident that they can potentially find a unique position in many medical and technological applications, such as sensors, flexible devices, and conductivity. To further explore materials that may also be easily reduced by *L. discolor*, silver nitrate was used for green synthesis. The silver nanoparticles were mostly spherical, with the appearance of a few rods and triangular nanoparticles. Furthermore, the nanoparticles showed high antioxidant and

antibacterial activity, suggesting their potential for treating a variety of oxidative stress-related diseases as well as pathogenic infections. Moreover, the cytotoxic effects of silver nanoparticles in comparison with the crude extracts was investigated by testing them against Caco-2 cells, wherein they manifested characteristics of cytotoxic nature against the intrinsically resistant human colorectal cancer cell line (Caco-2). This places *L. discolor* as a suitable candidate for future research on anticancer work.

At present, it is observed that the benefits of using plants for the synthesis nanoparticles are those of bioavailability of the phytochemicals that facilitate the reduction of silver ions. FTIR provided a clue to these mechanisms by enabling the confirmation of functional groups present on the surface of the nanoparticles. The confirmation of the presence of functional groups helped in the sustenance of the view that phytochemicals from *L. discolor* were responsible for the reduction of Cu, Au, and Ag ions in nanoparticle synthesis. This led to the further investigation of the metabolic profile of the extracts of *L. discolor* through conventional phytochemical screening, and Liquid chromatography mass spectrometry, to establish the diverse group of metabolites that are associated with various observed functional groups. This led to the identification of some major compound classes, flavonoids, fatty acids, phenolics, iridoids and terpenoids of the *Lannea* species and Anacardiaceae family. Most importantly, phenols, myricetin and other types of flavonoids have been identified as having chemotaxonomic qualities, confirming their significance within the family. The biological evaluation of *L. discolor* methanol extracts was also included in this study. The results showed that the plant extracts were highly active against an array of bacteria, which is observed to be due to the vast metabolic profile brought about using methanol as an extractant. While it is observed that methanol extracts bring about a rich abundance of compounds for identification and validation, it was important to further explore other suitable extraction solvents to do away with limitations that exist because of the concern of the safety of the solvents used and toxicity in cases where oral consumption is required. Considering this, acetone and water extracts were analysed to establish and contrast their extraction yield, and the appearance of metabolites. In both extracts different compound classes were observed through phytochemical screening. Additionally, of the identified compounds for both extracts, flavonoids and phenols were the major constituents. Moreover, acetone extracts demonstrated considerably high appearance of metabolites, significant Total phenolic content (TPC), Total flavonoid content (TFC), antioxidant and antibacterial activity compared to the water extracts. This suggests that the extraction solvent determines the results from certain analysis, and that its polarity can affect the phytochemical profile and the biological activity of the tested plant extracts.

The results presented in this study clearly demonstrate the feasibility of using *L. discolor* for the synthesis of nanoparticles. This study demonstrated that *L. discolor* extract mediated AuNPs synthesis is a greener alternative as it abides by the green environmentally friendly principles. The aims of the study were achieved as the results presented demonstrate that extracts from traditionally used medicinal plants like *L. discolor* can be utilised for green synthesis of AuNPs and essentially contribute to the

development of greener alternatives of nanoparticle synthesis in nanotechnology. Furthermore, the study outcomes will contribute to minimizing environmental pollution by finding use for nanoparticle synthesised here for possible biomedical and other industrial applications. It is also apparent that *L. discolor* boasts of a highly rich metabolic profile, consisting of some of the most important compounds as observed in recent research trends. The appearance of metabolites even when extracted by non-competitive solvents such as water, further validates the used of this plant as a traditional and complementary medicine.

8.2 Recommendations for future research

Although the study yielded valuable results, there were some few drawbacks. As such, based on the findings of this study, the following recommendations and additional research are proposed.

It seems the low acceptance and uncertainty of the risks associated with nanotechnology maybe the primary reason for a gap in its use in conjunction with traditional medicine. This calls for government/stakeholders to sensitize society through the establishment of regulatory measures by disseminating evidence-based data acquired from nanotechnology research, and societal sensitization through frequent consumer engagement and awareness.

This study showed the effect of temperature and variations in the solvent used to extract the plant parts. Nevertheless, the study can be also expanded to include the effect of the pH of the reaction medium on the synthesis by using suitable buffers. FTIR as a stand-alone technique may have limitations. Moreover, it can be also useful to study the chemical synthesis of nanoparticles from *L. discolor*, using other characterisation techniques such as nuclear magnetic resonance (NMR) to confirm the possible functional groups involved in the synthesis with greater accuracy, by comparing the NMR spectra of the extracts, the nanoparticles, and the supernatant after centrifugation.

In this study, challenges presented were the low yield of nanoparticles, which can be a setback for certain analytical techniques that may need more than a certain number of milligrams of sample material for accurate analysis, such as cytoxicity. Sufficient yield of the nanoparticles was not obtained from synthesis of the copper and gold nanoparticles, as such cytotoxicity of the nanoparticles against Caco-2 was only performed on silver nanoparticles as they had a better yield in their powder form. Further research can focus on optimizing continuous flow reactors for nanoparticle synthesis to enhance efficiency and yield. In this way, even the particle size of the nanoparticles could be maintained. Continuous flow synthesis would offer several advantages over divided batch processes, including improved control over reaction parameters, scalability, and reproducibility.

Further, it is observed that the plant extracts, AuNFs, AuNPs and AgNPs had potent antibacterial activity. As such, SEM and TEM can be used to directly observe the effects of the plant extracts, NFs and NPs on the cell morphology and integrity of bacteria at different concentrations.

Quantitative structure-activity relationship (QSAR) modelling techniques could be used to establish relationships between the properties of the metabolites identified in L. *discolor* and their biological activities, this would assist in further research for drug discovery.

It would be worthwhile to investigate the toxicity of both the plant extracts nanoparticles by using animal models. This would give a broad perception on the distribution, bioavailability of the nanoparticles, especially for would healing evaluations.

9. APPENDICES

9.1 APPENDIX A

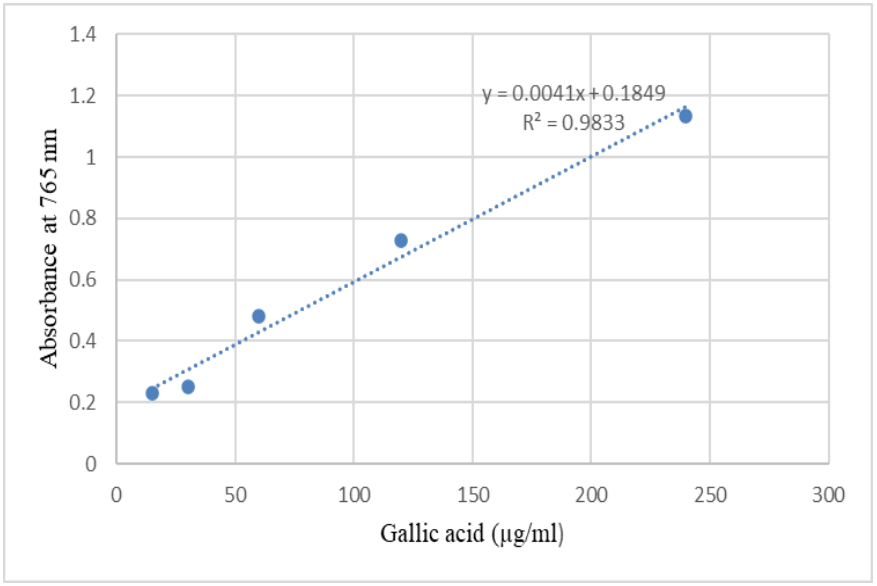


Figure A1. Standard curve generated from Total phenolic content for standard gallic acid. R^2 values represented mean data set of n=3. Regression and R^2 were generated using Excel.

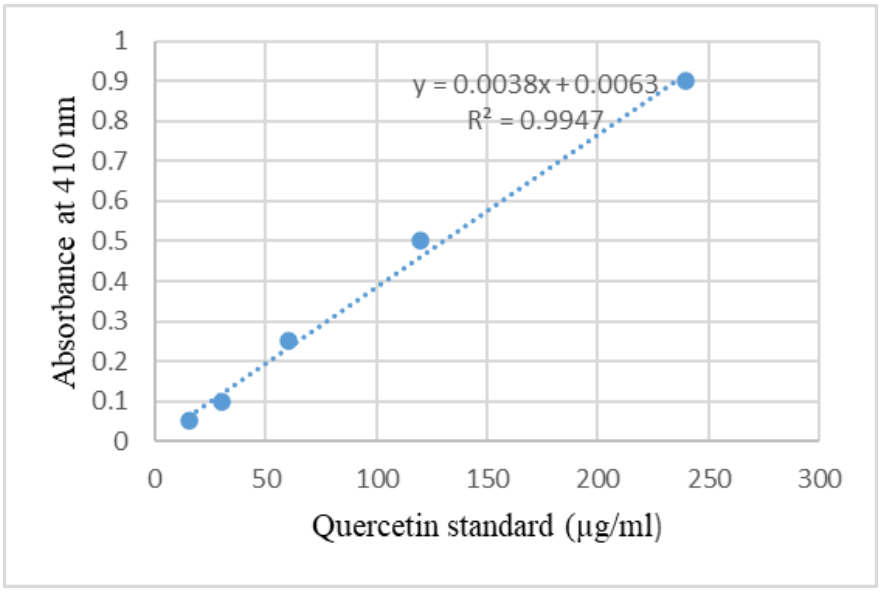


Figure A2. Standard curve generated from Total flavonoid content for standard quercetin. R^2 values represented mean data set of n=3. Regression and R^2 were generated using Excel.

9.2 APPENDIX B

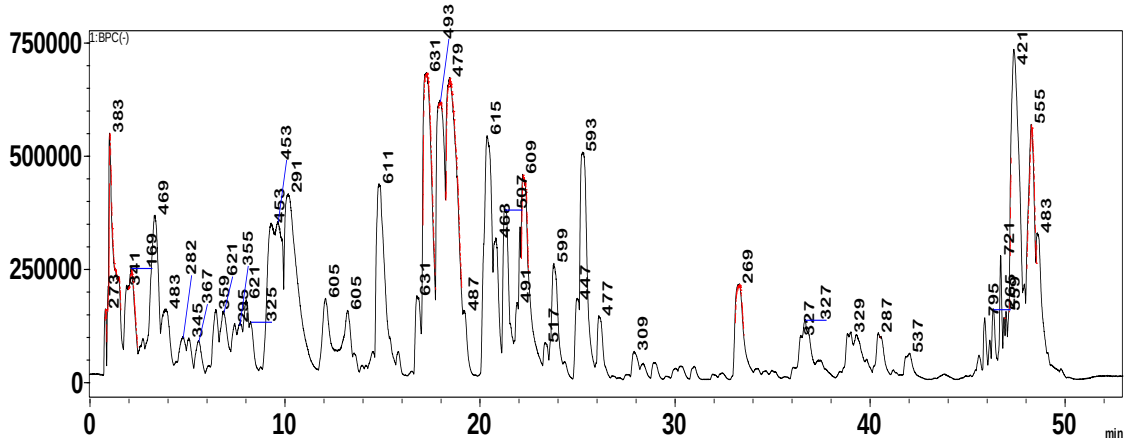


Figure B1 Base peak intensity chromatograms showing distribution of metabolites in the leaf water extract of *Lannea discolor.*

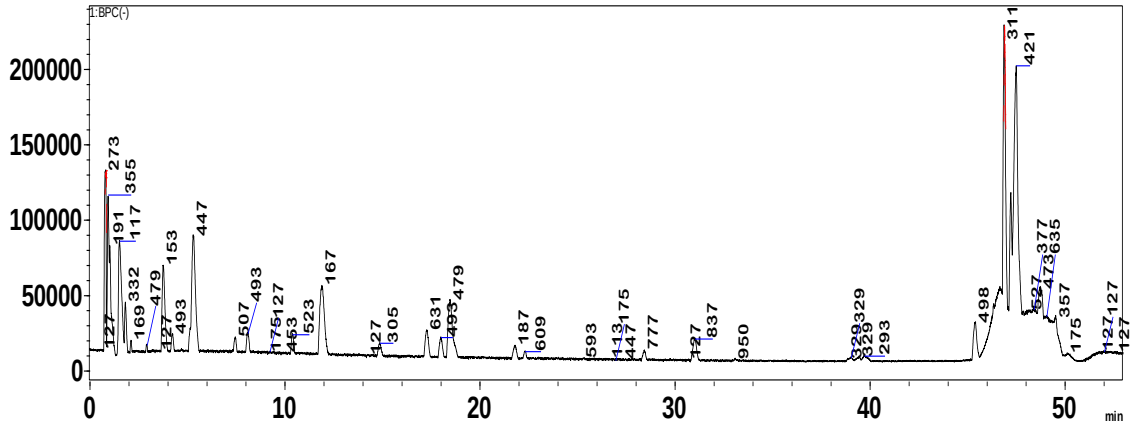


Figure B2 Base peak intensity chromatograms showing distribution of metabolites in the stem water extract of *Lannea discolor.*

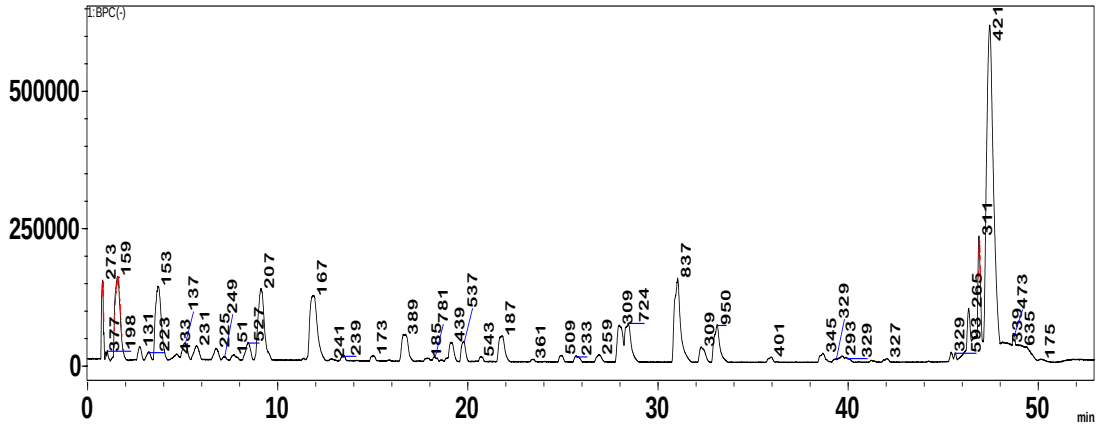


Figure B3 Base peak intensity chromatograms showing distribution of metabolites in the root water extract of *L. discolor.*

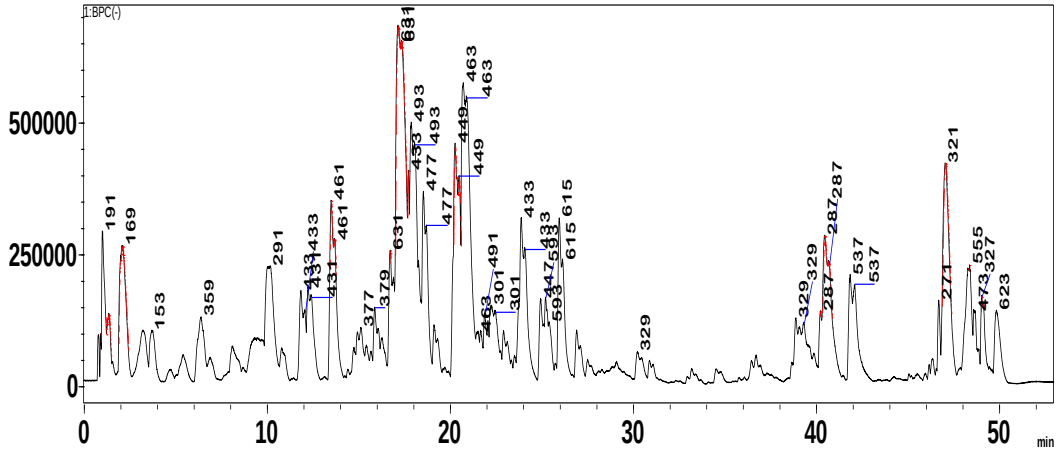


Figure B4 Base peak intensity chromatograms showing distribution of metabolites in the leaf acetone extract of *Lannea discolor.*

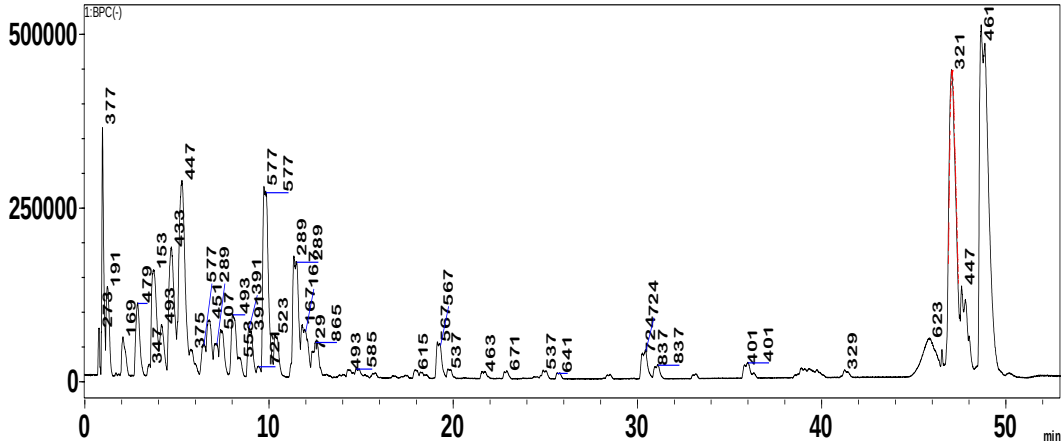


Figure B5. Base peak intensity chromatograms showing distribution of metabolites in the stem acetone extract of *Lannea discolor*.

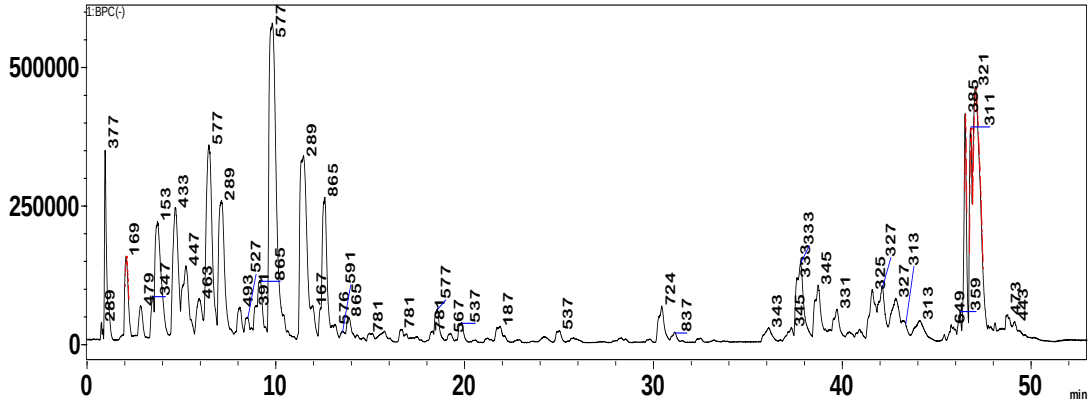
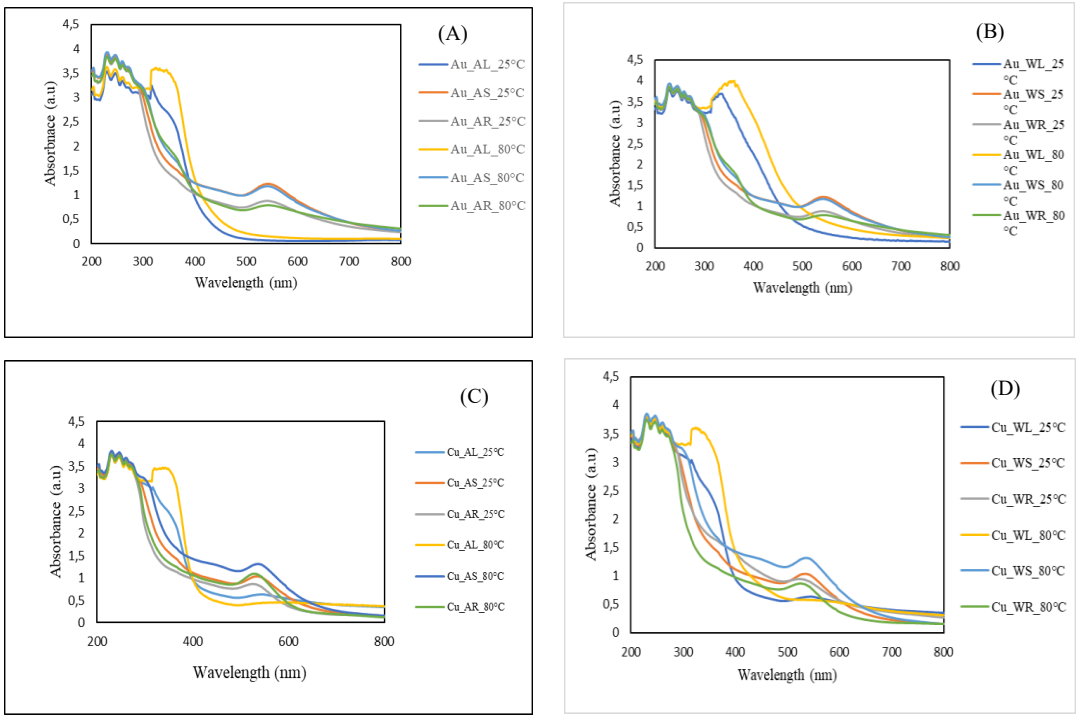


Figure B6 Base peak intensity chromatograms showing distribution of metabolites in the root acetone extract of *Lannea discolor.*

9.3 APPENDIX C



Sample keys : AL=acetone Leaf; AS=acetone stem; AR=acetone root; WL=water leaf; WS= water stem;WR=water root.

Figure C1 The UV-Vis data of AuNFs, NPs (A and B) and CuNPs (C and D). Legends denotes extracts from which nanoparticles were synthesised.

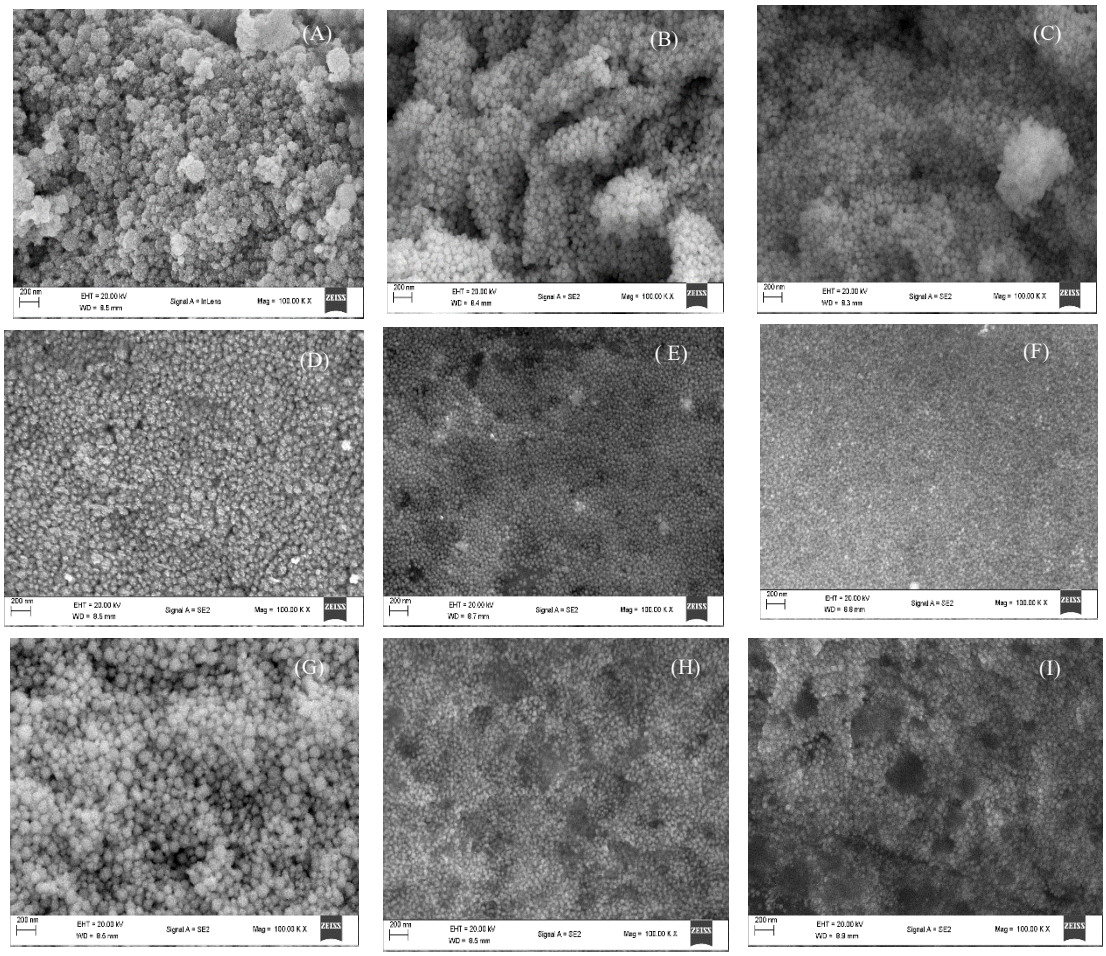


Figure C2 SEM micrographs of AuNFs and AuNPs synthesised from leaf, stem, and root methanol (A – C), acetone (D - F) and water (G – I) extracts, of *Lannea discolor*.

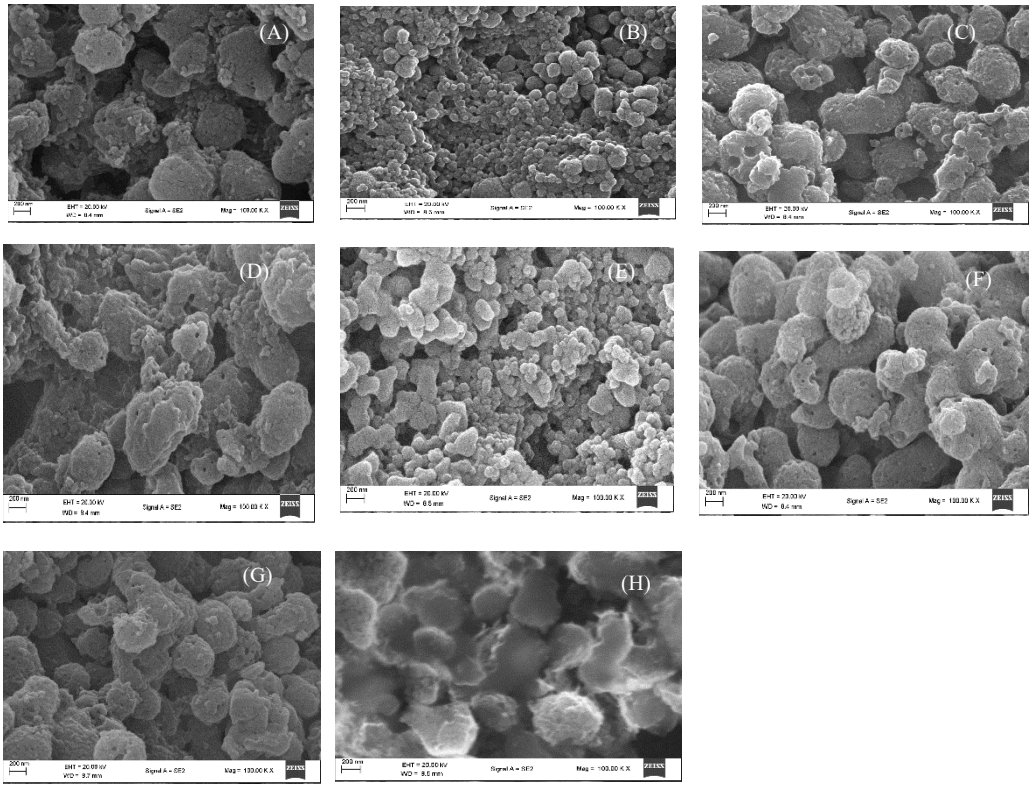


Figure C3 Representative SEM images of CuNPs from *Lannea discolor* leaf, stem, and root methanol (A – C), acetone (D - F) and water (G - H) extracts.

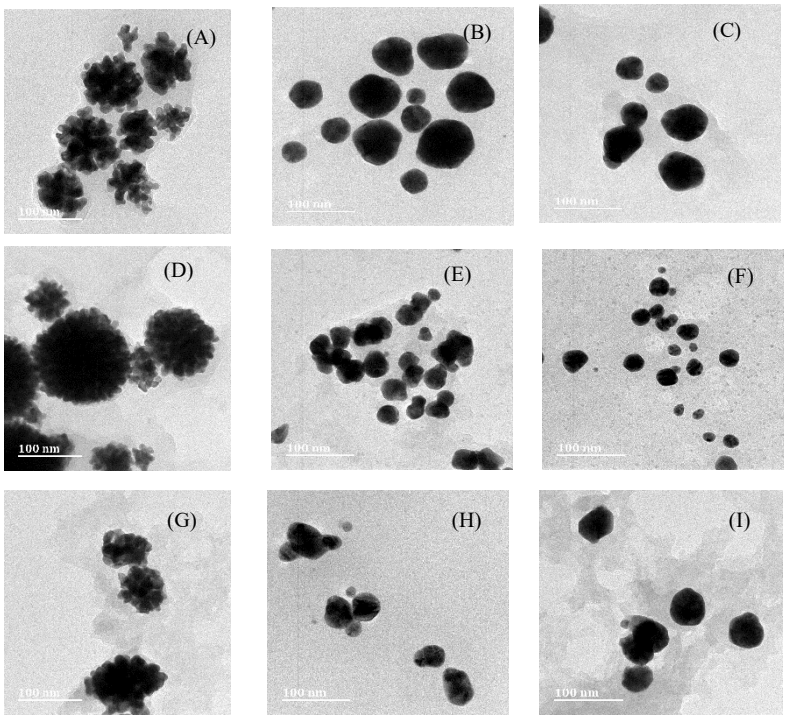


Figure C4 TEM images of the synthesised AuNFs and AuNPs. A, D and G show AuNFs synthesised from leaf methanol, acetone, and water extracts, respectfully. AuNPs synthesised from methanol, acetone, and water stem and roots extracts are shown in micrographs B, C, E, F, H, and I.

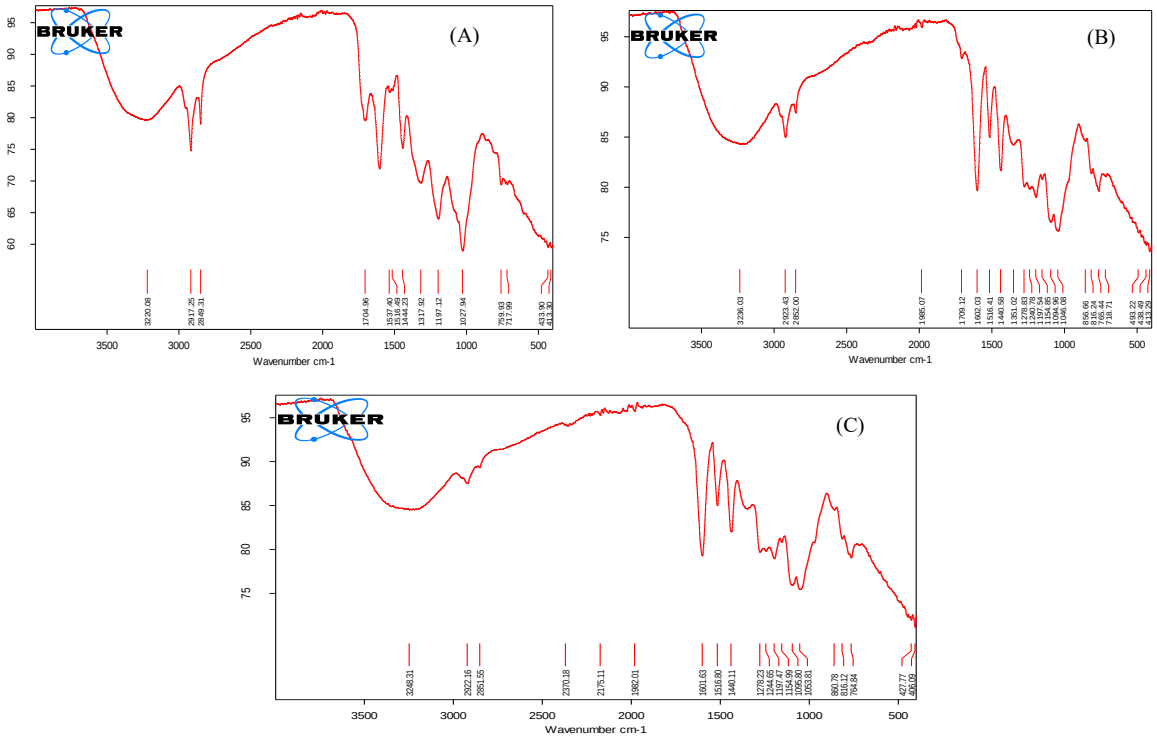


Figure C5 FTIR spectra of *Lannea discolor* leaf (A), stem (B) and root (C) crude methanol extracts.

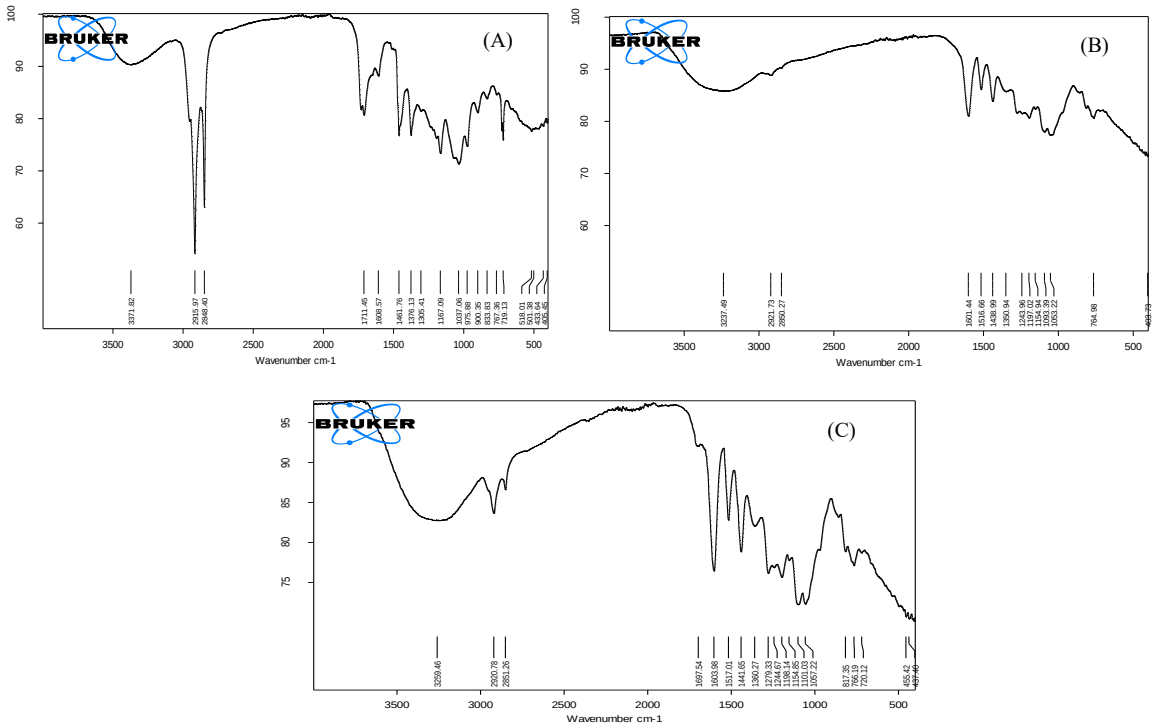


Figure C6 FTIR spectra of *Lannea discolor* leaf (A), stem (B) and root (C) crude acetone extracts.

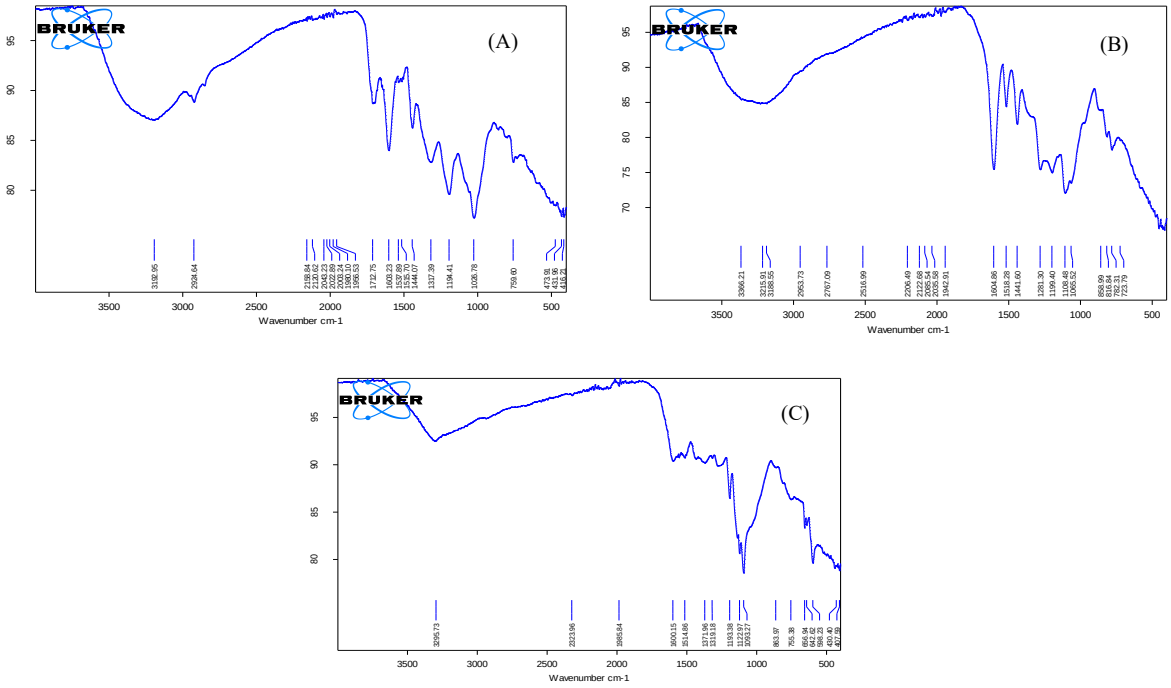


Figure C7 FTIR spectra of *Lannea discolor* leaf (A), stem (B) and root (C) crude water extracts.