SILVER NANOPARTICLES AND THEIR ANTIBACTERIAL ACTIVITY SYNTHESIZED USING SELECTED MEDICINAL PLANT EXTRACTS

KITHOKOI KILONZO JACKSON (B.Ed. Sci.)
I56/33673/2015

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Chemistry) in the School of Pure and Applied Sciences of Kenyatta University

MAY, 2019
DECLARATION

I hereby declare that this thesis is my original work and has not been presented for the award of a degree in any other University.

Jackson Kilonzo Kithokoi

Signature........................................ Date ................................................

This thesis has been submitted for examination with our approval as University Supervisors.

Prof. Wilson Njue  Signature..........................Date.............................

Department of Chemistry

Kenyatta University

Prof. Sauda Swaleh  Signature..........................Date.............................

Department of Chemistry

Kenyatta University
DEDICATION

This work is dedicated to Kithokoi’s family for their support and encouragement during my entire study period. God bless you
ACKNOWLEDGEMENTS

I thank the Almighty God for the grace He has given me, strength, good health and knowledge to accomplish my studies.

I sincerely thank my supervisors, Prof. Wilson Njue and Prof. Sauda Swaleh of Chemistry Department Kenyatta University for their kind and constant advice, encouragement and support during my research period.

Special thanks to Dr. John Maingi of the Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University for his guidance during my microbiological laboratory work. Special gratitude to Dr. Lawrence Ochoo from Physics Department Kenyatta University for his guidance and Prof. Martin Onani, University of the Western Cape for facilitating the EDX/HRTEM analysis.

I also take this opportunity to thank the entire staff of Chemistry and Biochemistry, Microbiology and Biotechnology Departments for their support during my research.

My dear parents, brothers and sisters, thank you for your love, prayers and support. God bless you.
TABLE OF CONTENTS

DECLARATION.................................................................................................................. ii
DEDICATION................................................................................................................... iii
ACKNOWLEDGEMENTS ................................................................................................... iv
TABLE OF CONTENTS .................................................................................................... v
LIST OF TABLES ................................................................................................................. x
LIST OF FIGURES ............................................................................................................. xi
PLATES ............................................................................................................................ xiii
ACRONYMS AND ABBREVIATIONS ............................................................................. xv
ABSTRACT ....................................................................................................................... xvi
CHAPTER ONE .................................................................................................................. 1
INTRODUCTION ................................................................................................................. 1
  1.1 Background Information ......................................................................................... 1
  1.2 Problem statement ................................................................................................. 4
  1.3 Justification ............................................................................................................. 4
  1.4 Hypotheses .............................................................................................................. 5
  1.5 Objectives ............................................................................................................... 5
    1.5.1 General objective ............................................................................................. 5
    1.5.2 Specific objectives ........................................................................................... 5
  1.6 Significance of the study ....................................................................................... 5
  1.7 Scope and limitations ............................................................................................. 6
CHAPTER TWO .................................................................................................................. 7
LITERATURE REVIEW ..................................................................................................... 7
  2.1 Biosynthesis of AgNPs ........................................................................................... 7
  2.2 Sonochemical synthesis of AgNPs .......................................................................... 7
2.3 Synthesis of AgNPs using plant extracts ................................................................. 8
   2.3.1 Adansonia digitata (baobab) .............................................................................. 11
   2.3.2 Prunus africana ................................................................................................. 12
   2.3.3 Bridelia micrantha ............................................................................................. 13
   2.3.4 Warburgia ugandensis ...................................................................................... 13
   2.3.5 Urtica dioica ....................................................................................................... 13
2.4 Antimicrobial activity of AgNPs ..................................................................................... 14
2.5 Characterization of AgNPs ............................................................................................. 14
CHAPTER THREE ................................................................................................................. 16
MATERIALS AND METHODS ............................................................................................... 16
3.1 Material, chemicals and apparatus ................................................................................. 16
   3.1.1 Sample collection ............................................................................................... 16
   3.1.2 Chemicals .......................................................................................................... 18
   3.1.3 Apparatus and instruments ............................................................................... 18
3.2 Sample preparation ......................................................................................................... 19
   3.2.1 Plant extraction .................................................................................................. 19
   3.2.2 Stationary biosynthesis of AgNPs ..................................................................... 19
   3.2.3 Synthesis of AgNPs over ultrasonic bath ................................................................ 19
3.3 Characterization and analysis .......................................................................................... 20
   3.3.1 UV-VIS spectroscopy ....................................................................................... 20
   3.3.2 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis ................................ 20
   3.3.3 High Resolution Transmission Electron Microscope (HRTEM) Analysis .......... 20
   3.3.4 High Resolution Scanning Electron Microscope (HRSEM) Analysis .............. 20
3.4 Anti-bacteria activity ...................................................................................................... 21
3.5 Data analysis .................................................................................................................. 21
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

4.2 AgNPs synthesized using Adansonia digitata leaves extract

4.2.1 Stationary synthesis of AgNPs

4.2.2 Ultrasound enhanced biosynthesis of AgNPs

4.2.3 High Resolution Transmission Electron Microscope (HRTEM) analysis

4.2.4 Selected Area Electron Diffraction (SAED) Analysis

4.2.5 Energy Dispersive X-ray (EDX) Analysis

4.2.6 Fourier Transform Infra-Red (FTIR) analysis of AgNPs

4.2.7 Antibacterial activity of AgNPs synthesized using A. digitata leaves extract

4.3 Synthesis of AgNPs using Adansonia digitata fruit pulp

4.3.1 Stationary synthesis of AgNPs

4.3.2 Ultrasound enhanced (sonication) formation of AgNPs

4.3.3 High Resolution Transmission Electron Microscope analysis

4.3.4 Energy Dispersive X-Ray (EDX) analysis

4.3.5 Fourier Transform Infra-Red (FTIR) analysis

4.3.6 Antibacterial activity of AgNPs synthesized using A. digitata fruit pulp

4.4 Synthesis of AgNPs using Prunus africana bark extract

4.4.1 Stationary biosynthesis of AgNPs using P. africana bark extract

4.4.2 High Resolution Transmission Electron Microscope (HRTEM) analysis

4.4.3 Energy Dispersive X-Ray (EDX) analysis

4.4.4 Ultrasound enhanced synthesis of AgNPs

4.4.5 High resolution Transmission Electron Microscope (HRTEM) analysis
4.4.6 Selected Area Electron Diffraction (SAED) Analysis .................................................45
4.4.7 Fourier Transform Infra-Red (FTIR) analysis ..........................................................46
4.4.8 Antibacterial activity of AgNPs synthesized using Prunus africana stem bark extract ........................................................................................................46
4.5 Biosynthesis of AgNPs using Prunus africana leaves extract ..................................49
  4.5.1 Stationary method ..........................................................................................................49
  4.5.2 Ultrasonic enhanced synthesis of AgNPs using P. africana leaves ..................50
  4.5.3 High Resolution Scanning Electron Microscope analysis ..................................51
  4.5.4 Fourier Transform Infra-Red (FTIR) analysis .........................................................53
  4.5.5 Antibacterial activity of AgNPs synthesized ...............................................................53
4.6 Synthesis of AgNPs using Bridelia micrantha bark extract ........................................56
  4.6.1 Stationary synthesis of AgNPs using ........................................................................56
  4.6.2 Ultrasonic enhanced synthesis of AgNPs using B. micrantha bark extract ....57
  4.6.3 High Resolution Transmission Electron Microscope (HRTEM) analysis ......58
  4.6.4 Scanning Area Electron Diffraction (SAED) Analysis ........................................60
  4.6.5 Energy Dispersive X-Ray (EDX) Analysis ...............................................................60
  4.6.6 Fourier Transform Infra-Red (FTIR) analysis .........................................................61
  4.6.7 Antibacterial activity of B. micrantha mediated synthesized AgNPs ..................62
4.7 Biosynthesis of AgNPs using W. ugandensis bark extract .........................................63
  4.7.1 Stationary biosynthesis of AgNPs using W. ugandensis bark extract ..........63
  4.7.2 Ultrasound enhanced synthesis of AgNPs using W. ugandensis bark extract .65
  4.7.3 High Resolution Transmission Electron Microscope (HRTEM) analysis ......66
  4.7.4 Scanning Area Electron diffraction (SAED) analysis ..............................................68
  4.7.5 The Energy Dispersive X-ray (EDX) analysis ............................................................68
  4.7.6 Fourier Transform Infra-Red (FTIR) analysis ............................................................69
4.7.7 Antibacterial activity of *W. ugandensis* mediated synthesized AgNPs ........ 70
4.8 Biosynthesis of AgNPs using *Urtica dioica* roots extract ........................................... 71
4.8.1 Ultrasound enhanced biosynthesis of AgNPs using *U. dioica* .................... 71
4.8.2 High Resolution Scanning Electron Microscope (HRSEM) Analysis ........ 73
4.8.3 Fourier Transform Infra-Red (FTIR) analysis ......................................................... 74
4.8.4 Antibacterial activity of AgNPs synthesized using *U. dioica* ......................... 74
4.9 Synthesis of AgNPs using *U. dioica* leaves ............................................................ 76
4.10 Comparison on the sizes and morphology of the AgNPs ................................... 77
4.11 Comparison on the antibacterial activity of AgNPs ............................................. 78
CHAPTER FIVE .................................................................................................................. 80
CONCLUSIONS AND RECOMMENDATIONS ................................................................. 80
5.1 Conclusions ............................................................................................................... 80
5.2 Recommendations .................................................................................................. 81
5.2.2 Areas for further research .................................................................................. 81
REFERENCES ..................................................................................................................... 82
APPENDIX ........................................................................................................................ 91
Samples ............................................................................................................................ 91
LIST OF TABLES

Table 2.1: List of plants and their biomolecules used on synthesis of AgNPs .............. 9
Table 4.1: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................ 30
Table 4.2: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 38
Table 4.3: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 47
Table 4.4: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 54
Table 4.5: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 62
Table 4.6: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 71
Table 4.7: Inhibition zones of AgNPs on *E. coli* and *S. aureus* ............................. 75
Table 4.8: Sizes, shapes and morphology of the AgNPs ........................................ 77
Table 4.9: Inhibition zones of the synthesized AgNPs on *E. coli* ............................. 78
Table 4.10: Inhibition zones of the synthesized AgNPs on *S. aureus* ......................... 79
LIST OF FIGURES

Fig. 4.1: The UV-VIS spectra of *A. digitata* leaves AgNPs (stationary)....................... 23
Fig. 4.2: UV-VIS spectra of *A. digitata* leaves AgNPs on sonication.......................... 24
Fig. 4.3: Size distribution of AgNPs synthesized using *A. digitata* leaves .................. 27
Fig. 4.4: EDX spectrum of synthesized AgNPs using *A. digitata* leaves ................... 28
Fig. 4.5: FTIR spectra of AgNPs synthesized using *A. digitata* leaves extract .......... 29
Fig. 4.6: UV-VIS spectra of *A. digitata* fruit pulp synthesized AgNPs .................... 32
Fig. 4.7: UV-VIS spectra of *A. digitata* fruit pulp mediate AgNPs on sonication ....... 34
Fig. 4.8: AgNPs distribution by size............................................................................. 36
Fig. 4.9: EDX spectrum of AgNPs ............................................................................... 36
Fig. 4.10: FTIR spectra of AgNPs *A. digitata* fruit pulp extract mediated AgNPs ....... 37
Fig. 4.11: UV-VIS spectra of *P. africana* bark extract mediated AgNPs.................. 40
Fig. 4.12: Size distribution of *P. africana* bark mediated AgNPs via stationary method. 42
Fig. 4.13: EDX spectrum of *P. africana* bark mediated AgNPs via stationary method... 42
Fig. 4.14: UV-VIS spectra of *P. africana* bark and AgNO₃ with time by sonication ....... 43
Fig. 4.15: FTIR spectra of *P. africana* bark extract mediated AgNPs....................... 46
Fig. 4.16: UV-VIS spectra of *P. africana* leaves mediated AgNPs, Stationary method .. 50
Fig. 4.17: UV-VIS spectra of *P. africana* leaves mediated AgNPs.............................. 51
Fig. 4.18: Particle size distribution of AgNPs determined from HRSEM micrograph. ... 52
Fig. 4.19: FTIR spectra of *P. africana* leaves extract mediated AgNPs.................... 53
Fig. 4.20: UV-VIS spectra on formation of AgNPs using *B. micrantha* bark ............. 57
Fig. 4.21: UV-VIS spectra on formation of AgNPs...................................................... 58
Fig. 4.22: Size distribution of AgNPs synthesized using *B. micrantha* under sonication 59
Fig. 4.23: EDX spectrum of AgNPs synthesized using *B. micrantha* under sonication. 61
Fig. 4.24: FTIR Spectrum of *B. micrantha* mediated synthesized AgNPs ............... 62
Fig. 4.25: UV-VIS spectra of AgNPs .......................................................................... 64
Fig. 4.26: UV-VIS spectra of *W. ugandensis* extract synthesized AgNPs on sonication. 66
Fig. 4.27: Size distribution of AgNPs synthesized using *W. ugandensis* .................... 67
Fig. 4.28: EDX spectrum of AgNPs ............................................................................ 69
Fig. 4.29: FTIR Spectrum of *W. ugandensis* mediated synthesized AgNPs .......... 70
Fig. 4.30: UV-VIS spectra of *U. dioica* roots extract synthesized AgNPs on sonication. 73
Fig. 4.31: FTIR Spectrum of *U. dioica* mediated synthesized AgNPs .......................... 74
Fig. 4.32: UV-VIS spectra ........................................................................................................ 77
PLATES

Plate 3.1: *Adansonia digitata* .......................................................... 16
Plate 3.2: *Prunus africana* ............................................................. 17
Plate 3.3: *Warburgia ugandensis* ..................................................... 17
Plate 3.4: *Bridelia micrantha* .......................................................... 18
Plate 4.1: Colour development.......................................................... 22
Plate 4.2: Colour development of *A. digitata* leaves extract and AgNO₃ on sonication... 24
Plate 4.3: HRTEM images of synthesized AgNPs sonication. ......................... 26
Plate 4.4: SAED micrograph for *A. digitata* leaves mediated AgNPs via sonication ..... 27
Plate 4.5: Antibacterial activity of *A. digitata* leaves AgNPs ........................ 31
Plate 4.6: Colour change of *A. digitata* fruit pulp extract and AgNO₃ solution .......... 32
Plate 4.7: Colour development .................................................................. 33
Plate 4.8: HRTEM and SAED micrographs of AgNPs .................................. 35
Plate 4.9: Zones of inhibition of AgNPs against *S. aureus* ............................ 39
Plate 4.10: *P. africana* bark extract and AgNO₃ solution ............................ 39
Plate 4.11: HRTEM images of AgNPs from *P. africana* bark extract ............... 41
Plate 4.12: Colour development of *P. africana* bark extract and AgNO₃ solution .... 43
Plate 4.13: HRTEM micrographs of AgNPs synthesized using *P. africana* bark extract 44
Plate 4.14: SAED image of *P. africana* bark mediated AgNPs via sonication ......... 45
Plate 4.15: Zones of inhibition of AgNPs against *S. aureus* ............................ 48
Plate 4.16: Zones of inhibition of AgNPs against *E. coli* ................................ 48
Plate 4.17: *P. africana* leaves extract in 0.001M AgNO₃ solution ...................... 49
Plate 4.18: *P. africana* leaves extract and 0.001M AgNO₃ solution .................. 50
Plate 4.19: HRSEM image of synthesized AgNPs synthesized using *P. africana* leaves 52
Plate 4.20: Zones of inhibition of against *S. aureus* ...................................... 55
Plate 4.21: Zones of inhibition of against *E. coli* ........................................ 55
Plate 4.22: *Bridelia micrantha* bark extract and AgNO₃ solution on stationary ....... 56
Plate 4.23: *B. micrantha* bark extract and AgNO₃ solution on sonication .......... 57
Plate 4.24: Transmission Electron Microscope micrographs of the *B. micrantha* ........ 59
Plate 4.25: SAED micrograph of AgNPs from *B. micrantha* under sonication .......... 60
Plate 4.26: Mixture of *Warburgia ugandensis* bark extract and AgNO₃ solution ...... 64
Plate 4.27: Colour change of *W. ugandensis* bark extract and AgNO₃ mixture ............... 65
Plate 4.28: HRTEM images of AgNPs synthesized using *W. ugandensis* .................. 67
Plate 4.29: SAED image of AgNPs synthesized using *W. ugandensis* extract ............. 68
Plate 4.30: Colour development of *U. dioica* roots extract and AgNO₃ mixture .......... 72
Plate 4.31: HRSEM micrograph of AgNPs from *U. dioica* leaves .......................... 73
Plate 4.32: Effect of AgNPs synthesized using *U. dioica* roots on *S. aureus* ............. 76
### ACRONYMS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. digitata</td>
<td>Adansonia digitata</td>
</tr>
<tr>
<td>AgNPs</td>
<td>Silver nanoparticles</td>
</tr>
<tr>
<td>B. micrantha</td>
<td>Bridelia micrantha</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDX</td>
<td>Energy Dispersive X-ray spectroscopy</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>HRTEM</td>
<td>High Resolution Transmission electron microscope</td>
</tr>
<tr>
<td>HRSEM</td>
<td>High Resolution Scanning Electron Microscope</td>
</tr>
<tr>
<td>MNPs</td>
<td>Metal nanoparticles</td>
</tr>
<tr>
<td>P. africana</td>
<td>Prunus africana</td>
</tr>
<tr>
<td>SAED</td>
<td>Selected Area Electron Diffraction</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface Plasmon Resonance</td>
</tr>
<tr>
<td>U. dioica</td>
<td>Urtica dioica</td>
</tr>
<tr>
<td>UV- Vis</td>
<td>Ultra Violet Visible Spectroscopy</td>
</tr>
<tr>
<td>W. ugandensis</td>
<td>Warburgia ugandensis</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
</tbody>
</table>
ABSTRACT

Studies have shown that medicinal plants can mediate synthesis of gold, copper, selenium, silver and titanium nanoparticles of various sizes and different morphology. The plants have an advantage over other green synthetic methods as they stabilize and cap the nanoparticles. In this study, silver nanoparticles (AgNPs) were synthesized using Kenyan medicinal plants namely: *Prunus africana* (*Muiri* in Kikuyu), *Adansonia digitata* (*Kiamba* in Kamba, *Mbuyu* in Swahili), *Bridelia micrantha* (*Mukwego* in Embu/Meru) *Warburgia ugandensis* (*Muthiga* in Kikuyu) and *Urtica dioica* (stinging nettle) known for treatment of various ailments. The synthesis was done on stationary and by use of ultrasound bath (sonicator). The formation of silver nanoparticles (AgNPs) was monitored by UV-VIS absorption spectroscopy where surface Plasmon resonance of the AgNPs was observed between 400 to 450 nm. The Selected Area Electron Diffraction (SAED) analysis showed the crystallinity of the silver nanoparticles. High Resolution Transmission Electron Microscope (HRTEM) analysis showed the silver nanoparticles formed were monodispersed, spherical and their size ranged from 13 nm to 45 nm. Energy Dispersive X-ray (EDX) analysis confirmed the synthesized nanoparticles were of pure silver. Fourier Transform Infra-Red (FTIR) was used to show the functional groups of biomolecules present in the plants responsible for reducing, capping and stabilization of the AgNPs. FTIR spectra showed that O-H stretch and –C=C- functional groups were responsible for the reduction and capping the AgNPs. The synthesized AgNPs were found to be toxic against *E. coli* and *S. aureus*. AgNPs mediated by *P. africana* leaves had the best antibacterial activity on *E. coli* of 19.06±1.00 mm. The lowest inhibition was shown by those synthesized using *P. africana* bark on stationary method (11.9±0.251 mm). AgNPs synthesized using *U. dioica* showed the highest activity against *S. aureus* followed by those synthesized using *W. ugandensis* with inhibition zones of 19.8±0.163 mm and 19.05±0.048 mm respectively. The study contributes towards application and documentation of some local Kenyan medicinal plants in the generation of useful silver nanoparticles in the fight against diseases.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

The field of nanotechnology is one of the most active areas of research in modern material science dealing with microscopic particles with dimensions ranging from 1 to 100nm (Saif et al., 2016). Nanotechnology has the potential to create new materials and certainly provide answers to world’s problems related to agriculture, nutrition, health, water and energy (Hassan et al., 2016). Metal nanoparticles properties depend on their size, shape, surface area, composition, crystallinity and structure (Sun and Xia, 2002). Metal nanoparticles with controlled shape and size form basis for advanced functional materials for electronic, medical sensor and optical devices (Gurunathan et al., 2009).

There are different approaches for the synthesis of metal nanoparticles which include physical, chemical and biological. Generally, the approaches for synthesis of metal nanoparticles are classified into two; Bottom - up and Top - down approach (Leela and Vivekanandan, 2008). In bottom - up, nanoparticles are formed by use of chemical and biological ways by self-assembly of atoms to form new nuclei which grow into a particle of Nano scale (Ahmed et al., 2016). In Top - down method; convenient bulk material is split into small particles by size reduction using various methods like grinding, milling, sputtering and laser ablation (Darroudi et al., 2011). The bottom - up approach involves either toxic or nontoxic processes. The toxic processes include laser pyrolysis, aerosol pyrolysis, spray pyrolysis, sol-gel process, vapor deposition and chemical/ electrochemical precipitation.
Chemical methods are costly and potentially harmful to the environment (Prabhu and Poulose, 2012). The chemical methods involve use of toxic chemicals like sodium borohydride (NaBH₄) and hydrazine as reducing agents. NaBH₄ can be a source of caustic salts and flammable gases, which contribute to various biological risks (Arya, 2010). Protecting the environment is a major concern to the chemists hence the need to use safe and ecofriendly methods for synthesis of metal nanoparticles (MNPs). Nontoxic processes, referred to as green synthesis is a feasible method being developed by researchers which embraces environmentally friendly techniques. The green synthesis technique for the assembly of metal nanoparticles involve use of plant extracts (Song and Kim, 2009b), fungi (Vigneshwaran et al., 2007), bacteria (Tsibakhashvil et al., 2010), molds (Elgorban et al., 2016), biodegradable polymers and sonicators (Perelshtein et al., 2008). Green synthesis of nanoparticles is on the limelight due to its various advantages over the chemical methods. The process is rapid, economical and ecofriendly (Mukherjee et al., 2008).

Metal nanoparticles have a wide range of applications including electronics, food industry, cosmetics and medicine. Plant mediated process have been developed for synthesis of gold, silver, titanium and copper nanoparticles. Gold nanoparticles synthesized from leaves extract of Nepenthes kalsian showed antifungal activity against Candida albicans and Aspergillus niger (Bhau et al., 2015). These synthesized nanoparticles have also been used in the treatment of cancer tumors. Cancer cells along with bacteria and viruses can be damaged by nanophotothermolys with laser and gold nanoparticles (Letfullin et al., 2006).

Titanium oxide nanoparticles have been synthesized from Catharanthus roseu, Ecliptaprostrat and Nyctanthes Arbor-Tristis and Planomicrobium sp (Krishnasamyet et
The synthesized titanium oxide nanoparticles showed antimicrobial activity against *Bacillus subtilis* and *Aspergillus niger* (Malarkodi *et al.*, 2013).

*Areva lanata* leaves extract was used to synthesize copper nanoparticles. These copper nanoparticles showed good bacterial activity against *E. coli, S. aureus, Bacillus cereus* and *Pseudomonas aeruginosa* (Hariprasad *et al.*, 2016).

Metal nanoparticles have been synthesized via sonochemistry. The method is used to facilitate chemical reactions and processes by applying ultrasound radiation (20KHz-10MHz) where metal nanoparticles of different sizes are formed (Esmaeili-Zare *et al.*, 2012). The physical phenomenon in sonochemistry involves cavitation and nebulization. Cavitation involves formation, growth and implosive of a bubble in liquid which creates conditions suitable for synthesis of a wide variety of nanostructures (Bang and Suslick, 2010). Several metal nanoparticles have been synthesized using sonochemical methods. Silver nanoparticles were prepared from silver nitrate (AgNO₃) solution by subjecting AgNO₃ solution to intense ultrasound flow (Mănoiu and Aloman, 2010).

Silver nanoparticles exhibit strong antiseptic, antibacterial, antifungal and antiviral properties thus making them to be of great interest in the medicinal field (Franci *et al.*, 2015). The aim of the study was to synthesize silver nanoparticles from silver nitrate solution mediated by selected Kenyan medicinal plants; *Prunus africana, Adansonia digitata, Bridelia micrantha, Warburgia ugandensis* and *Urtica dioica* and test for antibacterial activity.
1.2 Problem statement

Silver nanoparticles properties are unique as they depend on size, shape, and morphology. Different approaches can be used to synthesize AgNPs which include: reduction in solution, radiation assisted, thermal decomposition of silver compounds and microwave assisted. These conventional methods are not environmentally friendly as they involve use of toxic and hazardous chemicals which produce harmful by products (Prabhu and Poulose, 2012).

Plant mediated synthesis of metal nanoparticles does not involve use of toxic chemicals. It is fast, clean, can be scaled up and compatible for pharmaceutical and other biomedical applications. Silver nanoparticles synthesized using plant extracts vary in size and shape depending on the plant extract used (Song and Kim, 2009a). Their applications depends on their size and shape (Pal et al., 2007). Due to the various applications, there is need to explore new and ecofriendly ways of their synthesis using Kenyan plants with known medicinal value and then test for antibacterial activity.

1.3 Justification

AgNPs have been used in medicinal field due to their antimicrobial and anti-inflammatory activity. The conventional methods for their synthesis are not environmentally friendly. They involve the use of toxic and hazardous chemicals resulting in environmental degradation. Plants contain phytochemicals which offer a friendly environment in the synthesis of metal nanoparticles. Different plants can mediate in the formation and stabilization of metal nanoparticles of different sizes and shape, hence different properties and applications. The rate of formation and size control can be done through use of ultrasound. AgNPs have been synthesized using various plant extracts. However, Kenyan
medicinal plants have not been studied for the synthesis of such nanoparticles. Hence, there was need to research and contribute knowledge on the generation of novel silver nanoparticles using Kenyan medicinal plant extracts and test their activity against microbes.

1.4 Hypotheses
i. AgNPs can be synthesized using selected medicinal plant extracts.
ii. There is a difference in size and morphology of AgNPs synthesized using different plant extracts.
iii. The biosynthesized AgNPs exhibit significant difference in antibacterial activity.

1.5 Objectives
1.5.1 General objective
To explore use of plant extracts in biosynthesis of AgNPs and evaluate the antibacterial activity of those synthesized AgNPs.

1.5.2 Specific objectives
i. To synthesize silver nanoparticles using Adansonia digitata, Prunus africana, Bridelia micrantha, Warburgia ugandensis and Urtica dioica plant extracts.
ii. To characterize the biosynthesized AgNPs by UV – Vis, EDX, SAED, HRSEM, HRTEM and FTIR spectroscopy.
iii. To evaluate the antibacterial activity of biosynthesized AgNPs against E. coli and S. aureus.

1.6 Significance of the study
Metal nanoparticles, especially silver, selenium and gold are commonly used in the field of biomedical studies, electronic and medicine. The conventional methods for their synthesis are non-environmentally friendly. Metal nanoparticles are the current focus of
research in many fields especially in biomedical applications and is opening new frontier of research in the diagnostic and treatment of complex diseases. This study will contribute significantly to the generation of novel AgNPs useful in the control and management of infectious diseases.

1.7 Scope and limitations

- The study only focused on the susceptibility of bacteria on synthesized AgNPs, the mechanism for antibacterial activity was not determined.

- Only a few selected Kenyan medicinal plant extracts were used in the synthesis of AgNPs.
CHAPTER TWO

LITERATURE REVIEW

2.1 Biosynthesis of AgNPs

The synthesis of AgNPs is in the limelight because of their diverse range of application in bioengineering, cosmetic (Keller et al., 2013), food packaging (Duncan, 2011), medical industry (Prabhu and Poulase, 2012) and water treatment (Bystrzejewska-Piotrowska et al., 2009). The AgNPs properties depend on their size, shape, morphology and distribution. The size of those synthesized by biological methods varies between 1 and 600 nm (Rudramurthy et al., 2016).

2.2 Sonochemical synthesis of AgNPs

Sonication is a process in which ultrasound waves (>20 kHz) are used to agitate particles in solution by use of probes or ultrasound bath. Sonication method has been reported in green synthesis of AgNPs where an aqueous extract of Portulaca oleracea leaves was used to synthesize AgNPs using ultrasonic bath. X-ray diffraction (XRD) showed that crystals were formed and Scanning Electron Microscope (SEM) determined the size of AgNPs formed which was less than 60 nm (Firdhouse and Lalitha, 2012).

This novel approach was applied to synthesize AgNPs using starch in an ultrasonic bath. The UV-VIS analysis of the Colloidal AgNPs showed an increase in absorption from 420 to 440nm with increase in starch quantity meaning that when starch quantity was increased the amount of AgNPs synthesized also increased. Transmission Electron Microscope (TEM) followed by Selected Area Diffraction (SAED) pattern analysis indicated formation of spherical, polydisperse, amorphous AgNPs of diameter ranging from 23 to 97 nm.
A study by Yin, 2002 showed that, increase in temperature during sonication leads to a decrease in the size of the AgNPs.

*Camellia sinensis* fortified with lemon and honey was also used to synthesize AgNPs by ultra-sonication (Kothai and Jayanthi, 2014). The extract acted both as a reducing and a capping agent. The reaction rate was found to be increased by ultrasonic irradiation (Kothai and Jayanthi, 2014).

### 2.3 Synthesis of AgNPs using plant extracts.

Green synthesis of novel metal nanoparticles using plant extracts and their applications has been reported (Firdhouse and Lalitha, 2015). The plant extracts can be used as reducing and capping agents in the synthesis of metal nanoparticles. Biomolecules like proteins, alkaloids, flavonoids, phenolic compounds, vitamins, polysaccharides and terpenoids have been used in reducing, capping and stabilization of the AgNPs (Gebru *et al.*, 2013). Plant biosynthesized nanoparticles are more stable and are produced faster than those synthesized by microorganisms (Firdhouse and Lalitha, 2015).

*Ocimum sanctum* leaves extract was also used to reduce silver ions into spherical crystalline AgNPs of sizes between 4 and 30 nm (Pavani *et al.*, 2013). Bio-reduction was achieved due to presence of ascorbic acid in the leaves which shows it is a good reducing agent. It has been confirmed that, AgNPs exhibited antibacterial activity against *E. coli* and *S. aureus* (Singhal *et al.*, 2011).

AgNPs have been biosynthesized by use of *Boerhaavia diffusa* plant extract as a mediating agent by applying stationary method (Nakkala *et al.*, 2014). The TEM analysis showed an average particle size of 25 nm of AgNPs having face centered cubic structure with spherical
shape. The nanoparticles were tested for antibacterial activity against three fish bacterial pathogens *Pseudomonas fluorescens*, *Flavobacterium branchiophilum* and *Aeromonashy drophila*. The findings showed a higher sensitivity towards *Flavobacterium branchiophilum* than the other two bacteria (Nakkala et al., 2014).

*Abutilon indicum* extract was also used to synthesize spherical shaped AgNPs (Mata et al., 2015). The analysis by Mata, 2015 indicated that the nanoparticles were spherical and uniformly distributed having size of 8 to 40 nm. The synthesized nanoparticles showed high antimicrobial activity against *S. typhi*, *E. coli*, *S. aureus* and *B. subtilis* microorganisms.

AgNPs have also been synthesized using an aqueous solution of *Pulicaria glutinosa* extract, which was used as a bio-reductant. The particles obtained were pure and crystalline with face-centered cubic structure. The results showed the size of the particles depended on the concentration of the plant extract (Khan et al., 2013).

Various studies have shown that, different plant extracts produce AgNPs of different sizes and shapes, and in certain cases they produce mixed shapes as shown in table 2.1 (Kuppusamy et al., 2016).

**Table 2.1: List of plants and their biomolecules used in synthesis of AgNPs**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Part</th>
<th>Size and shape</th>
<th>Biocomponents</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Albizia adianthifolia</em></td>
<td>Leaf</td>
<td>4–35 nm; spherical</td>
<td>Saponins, proteins, and sugars</td>
<td>Govender et al., 2013</td>
</tr>
<tr>
<td><em>Allium sativum</em></td>
<td>Clove</td>
<td>7.3 ± 4.4 nm; spherical</td>
<td>Proteins Sucrose and fructose</td>
<td>Rastogi and Arunachalam, 2011</td>
</tr>
<tr>
<td><em>Allophylus cobbe</em></td>
<td>Leaf</td>
<td>2–10 nm; spherical</td>
<td>Proteins</td>
<td>Gurunathan et al., 2014</td>
</tr>
<tr>
<td>Plant</td>
<td>Part</td>
<td>Diameter (nm)</td>
<td>Group</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>--------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td><em>Aloe vera</em></td>
<td>Leaf</td>
<td>34–102; spherical</td>
<td>Carbonyl group</td>
<td>Moosa et al., 2015</td>
</tr>
<tr>
<td><em>Amaranthus gangeticus</em></td>
<td>Leaf</td>
<td>11–15; globular</td>
<td>Amino acids</td>
<td>Kolya et al., 2015</td>
</tr>
<tr>
<td><em>Amaranthus dubius</em></td>
<td>Leaf</td>
<td>40–50; spherical</td>
<td>Proteins</td>
<td>Firdhouse and Lalitha, 2013</td>
</tr>
<tr>
<td><em>Anacardium occidentale</em></td>
<td>Leaf</td>
<td>6–17</td>
<td>Proteins, aromatic amines, and polyphenols</td>
<td>Sheny et al., 2011</td>
</tr>
<tr>
<td><em>Andrographis paniculata</em></td>
<td>Leaf</td>
<td>28; cubic and hexagonal</td>
<td>Hydroxyflavones and catechins</td>
<td>Sulochana et al., 2012</td>
</tr>
<tr>
<td><em>Annona squamosa</em></td>
<td>Peel Leaf</td>
<td>20–60; irregular spherical</td>
<td>Hydroxy group, Phenolic group, proteins, and carbohydrates</td>
<td>Kumar et al., 2012</td>
</tr>
<tr>
<td><em>Anthemis tinctoria</em></td>
<td>Leaf, stem &amp; flower</td>
<td>34.77</td>
<td>Alcoholic and phenolic groups</td>
<td>Khoshkhoo and Nematollahi, 2016</td>
</tr>
<tr>
<td><em>Arbutus andrachne</em></td>
<td>Leaf</td>
<td>107.8 ± 0.8; spherical</td>
<td>Proteins, phenols and hydroxyl group</td>
<td>Erdoğan et al., 2016</td>
</tr>
<tr>
<td><em>Argemone mexicana</em></td>
<td>Leaf</td>
<td>2–6; spherical</td>
<td>Metabolic fluxes and ascorbates or catechol/protocatechuic acid, alkaloids</td>
<td>Jha and Prasad, 2014</td>
</tr>
<tr>
<td><em>Astragalus gummifer</em></td>
<td>Gum tragacanth</td>
<td>13.1± 18.0; spherical</td>
<td>Proteins</td>
<td>Kora and Arunachalam, 2012</td>
</tr>
<tr>
<td><em>Avicennia alba</em></td>
<td>Leaf</td>
<td>Different</td>
<td>Alcoholic and phenolic compounds</td>
<td>Nagababu and RAO, 2016</td>
</tr>
<tr>
<td><em>Ocimum tenuiflorum</em> (green)*</td>
<td>Leaf</td>
<td>30.56–82.63; spherical (green) and 39.95–53.47 nm; spherical (purple)</td>
<td>Carbonyl group and flavonoids</td>
<td>Anuradha et al., 2014</td>
</tr>
<tr>
<td><em>Odina wodier</em></td>
<td>Leaf</td>
<td>5–30; spherical</td>
<td>Unsaturated carbonyl groups</td>
<td>Malathi and Ganesan, 2015</td>
</tr>
<tr>
<td><em>Olea europaea</em></td>
<td>Leaf</td>
<td>10–30; cubical and uniform</td>
<td>Proteins</td>
<td>Awwad et al., 2012</td>
</tr>
<tr>
<td><em>Papaver somniferum</em></td>
<td>Seed</td>
<td>3.2–7.6; spherical</td>
<td>Alkaloids</td>
<td>Vijayaraghavan et al., 2012</td>
</tr>
<tr>
<td><em>Parthenium hysterophorus</em></td>
<td>Leaf</td>
<td>10; spherical</td>
<td>Polyols, hydroxyflavones, catechins</td>
<td>Kumar, 2012</td>
</tr>
<tr>
<td><em>Pedilanthus tithymaloides</em></td>
<td>Leaf</td>
<td>15–30; spherical</td>
<td>Proteins and enzymes</td>
<td>Sundaravadivelan and Nalini, 2012</td>
</tr>
</tbody>
</table>
AgNPs of different sizes and shapes have different antimicrobial activities (Pal et al., 2007). The selection of Kenyan plants for this study was based on availability of plant materials and their medicinal use as antioxidants, anti-inflammatory, antidiabetic and anticancer agents (Kokwaro, 2009; Kweifio-Okai, 1991; Núñez-Sellés, 2005). There is also limited information on green synthesis of AgNPs using these Kenyan medicinal plants and their antibacterial activity. The selected plants were: *Adansonia digitata*, *Prunus Africana*, *Warburgia ugandensis*, *Bridelia micrantha* and *Urtica dioica*.

### 2.3.1 *Adansonia digitata* (baobab)

*Adansonia digitata* (baobab) also called *kiamba* (Kikamba), Mbuyu (Swahili) is found in lower Eastern and Coastal regions in Kenya. *A. digitata* fruit contains vitamin C (ascorbic acid), vitamin A, calcium, iron, and carbohydrates. The fruit is used as a source of food, medicine, and traditional therapy. The outer rind of the fruit is used in making fibers, clothes, house hold items, and other objects. The seeds are used in making oil, which is used in cooking and manufacturing soap and detergents.
acid), higher than that found in citrus fruits (Irvine, 1952), carbohydrates, polyphenols, and has high antioxidant capacity (Ibrahima et al., 2013). The young leaves are rich in calcium, iron, zinc and vitamin C (Gebauer et al., 2002). The leaves are used in soups and eaten as vegetables (Irvine, 1952). The seeds have been found to be rich in proteins (Osman, 2004). The presence of these phytochemicals made it suitable as a mediating agent in the synthesis of AgNPs (Kumar et al., 2016). Antibacterial activity of baobab leaves, fruit pulp, and stem bark has been done on E. coli and S. aureus. The inhibition zones shown by the leaves extract and fruit pulp on E. coli were 6.50 mm and 7.0 mm respectively, while for S. aureus the inhibition zones were 7.5 mm and 23.0 mm. A. digitata fruit pulp was used to synthesize spherical AgNPs of size 3 to 57 nm which showed antibacterial activity against of E. coli and S. aureus (Kumar et al., 2016). The inhibition zones obtained by Kumar et al., (2016) were 22 mm for E. coli and 15.9 mm for S. aureus. A. digitata leaves AgNPs showed inhibition zones of 22 mm on E. coli and 11.1 mm on S. aureus (Kumar et al., 2015).

2.3.2 Prunus africana

Prunus Africana, Mwiria (Meru) commonly known as African cherry has a wide distribution in Africa growing in mountainous regions. In Kenya it grows predominantly in central Kenya. Prunus africana has been reported to treat many disorders including fevers, malaria, stomach pain, kidney disease, gonorrhea (Iwu, 2014) and it is being investigated for treatment of prostate cancer (Shenouda et al., 2007). It has several bio components: fatty acids, esters and alkanols (Kadu et al., 2012). Biosynthesis of AgNPs using P. africana plant extract has not been reported, though presence of alkanols suggest it can be used as reducing, capping and stabilizing agent in synthesis of silver nano particles (Song et al., 2009).
2.3.3 *Bridelia micrantha*

*B. micrantha* known as *Mukoigo* (Kikuyu), *Odugu-Kulo* (Luo) is a medium-tall tree of up to 20 meters in height. It belongs to the family of Euphorbiaceae. It is commonly called Coast gold leaves, traditionally used in Asia and Africa for treatment of various ailments like bronchitis, anemia and sexually transmitted diseases (Kokwaro, 2009; Munayi, 2016). A study by Munayi, (2016) indicated that *B. micrantha* can be used in the treatment of diabetes mellitus, sphyllis, tape worm, abdominal pain, headache, pneumonia, sore eyes and coughs. The analysis phytochemicals analysis showed that it contains various secondary metabolites like flavonoids, saponins, lignans and triterpenes (Nguyen *et al.*, 2009).

2.3.4 *Warburgia ugandensis*

*Warburgia ugandensis* also known as Ugandan greenheart, *Muthiga* (Kikuyu) contains alkaloids, terpenoids and sesquiterpenoids (Abuto, 2016). The bark is used as a remedy for toothache, stomachache, coughs and fever (Wube *et al.*, 2010). *W. ugandensis* plant extracts have shown antibacterial activity to *E. coli* and *S. aureus* but their synergistic effect with AgNPs on these pathogens has not been studied.

2.3.5 *Urtica dioica*

*Urtica dioica* known as *Thabai* (Kikuyu) is herbaceous perennial flowering plant in the family of Urticaceae commonly known as stinging nettle or common nettle. Traditionally, it is used to treat painful muscles and joints, arthritis, gout and anemia (Upton, 2013). The stinging nettle roots have been used for prostate ailments and irritable bladder. They have also shown anesthetic, analgesic and anti-arthritic properties (Gulcin *et al.*, 2004). Moreover, it contains metabolites like alkaloids, phenols, amino acids and proteins (Jyoti
et al., 2016). Spherical AgNPs with diameter 20 to 30 nm have been synthesized using *U. dioica* leaves (Jyoti et al., 2016) though the roots extract have not been investigated. Synthesized AgNPs using *U. dioica* leaves showed antibacterial activity against Gram positive and gram-negative bacteria (Jyoti et al., 2016).

### 2.4 Antimicrobial activity of AgNPs

AgNPs exhibit potential antibacterial effects against *Bacillus subtilis, S. aureus, Pseudomonas aeruginosa, E. coli* and *Treponema pallidum* (Rudramurthy et al., 2016). The activity on the bacteria depends on the size, shape and morphology of the AgNPs. AgNPs can release Ag⁺ which can interact with thiol groups of many enzymes and deactivate them (Matsumura et al., 2003). Silver ions work by suppression of respiratory enzymes and electron transport components which interfere with DNA functions (Li et al., 2006).

The antibacterial activity of the AgNPs varies between Gram negative and Gram-positive bacteria. The difference in inhibition zones between Gram-negative and Gram-positive bacteria is attributed to the thick peptidoglycan layer of Gram-positive bacteria compared to Gram-negative which have cell membrane, thus the AgNPs are able to penetrate through the thin peptidoglycan layer of Gram negative bacteria easily than the thick peptidoglycan of Gram-positive bacteria (Yugandhar and Savithamma, 2015).

### 2.5 Characterization of AgNPs

AgNPs can be characterized using X-ray diffraction (XRD), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM) and Energy Dispersive X-ray (EDX). UV-VIS spectroscopy is used to monitor formation of AgNPs due to colour development. The colour is due to silver Plasmon resonance with spectrum ranging
between 400 to 450 nm. Shift in the wavelength indicates change in the size of AgNPs. Furthermore, an increase in optical density (OD) during synthesis shows increase in the concentration of the nanoparticles (He et al., 2005). Broad peaks indicate the AgNPs are polydisperse. Broadening of the peak during synthesis of AgNPs indicate agglomeration of the nanoparticles (Henglein, 1993).

Transmission Electron microscope (TEM) is a microscopic technique in which a beam of electrons is transmitted through a specimen to form an image. TEM shows the shape, size and morphology of the AgNPs (Sun and Xia, 2002).

Energy Dispersive X-Ray (EDX) spectroscopy is an analytical technique used for the elemental analysis. Its fundamental principle is that each element has a unique atomic structure allowing a unique set of peaks on its electromagnetic spectrum. In the analysis of AgNPs, EDX is used to identify the elemental silver and depict their crystalline nature (Naik et al., 2002). Fourier Transform Infra-Red (FTIR) has been used to show the presence of functional groups of the phytochemicals used to reduce, cap and stabilize the AgNPs.
CHAPTER THREE

MATERIALS AND METHODS

3.1 Material, chemicals and apparatus

3.1.1 Sample collection

Leaves, fruits and stem barks of the plants under study were collected from their natural habitats. The *A. digitata* (Plate 3.1) fruits and leaves were collected from the lower part of Makueni County South Eastern Kenya. The leaves and stem bark of *Prunus africana* (Plate 3.2) *Bridelia micrantha* (Plate 3.3), *Warburgia ugandensis* (Plate 4.4) and *Urtica dioica* (roots, leaves) were obtained from Manyatta constituency, Embu County Kenya.

Plate 3.1: *Adansonia digitata* (boabob)
Plate 3.2: *Prunus africana* (Photo by Wilson Njue)

Plate 3.3: *Warburgia ugandensis* (Photo by Wilson Njue)
The plant specimens were identified by a taxonomist from Department of Plant Sciences and voucher specimen deposited at the herbarium in Kenyatta University.

3.1.2 Chemicals

Potassium bromide, Muller Hinton, Nutrient agar, sabouraud dextrose agar and silver nitrate were of analytical grade obtained from Sigma Aldrich (Germany).

3.1.3 Apparatus and instruments

Sonicator (WUC-A03H), UV-Visible spectrometer (Specord 200 analytik Jena), Fourier transform infrared spectrophotometer (Shimadzu IRT racer-100), Energy Dispersive X-ray (EDX), High Resolution Transmission Electron Microscope (HRTEM FEI Technai F20),
grinder (Retsch 200), Whatman filter papers No-1, clean glassware and petri dish were used in this study.

3.2 Sample preparation

The plant leaves, stem bark and roots were cleaned with tap water followed by distilled water then air dried. They were chopped then pulverized to a fine powder. The dry fruit pulp from *A. digitata* dry seeds was scrapped off using a stainless knife and then ground to obtain a fine powder.

3.2.1 Plant extraction

About 10 g of *A. digitata* leaves powder was added to 100mL of distilled water in 250 mL conical flask and then heated at 60 °C in a water bath for 4 hours. The cool mixture was filtered using Whatman paper No.1 to obtain a clear filtrate, centrifuged for 10 minutes to remove all the fine plant particles and stored at -4°C. The same procedure was followed for the other plant materials.

3.2.2 Stationary biosynthesis of AgNPs

The procedure by Singhal *et al.*, (2011) was adapted but with slight modification for the synthesis. Silver nitrate solution was mixed with the plant extract in the ratio of 9:1 in a conical flask and kept at 25 °C. Reduction of pure Ag⁺ to Ag⁰ was monitored by visual observation of colour change of the solution and measuring UV-Visible spectrum at regular intervals.

3.2.3 Synthesis of AgNPs over ultrasonic bath

Ultrasound enhanced (sonication) synthesis was performed as per the method described by Mason, (1997). Ultrasonic bath was used instead of ordinary water bath to facilitate the reaction process. The plant extract (10 mL) was mixed with 90 mL of 0.001M AgNO₃
solution in a 250 mL conical flask then immersed in the ultrasonic bath. The progress of the reaction was then monitored regularly.

3.3 Characterization and analysis

3.3.1 UV-VIS spectroscopy

UV-VIS spectroscopy was used to monitor the formation of the AgNPs. The prepared nanoparticles solution was diluted 5 times with distilled water then put in quartz UV-VIS cuvette. Scanning was done between 300 to 800 nm. Distilled water was used as the blank.

3.3.2 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis

FT-IR measurements were done so as to determine the functional groups of biomolecules capping and stabilizing the AgNPs. The sample solution containing the nanoparticles was centrifuged at 5,000 rpm for 20 minutes then filtered. The solid matter obtained was then ground with potassium bromide (KBr) then formed into pellets. The pellet was used for FTIR analysis.

3.3.3 High Resolution Transmission Electron Microscope (HRTEM) Analysis

HRTEM was used to determine size, shape and morphology of the AgNPs. The samples for HRTEM analysis were prepared by drop coating synthesized AgNPs solution on to carbon-coated copper HRTEM grids (Woehrle et al., 2006). Energy dispersive X-Ray and Selected Area Electron diffraction used in the study were coupled with the HRTEM.

3.3.4 High Resolution Scanning Electron Microscope (HRSEM) Analysis

High Resolution Scanning Electron Microscope (HRSEM) analysis was done using a Zeiss field Emission Gun SEM operated with SMART SEM version 5.04 software. Samples were placed on HRSEM to determine shape and size of the nanoparticles.
3.4 Anti-bacteria activity

The antibacterial activity was done using paper disc diffusion technique (Ruparelia et al., 2008) on Gram positive (*S. aureus*) and gram negative (*E. coli*) bacteria. The test bacterial strains were sub cultured for 24 hours. The concentration of the bacteria was determined by comparing its turbidity with McFarland solution. The inoculum (\(1.5 \times 10^8\) colony forming units/ml) was swabbed on the nutrient agar in sterile petri dishes. Paper discs (6mm) impregnated with AgNPs were placed on the same petri dishes then incubated for 24 hours at 37 °C. Zones of inhibition caused by AgNPs were measured after 24 hours of incubation. The magnitude of antibacterial effect against, *E. coli* (ATCC No.25922) and *S. aureus* (ATCC No14028) was determined based on the inhibition zone measured (Gebru et al., 2013). The AgNPs exhibiting activity had their minimum inhibitory concentration (MIC) determined. Vancomycin was used as the positive control for *S. aureus* and Ciprofloxacin for *E. coli*. Distilled water was used as the negative control.

3.5 Data analysis

Data analysis was done using by One-way anova using the SPSS data software. The sizes of the synthesized AgNPs were measured and the average size determined. Post hoc analysis was done using Turkey at 95% confidence level to determine if there was significant difference in the average sizes of the AgNPs synthesized from different plant extracts. Inhibition zones were measured in triplicates and the mean determined. One-way anova was done using Turkey at 95% confidence level to determine if the inhibition zones caused by AgNPs synthesized using different plants were significantly different.
CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

The formation of AgNPs was monitored by visual assessment of the colour change of the solution and by use of UV-VIS spectrophotometer. The characterization of the synthesized AgNPs was done using Energy Dispersive X-ray (EDX), Scanning Electron Area Diffraction (SAED) and High-Resolution Transmission Electron Microscope (HRTEM).

4.2 AgNPs synthesized using *Adansonia digitata* leaves extract

4.2.1 Stationary synthesis of AgNPs

*A. digitata* leaves extract and the AgNO₃ solution changed from yellow to brown then dark brown in 60 minutes as shown in Plate 4.1 indicating formation of AgNPs. A similar colour change was observed on the biosynthesis of AgNPs using *A. digitata* leaves by Kumar *et al.*, (2015). The dark-Brown colour was due to the excitation of surface Plasmon vibrations of AgNPs (Ahmad *et al.*, 2010). This is about combination vibrations of electrons of the AgNPs in resonance with the light wave. The specific oscillations depend on the shape and size of the particles (Henglein, 1993).

Plate 4.1: Colour development of *A. digitata* leaves extract and AgNO₃ on stationary at (i) 0 (ii) 15 (iii) 30 (iv) 45 and (v) 60 minutes
The UV-VIS spectrum (Fig. 4.1) showed absorbance peaks at $\lambda_{\text{max}}$ 450 nm, characteristic of AgNPs similar to what was reported earlier by Henglein, (1993) on his study on Physiochemical properties of metal nanoparticles in solution. Kumar et al., (2015) on their study on biosynthesis of AgNPs observed absorbance peaks at $\lambda_{\text{max}}$ 434 nm. The absorbance increased with time indicating increase in the concentration of nanoparticles. A similar observation was reported by Smitha et al., (2008) on their studies on surface Plasmon resonance (SPR) and photoluminescence of AgNPs synthesized using citrate. The variation of absorbance with concentration of nanoparticles are directly proportional to each other as explained by the Beer lamberts law. The optical density increased with time up to 80 minutes. This was the optimum time taken for the synthesis of the AgNPs via stationary method.

![Graph](image)

**Fig. 4.1:** The UV-VIS spectra of *A. digitata* leaves mediated AgNPs (stationary)

**4.2.2 Ultrasound enhanced biosynthesis of AgNPs**

The mixture of the *A. digitata* leaves extract and AgNO$_3$ solution in a conical flask on ultrasonic bath changed from pale yellow to light brown, red brown then dark brown in 20 minutes (Plate 4.2). The change in colour took a shorter time than under stationary method.
There are no previous reported studies done on the synthesis of AgNPs using *A. digitata* leaves under ultrasonic bath.

**Plate 4.2**: Colour development of *A. digitata* leaves extract and AgNO₃ on sonication at (i) 0 (ii) 5 (iii) 10 and (iv) 20 minutes on ultrasonic bath (sonicator)

UV-VIS absorption spectra (Fig 4.2) showed absorption peaks between $\lambda_{\text{max}}$ 425nm to 448nm.

**Fig. 4.2**: UV-VIS spectra of *A. digitata* leaves AgNPs on sonication
The peaks at 5 and 10 minutes were broad indicating that the AgNPs formed were polydisperse similar to the study of optical properties and ultrafast dynamics of metallic nanocrystals (Link and El-Sayed, 2003). The other peaks from 15 to 30 minutes were narrow, an indication of monodisperse nanoparticles. The absorbance intensity increased steadily up to 30 minutes indicating increase in the concentration of silver nanoparticle with time. Increase in absorption peaks with time was also noted in the study on biosynthesis of AgNPs using (Diopyros kaki leaves), where absorption peaks at $\lambda_{\text{max}}$ 430nm increased intensity up to 480 minutes (Song and Kim, 2009a).

The blue shift in wavelength from 448 nm to 425 nm indicated decrease in size of the synthesized AgNPs under sonication. Similar observation on decrease in size of AgNPs under sonication was also made by Petit, (1993) during his study on In situ synthesis of silver nanocluster. The decrease in size of the AgNPs during synthesis can be attributed to ultrasound cavitation phenomenon during sonication (Yin et al., 2002) and also the decrease in concentration of the reactants with time (Prathna et al., 2011).

### 4.2.3 High Resolution Transmission Electron Microscope (HRTEM) analysis

Images of HRTEM micrographs on synthesized AgNPs using A. digitata leaves via sonication method presented in Plate 4.3 (Image A-E) depicted the synthesized AgNPs were spherical and evenly distributed. The images showed that, there was no agglomeration of the AgNPs synthesized. Thus A. digitata leaves biomolecules reduced Ag$^+$ to Ag$^0$ and formed stable nanoparticles with no clustering. The surface of the spherical nanoparticle was non-uniform as indicated in Plate 4.3 image F.
Plate 4.3: HRTEM images of synthesized AgNPs using A. digitata leaves via sonication

The sizes of the synthesized AgNPs obtained from HRTEM analysis are portrayed in the graph in Fig 4.3. The sizes ranged from 5 to 24 nm with the highest range of nanoparticles being 10 to 14 nm. The result showed that the synthesized nanoparticles were monodispersed which concurs with UV-VIS analysis. The average size of the synthesized AgNPs using A. digitata leaves was 13.2±2.46 nm. The AgNPs synthesized in this study were smaller (5 to 24 nm) compared to what Kumar et al., (2015) reported (3 to 57 nm).
Fig. 4.3: **Size distribution of AgNPs synthesized using *A. digitata* leaves extract via sonication**

4.2.4 Selected Area Electron Diffraction (SAED) Analysis

The Selected Area Electron Diffraction (SAED) micrograph (Plate 4.4) depicted discrete shiny spots of AgNPs thus confirming the AgNPs formed were crystalline.

Plate 4.4: SAED micrograph for *A. digitata* leaves mediated AgNPs via sonication

4.2.5 Energy Dispersive X-ray (EDX) Analysis

Energy Dispersive X-ray (EDX) spectroscopy was used to show the elemental composition of the sample. The EDX confirmed that the nanoparticles formed were pure silver. Fig. 4.4 potrays an EDX spectrum with peaks at around 3.0 KeV which are due to elemental silver.
4.2.6 Fourier Transform Infra-Red (FTIR) analysis of AgNPs

The FTIR spectrometer was used to identify the functional groups of the plant biomolecules responsible for reduction and capping of the AgNPs. FTIR spectrum for AgNPs biosynthesized using *A. digitata* leaves extract is shown in Fig 4.5. The broad peak at 3268 cm\(^{-1}\) was due to O-H stretch and at 1637 cm\(^{-1}\) for C = C stretching. The functional groups could be due to phenolic compounds capping and stabilizing the AgNPs. Similar functional groups were reported by Kumar *et al.*, (2015).
Fig. 4.5: FTIR spectra of AgNPs synthesized using A. digitata leaves extract

4.2.7 Antibacterial activity of AgNPs synthesized using A. digitata leaves extract

Results of antibacterial activity showed clear hollows on plates with nutrient agar swabbed with *E. coli* and *S. aureus*. AgNPs exhibited inhibition zone of 17.3±0.208 mm towards Gram-negative bacteria *E. coli* against the positive control with inhibition zone of 33.4±0.443 mm. The AgNPs showed inhibition of 13.0±0.082 mm towards Gram positive bacteria *S. aureus* against an inhibition zone of 22.5±0.988 of positive control. Zones of inhibition are shown in table 4.1.
Table 4.1: Inhibition zones of AgNPs synthesized using A. digitata leaves extract on E. coli and S. aureus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Zones of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>A. digitata leaves AgNPs</td>
<td>Stationary</td>
<td>17.3±0.208&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. digitata leaves AgNPs</td>
<td>Sonication</td>
<td>17.1±0.130&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>33.4±0.443&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt; solution</td>
<td></td>
<td>11.26±0.205&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. digitata leave extract</td>
<td></td>
<td>6&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

AgNPs synthesized via stationary method had inhibition zones of 17.3±0.208 mm on E. coli which is not significantly different from the inhibition caused by AgNPs synthesized on sonication method. The inhibition zones on S. aureus caused by AgNPs were significantly different at 95% confidence level. Studies done by Marutikesavakumar <i>et al.</i>, (2015) showed AgNPs synthesized using A. digitata leaves of size 5 to 10 nm had antibacterial activity of 24.1±0.60 mm on E. coli. The higher antibacterial activity in his study was due to the smaller size of 5 to 10 nm of the AgNPs synthesized. AgNPs can penetrate through the bacteria cell membrane and interact with compounds containing sulphur and phosphorous such as proteins and DNA thus making the bacteria loose viability that eventually leads to death (Matsumura <i>et al.</i>, 2003). The A. digitata leaves extract showed inhibition zones of 6 mm which was small compared to the inhibition zones of the
AgNPs. Comparison of the inhibition zones caused by AgNPs and those of the standard showed that, both bacteria were susceptible to the AgNPs.

The minimum Inhibitory concentration was determined by doing replicate tests. For *E. coli* it was - 0.125mM AgNPs while for *S. aureus* it was 0.25mM AgNPs. The clear hollows in Plate 4.5 shows some zones of inhibition due to different concentrations of AgNPs on *S. aureus*; 1-1mM AgNPs, 2-0.75mM AgNPs, 3-0.5mM AgNPs and 4-0.25mM AgNPs.

**Plate 4.5:** Antibacterial activity of *A. digitata* leaves AgNPs on (A) *S. aureus*, (B) *E. coli*

### 4.3 Synthesis of AgNPs using *Adansonia digitata* fruit pulp

#### 4.3.1 Stationary synthesis of AgNPs

The *A. digitata* fruit pulp extract and 0.001 M silver nitrate solution changed from cream to yellow in 15 minutes then turned to brown after 30 minutes as shown in Plate 4.6.
Plate 4.6: Colour change of *A. digitata* fruit pulp extract and AgNO$_3$ solution at 0 (ii) 15 and (iii) 30 minutes

The formation of AgNPs was monitored by UV-VIS spectrometer at different time intervals and had Plasmon resonance peaks at $\lambda_{\text{max}}$ 425 nm as shown in Fig. 4.6. The absorption of radiation increased steadily with time where maximum absorption was observed after 35 minutes of interaction. The peaks formed were narrow an indication of monodispersed AgNPs. Similar observation was reported on a the study on synthesis of AgNPs using *A. digitata* fruit pulp by Kumar *et al.*, (2016).

Fig. 4.6: UV-VIS spectra of *A. digitata* fruit pulp synthesized AgNPs by keeping the solution stationary
4.3.2 Ultrasound enhanced (sonication) formation of AgNPs

The *A. digitata* fruit pulp extract and 0.001M AgNO$_3$ solution changed from milky to brown after thirty minutes on sonication as shown in Plate 4.7. No further colour change was observed after 30 minutes, an indication of complete formation of AgNPs.

![Plate 4.7: Colour development at (i) 0 (ii) 30 minutes of sonication](image)

Figure 4.7 below shows the UV VIS spectra obtained during synthesis of AgNPs using *A. digitata* fruit pulp extract via sonication method. The AgNPs formed showed peaks ranging from between 425nm to 445 nm. The absorbance intensity increased steadily up to 30 minutes, thereafter, no further observable change indicating the completion of the reaction. With an increase in time, the absorption shifted to shorter wavelength, from 445 nm to 425 nm (blue shift), indicating decrease in the size of the AgNPs (Khalil *et al.*, 2014). Similar observation of blue shift was reported by Khalil, (2014) when synthesizing AgNPs using olive oil extract by use of sonicator bath. Decrease in size during sonication is attributed to cavitation collapse on or near the surface of AgNPs which tends to break the particles apart during sonication (Zhu *et al.*, 2000).
The synthesis AgNPs was faster when sonication method was used as the average time taken for the synthesis was 30 minutes. Synthesis of AgNPs using A. digitata using ultrasonic bath has not been reported before.

4.3.3 High Resolution Transmission Electron Microscope analysis

HRTEM analysis of AgNPs synthesized using A. digitata fruit pulp via stationary method showed spherical AgNPs (Plate 4.8 Image A-F). The synthesized nanoparticles had a non-uniform surface but were stable and well dispersed. The SAED micrograph showed shinny spots (Plate 4.8 Image H) an indication that the AgNPs synthesized were crystalline. Similar results were obtained by Kumar et al., (2016) on the studies of biological synthesis of AgNPs using A. digitata fruit pulp but were smaller in size of 7 to 10 nm.
Plate 4.8: HRTEM and SAED micrographs of AgNPs

The size of synthesized AgNPs using *A. digitata* fruit pulp ranged from 6 nm to 30 nm with modal class being 21 to 25 nm. This confirms the UV-VIS analysis that the synthesized nanoparticles were monodispersed. The data on their size obtained from HRTEM is shown in the graph in Fig 4.8. The result confirmed that *A. digitata* fruit pulp can be used to synthesize spherical nanoparticles of average diameter of 19.8±5.93 nm.
4.3.4 Energy Dispersive X-Ray (EDX) analysis

The EDX analysis in Fig 4.9 showed strong signals at 3.0 Kev characteristic of AgNPs.
4.3.5 Fourier Transform Infra-Red (FTIR) analysis

FTIR spectra for AgNPs synthesized using *A. digitata* fruit pulp extract (Fig 4.10) showed broad transmittance peak at 3436 cm\(^{-1}\) assigned to O-H bonds of phenols. Peaks at 2923 cm\(^{-1}\) for C-H stretching. The peak at 1733 cm\(^{-1}\) is associated with C=O of an acid or saturated ester. Peak at 1637 cm\(^{-1}\) –C=C-bond stretch probably for alkene. Peak at 1456 cm\(^{-1}\) for CH\(_3\) bending modes of end ethyl groups of proteins. Peak at 1091 cm\(^{-1}\) is attributed to C-O stretching of alcohols. The analysis suggests that proteins and phenols present in the *A. digitata* fruit pulp were responsible for capping and stabilizing the synthesized AgNPs (Kumar et al., 2016).

![FTIR spectra of A. digitata fruit pulp extract mediated AgNPs](image)

**Fig. 4.10:** FTIR spectra of *A. digitata* fruit pulp extract mediated AgNPs

4.3.6 Antibacterial activity of AgNPs synthesized using *A. digitata* fruit pulp

AgNPs synthesized using *A. digitata* fruit pulp via stationary method exhibited antibacterial activity against *E. coli* with inhibition zone of 17.9 ± 0.081 mm and those synthesized via sonication had inhibition zones of 16.2 ± 0.124 mm compared to the standard which had inhibition zone of 33.4 ± 0.443 mm. AgNPs synthesized on stationary exhibited antibacterial activity of 15.3 ± 0.458 mm while those synthesized via sonication had inhibition zone of 14.1 ± 0.086 mm on Gram positive bacteria (*S. aureus*). The
inhibition zones were smaller than those observed by Kumar et al., (2016) a similar investigation which had an inhibition zones of 22.5 ± 0.998 mm on E. coli and 15.9 ± 1.09 on S. aureus. This can be attributed to bigger size of AgNPs synthesized in this study (6 to 30 nm) compared to what Kumar et al., (2016) obtained (3 to 7 nm). Small sized AgNPs have more antibacterial effect than larger ones because they have large surface area to interact with the pathogens (Vanaja and Annadurai, 2013). The fruit pulp extract showed an inhibition zone of 6 mm. S. aureus was susceptible to the synthesized AgNPs while E. coli was average. The inhibition zones were recorded in table 4.2.

Table 4.2: Inhibition zones of AgNPs synthesized using A. digitata fruit pulp extract on E. coli and S. aureus

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>A. digitata fruit pulp extract AgNPs</td>
<td>Stationary</td>
<td>17.9±0.081&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. digitata fruit pulp extract AgNPs</td>
<td>Sonication</td>
<td>16.2±0.124&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>33.4±0.443&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt; solution</td>
<td></td>
<td>11.26±0.205&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>A. digitata fruit pulp extract</td>
<td></td>
<td>6&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable
i. Means in the above table followed by same letter are not significantly different at 95% confidence level.
ii. Means in the above table followed by different letter are significantly different (Tukey’s test P< 0.05) p values

The minimum Inhibitory concentration was determined by doing replicate tests. For E. coli it was 0.125 mM AgNPs while for S. aureus 0.25 mM AgNPs. The clear hollows in Plate 4.9 shows some zones of inhibition at different concentrations of AgNPs.
The clear hollows show zones of inhibition of different concentrations of AgNPs on *S. aureus*; 1-1 mM AgNPs, 2- 0.75 mM AgNPs, 3- 0.5 mM AgNPs, 4- 0.25 mM AgNPs and E- the plant extract.

### 4.4 Synthesis of AgNPs using *Prunus africana* bark extract

#### 4.4.1 Stationary biosynthesis of AgNPs using *P. africana* bark extract

Visual observation of a mixture of *P. africana* stem bark extract and silver nitrate solution when kept stationary, showed colour change from yellow to orange in 60 minutes then finally to brown after 120 minutes (Plate 4.10).
The UV-VIS spectra of the reaction media showed an absorption peak at $\lambda_{\text{max}}$ 448 nm as presented in Fig. 4.11. Broadening of the peak indicated the nanoparticles formed were polydispersed (Ramteke et al., 2012). The absorbance intensity increased with time and a maximum peak obtained after 4 hours.

![UV-VIS spectra of AgNPs using P. africana bark extract, stationary](image)

**Fig. 4.11:** UV-VIS spectra of AgNPs using *P. africana* bark extract, stationary

### 4.4.2 High Resolution Transmission Electron Microscope (HRTEM) analysis

The results of HRTEM microscopy investigation of the synthesized AgNPs using *Prunus africana* bark via stationary are presented in Plate 4.11. The HRTEM images (Plate 4.11 image A-E) showed spherical nanoparticles and were in the size range of 26 to 45 nm. The images showed the AgNPs were evenly distributed. Plate 4.11 Image F showed the surface of the nanoparticles was not uniform.
Plate 4.11: HRTEM images of *P. africana* bark extract mediated AgNPs via stationary method

The values of the sizes of the AgNPs obtained from the HRTEM are represented in Fig. 4.12. The sizes of the synthesized AgNPs ranged from 26 to 45 nm with a high number ranging from 36 to 40 nm. This shows the AgNPs synthesized using *P. africana* bark were monodispersed. This is the first time study on biosynthesis of AgNPs using *P. africana* is being reported.
4.4.3 Energy Dispersive X-Ray (EDX) analysis

The Energy Dispersive X-ray confirmed the presence of pure AgNP as shown in Fig. 4.13.
4.4.4 Ultrasound enhanced synthesis of AgNPs

The colour of the *P. africana* bark extract and AgNO$_3$ mixture changed from orange then darkened with time up to 120 minutes on sonication (Plate 4.12), thereafter no colour change was observed. The colour intensity increased with time due to formation of AgNPs. (Rai *et al.*, 2009)

Plate 4.12: Colour development of *P. africana* bark extract and AgNO$_3$ solution on sonication

The reaction was also monitored by UV-VIS spectrometer as shown in Fig. 4.14. The absorption peak was centered at $\lambda_{\text{max}}$ 425 nm characteristic of surface Plasmon resonance of AgNPs. The absorption intensity increased steadily up to 180 minutes and after that it remained the same. All the peaks were at 425 nm indicating the nanoparticles formed are of uniform size (Sondi *et al.*, 2003)

Fig. 4.14: UV-VIS spectra of *P. africana* bark and AgNO$_3$ with time by sonication
4.4.5 High resolution Transmission Electron Microscope (HRTEM) analysis

HRTEM micrographs analysis of *P. africana* AgNPs synthesized via sonication method showed evenly distributed spherical nanoparticles (Plate 4.13). The sizes ranged from 18 nm to 35 nm with mode being in the range of 20 to 25 nm and average size of 23±3.06 nm. From HRTEM analysis, AgNPs synthesized via sonication method were smaller than those synthesized via stationary method. The observation also concurs with UV-VIS analysis because AgNPs synthesized via stationary method had absorbance peaks at $\lambda_{\text{max}}$ 448 nm while those synthesized via sonication had absorbance peaks at $\lambda_{\text{max}}$ 425 nm. From studies done by Suriyakalaa *et al.*, (2013), UV-VIS absorbance of AgNPs increase from 400 nm to 450 nm with increase in size of the nanoparticles.

![HRTEM micrographs of AgNPs synthesized using *P. africana* bark extract via sonication method](image)

Plate 4.13: HRTEM micrographs of AgNPs synthesized using *P. africana* bark extract via sonication method
4.4.6 Selected Area Electron Diffraction (SAED) Analysis

The SAED micrograph presented in Plate 4.14 showed discrete shiny spots affirming the synthesized nanoparticles were crystalline.

Plate 4.14: SAED image of AgNPs synthesized using P. africana bark via sonication

From HRTEM analysis, AgNPs synthesized via sonication were smaller (23±3.06 nm) than those synthesized via stationary (36.75±3.6 nm) method. The results showed that, the size of the synthesized AgNPs using the two methods were significantly different since t calculated (3.307) was higher than t tabulated (2.06) at confidence level 95%. The observation also concurs with UV-VIS analysis because AgNPs synthesized using P. africana bark via stationary method had absorption peaks at $\lambda_{\text{max}}$ 448 nm while those synthesized via sonication had absorption peaks at $\lambda_{\text{max}}$ 425 nm. From studies done by Suriyakalaa, (2013), UV-VIS absorbance of AgNPs increase from 400 nm to 450 nm with increase in size of the nanoparticles.
4.4.7 Fourier Transform Infra-Red (FTIR) analysis

The FTIR spectra for AgNPs synthesized using *P. africana* bark (Fig. 4.15) showed peaks at 3275 cm\(^{-1}\) attributed to O-H stretching, and 1632 cm\(^{-1}\) which could be attributed to -C=C-stretching. The above data indicates the involvement of phenolic compounds as reducing and capping agents of AgNPs.

![FTIR spectra of P. africana bark extract mediated AgNPs](image)

*Fig. 4.15: FTIR spectra of *P. africana* bark extract mediated AgNPs*

4.4.8 Antibacterial activity of AgNPs synthesized using *Prunus africana* stem bark extracts

AgNPs synthesized using *P. africana* stem bark exhibited antibacterial activity against *E. coli* and *S. aureus* pathogens. On the Gram-positive bacteria (*S. aureus*), the *P. africana* stem bark mediated AgNPs by sonication showed inhibition of 16.03±0.204 mm which was significantly different from AgNPs synthesized via stationary which had inhibition zones of 16.97±0.206 mm. The positive control for *S. aureus* was vancomycin which had an inhibition zone of 22.5±0.443 mm. Inhibition zones indicate *S. aureus* was susceptible to AgNPs. On the Gram-negative bacteria (*E. coli*), the *P. africana* stem bark synthesized AgNPs via sonication showed inhibition zones of 14.2±0.208 mm and stationary showed
inhibition zones of 11.9±0.251 mm. The inhibition zones were significantly different at 95% confidence level. Positive control for \textit{E. coli} was ciprofloxacin which had an inhibition of 34.2±0.443 mm. \textit{E. coli} was both susceptible to AgNPs according to Interpretation of zones of inhibition using Bauer-Kirby Antibiotic Susceptibility testing (Bauer \textit{et al.}, 1966). However, more studies should be done to find the most effective concentration of AgNPs. The negative control (water) showed no inhibition. The silver nitrate solution showed inhibition zones of diameter 11.26±0.205 mm on \textit{E. coli} and 10.13±0.206 mm for \textit{S. aureus} which were smaller compared to the inhibition zones caused by AgNPs on the same pathogens. The \textit{P. africana} bark showed an inhibition of 9.03±0.128 mm on \textit{E. coli} and 7.97±0.124 mm on \textit{S. aureus}. The inhibition zones measured after 24 hours were recorded in table 4.3

\textbf{Table 4.3: Inhibition zones of AgNPs on \textit{E. coli} and \textit{S. aureus}}

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method for AgNPs preparation</th>
<th>Zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\textit{E. coli}</td>
</tr>
<tr>
<td>\textit{P. africana} bark AgNPs</td>
<td>Sonication</td>
<td>14.2±0.208 \textsuperscript{d}</td>
</tr>
<tr>
<td>\textit{P. africana} bark AgNPs</td>
<td>Stationary</td>
<td>11.9±0.251 \textsuperscript{e}</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>33.4±0.443 \textsuperscript{a}</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>-</td>
<td>N/A</td>
</tr>
<tr>
<td>Pure bark extract</td>
<td>-</td>
<td>9.03±0.128 \textsuperscript{h}</td>
</tr>
<tr>
<td>AgNO\textsubscript{3} solution</td>
<td>-</td>
<td>11.26±0.205 \textsuperscript{f}</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6\textsuperscript{j}</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

iii. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

iv. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

Some of the zones of inhibition of synthesized AgNPs on \textit{S. aureus} are displayed in Plate 4.15
Plate 4.15: Zones of inhibition of AgNPs against *S. aureus*

(A):- Inhibition zones from AgNPs synthesized by *P. africana* stem extract via stationary and (B):- via sonication

The clear hollows show inhibition zones caused by AgNPs of different concentrations; 1:- 1 mM AgNPs, 2:- 0.75 mM AgNPs, 3:- 0.5 mM AgNPs, 4:- 0.25 mM AgNPs and E - respective plant extract, S- vancomycin

On the antibacterial activity on *E. coli*, the ciprofloxacin (positive control) showed large clear hollow at the center. Zones of inhibition of the synthesized AgNPs on *E. coli* are shown in Plate 4.16

Plate 4.16: Zones of inhibition of AgNPs against *E. coli*

(A):- Zones of inhibition of AgNPs synthesized using *P. africana* stem bark extract via stationary and (B):- via sonication
The Minimum Inhibitory Concentration (MIC) for *S. aureus* was 0.25 mM for stem bark synthesized AgNPs. The Minimum inhibitory concentration for *E. coli* was 0.25 mM for the synthesized AgNPs.

4.5 Biosynthesis of AgNPs using *Prunus africana* leaves extract

4.5.1 Stationary method

The *P. africana* leaves extract and silver nitrate solution changed from yellow to orange then dark brown in 120 minutes (Plate 4.17) indicating formation of AgNPs.

![Plate 4.17: *P. africana* leaves extract in 0.001M AgNO₃ solution reaction mixture at (i) 0 minute (ii) 60 minutes (iii) 120 minutes](image)

UV-VIS spectra showed absorption peaks at $\lambda_{\text{max}}$ 434 nm and 437 nm (Fig 4.16). The absorbance increased steadily up to five hours, after which there was no further change. Increase in absorbance with time was due to an increase in concentration of the AgNPs. Broadening of the UV-VIS peaks indicated the nanoparticles were polydispersed as shown in the study of the synthesis of AgNPs using papaya fruit extract (Jain *et al.*, 2009).
Fig. 4.16: UV-VIS spectra of AgNPs synthesized using *P. africana* leaves, Stationary method

4.5.2 Ultrasonic enhanced synthesis of AgNPs using *P. africana* leaves

The *P. africana* leaves extract and silver nitrate solution changed from yellow to orange within 30 minutes then to dark brown after 60 minutes under sonication (Plate 4.18) indicating formation of AgNPs.

Plate 4.18: *P. africana* leaves extract and 0.001M AgNO₃ solution at (i) 0 minute (ii) 30 minutes (iii) 60 minutes
The spectra of reaction mixture at different times is shown in Fig. 4.17. The wavelength of maximum absorption was at $\lambda_{\text{max}}$ 425 nm, due to surface Plasmon resonance AgNPs. The absorption peak increased in intensity up to 180 minutes and remained the same thereafter an indication that the reaction was complete. The increase in the intensity of the peak indicated increase in the concentration of the AgNPs.

![UV-VIS spectra of AgNPs from P. africana leaves at different time intervals](image)

**Fig. 4.17: UV-VIS spectra of AgNPs from P. africana leaves at different time intervals**

### 4.5.3 High Resolution Scanning Electron Microscope analysis

High Resolution Scanning Electron Microscope (HRSEM) analysis was done using a Zeiss field Emission Gun SEM operated with SMART SEM version 5.04 software on AgNPs synthesized using *P. africana* leaves. The HRSEM analysis showed evenly distributed spherical nanoparticles (Plate 4.19).
Plate 4.19: HRSEM image of synthesized AgNPs synthesized using *P. africana* leaves

Fig 4.18 shows the particle size histogram derived from the SEM image. The size of the nanoparticles ranges from 10 to 34 nm with average size of the nanoparticles at 22.33±3.078 nm.

Fig. 4.18: Particle size distribution of AgNPs determined from HRSEM micrograph

Both HRTEM and HRSEM analysis on AgNPs from *P. africana* showed they are spherical in shape and monodispersed. There was a significant difference in their average sizes.
4.5.4 Fourier Transform Infra-Red (FTIR) analysis

The FTIR spectra for AgNPs synthesized using *P. africana* leaves (Fig. 4.19) showed peaks at 3436 cm\(^{-1}\) attributed to O - H stretching, 2925 cm\(^{-1}\) of C - H interlayer stretching, -1627 cm\(^{-1}\) and 1329 cm\(^{-1}\) could be attributed to C=\(\equiv\)C- stretching for benzene ring and absorption at 1075 cm\(^{-1}\) could be C-O stretch. The absorption could be due to functional groups of phenolic compounds involved in capping and stabilizing the AgNPs.

![FTIR spectra of *P. africana* leaves extract mediated AgNPs](image)

**Fig. 4.19:** FTIR spectra of *P. africana* leaves extract mediated AgNPs

4.5.5 Antibacterial activity of AgNPs synthesized

AgNPs synthesized using *P. africana* exhibited antibacterial activity on *E. coli* and *S. aureus* pathogens. On the Gram-positive bacteria *S. aureus*, the *P. africana* mediated AgNPs showed inhibition of 17±0.408 mm and 17.06±0.178 mm. These inhibition zones of AgNPs synthesized via stationary and sonication method had no significant difference. The positive control had an inhibition zone of 22.5±0.988 mm. The *S. aureus* were susceptible to AgNPs. The Minimum Inhibitory Concentration (MIC) for *S. aureus* was 0.25 mM.
On the Gram-negative bacteria, the *P. africana* synthesized AgNPs showed inhibition zone of 17±0.141 mm and 19.06±0.100 mm for stationary and sonication AgNPs respectively, compared to the standard which had an inhibition zone of 33.4±0.443. Inhibition zones indicate that *E. coli* was both susceptible and intermediate to AgNPs according to interpretation of zones of inhibition using Bauer-Kirby Antibiotic Susceptibility testing (Bauer *et al.*, 1966). The Minimum inhibitory concentration for *E. coli* was 0.125 mM for the synthesized AgNPs. The negative control (water) did not show any inhibition. The *P. africana* leaves extract showed an inhibition of 10.23±0.015 mm on *E. coli* and 8.36±0.125 mm on *S. aureus*. Zones of inhibition measured were recorded in table 4.4

**Table 4.4: Inhibition zones of AgNPs on *E. coli* and *S. aureus***

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method for AgNPs preparation</th>
<th>Zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>P. africana</em> leaf AgNPs</td>
<td>Sonication</td>
<td>19.06±0.100&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. africana</em> leaf AgNPs</td>
<td>Stationary</td>
<td>17±0.141&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>-</td>
<td>33.4±0.443&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>N/A</td>
<td>22.5±0.988&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative control</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pure leaf extract</td>
<td>10.23±0.015&lt;sup&gt;f&lt;/sup&gt;</td>
<td>8.36±0.125&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>AgNO&lt;sub&gt;3&lt;/sub&gt; solution</td>
<td>-</td>
<td>11.26±0.205&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

1. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

2. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

The observed results concur with literature that AgNPs can release Ag<sup>+</sup> which may interact with thiol groups of enzymes and deactivate them (Matsumura *et al.*, 2003). Silver ions work by suppression of respiratory enzymes and electron transport components which
interfere with DNA functions (Li et al., 2006). Some of the zones of inhibition of synthesized AgNPs on *S. aureus* are displayed in Plate 4.20

(A) AgNPs synthesized using *P. africana* leaves extract via sonication (B) via standing

The clear hollows show inhibition zones caused by AgNPs of different concentrations; 1:- 1 mM AgNPs, 2:- 0.75 mM AgNPs, 3:- 0.5 mM AgNPs, 4:- 0.25 mM AgNPs and E - *P. africana* leaves extract, S- vancomycin

Some zones of inhibition against *E. coli* are shown on Plate 4.21. The clear hollows indicate the inhibition zones.

Plate 4.21: Zones of inhibition of against *E. coli*

Plate 4.21 shows inhibition zones caused by AgNPs of different concentrations; 1:-1mM AgNPs, 2:- 0.75 mM AgNPs, 3:- 0.5mM AgNPs, 4:- 0.25mM AgNPs on *E.coli*
4.6 Synthesis of AgNPs using *Bridelia micrantha* bark extract

4.6.1 Stationary synthesis of AgNPs using

The *B. micrantha* stem bark extract and silver nitrate solution changed from red brown to dark brown after 120 minutes indicating formation of AgNPs (Plate 4.22).

![After 120 minutes](image)

Plate 4.22: *Bridelia micrantha* bark extract and AgNO$_3$ solution on stationary at (i) 0 minute (ii) 120 minutes

The UV-VIS spectra shown in Fig 4.20 represents absorption spectra of AgNPs at different time intervals of their formation. The maximum peak of absorption appeared at $\lambda_{\text{max}}$ 426 nm an indication of formation of AgNPs. The absorbance peaks were broad, an indication the synthesized nanoparticles could be polydisperse. Broad absorption peaks of the UV-VIS spectra is an indication of polydispersed AgNPs as observed in the study of Green synthesis of robust, AgNPs using garlic extract (Von White *et al.*, 2012). *B. micrantha* has not been used to synthesize AgNPs before.
Fig. 4.20: UV-VIS spectra on formation of AgNPs using *B. micrantha* bark extract when stationary

4.4.2: Ultrasonic enhanced biosynthesis of AgNPs using *B. micrantha* bark extract

The reaction medium of *B. micrantha* stem bark extract and silver nitrate solution changed from red brown to dark brown in 60 minutes an indication of formation of AgNPs (Plate 4.23).

Plate 4.23: *B. micrantha* bark extract and AgNO₃ solution on sonication (i) 0 minute (ii) 60 minutes of sonication

The UV-VIS absorption spectra of the synthesized nanoparticles using *B. micrantha* bark extract showed peaks at $\lambda_{\text{max}}$ 431 nm throughout the sonication period (Fig. 4.21). The UV-VIS peaks at the same wavelength (431 nm) indicated that, the nanoparticles were dispersed in the solution with no ability to aggregate. Similar observation was made on the
study of photo-chemically grown AgNPs with wavelength-controlled size and shape (Callegari et al., 2003). The absorbance or optical density increased with time up to 90 minutes. Increase in optical density of the solution suggest conversion of Ag$^+$ to Ag$^0$ forming nanoparticles (Maillard et al., 2003). Maximum absorbance was obtained after 90 minutes indicating that, no further reaction is taking place. The UV-VIS spectra for AgNPs of B. micrantha on stationary had peaks at $\lambda_{max}$ 426 nm while on sonication absorbance peaks were at $\lambda_{max}$ 431 nm. This showed that the size of the synthesized nanoparticles via stationary and sonication using B. micrantha had almost the same size though the rate of formation via sonication was faster (90 minutes) than stationary (240 minutes).

Fig. 4.21: UV-VIS spectra on formation of AgNPs using B. micrantha bark extract on sonication

4.6.3 High Resolution Transmission Electron Microscope (HRTEM) analysis

The High-Resolution Transmission Electron Microscope of the images of the synthesized AgNPs using B. micrantha bark via sonication are shown in Plate 4.24. The sizes of the AgNPs vary from 10 to 26 nm. They had non-uniform surface, quasi spherical nanoparticles and were evenly distributed.
Plate 4.24: Transmission Electron Microscope micrographs of the *B. micrantha* mediated AgNPs via sonication method

The size distribution of the AgNPs determined from HRTEM analysis are shown in the Fig 4.22. The size ranges from 10 to 25 nm with mode range being 14 to 17 nm. The average diameter of the nanoparticles was 16.07±3.192 nm. The narrow range of the size indicated the nanoparticles were monodispersed.

![Histogram of AgNPs sizes](image)

**Fig. 4.22:** Size distribution of AgNPs synthesized using *B. micrantha* under sonication
4.6.4 Scanning Area Electron Diffraction (SAED) Analysis

The SAED image showed discrete shiny rings confirming crystalline nature of the AgNPs (Plate 4.25).

Plate 4.25: SAED micrograph of B. micrantha mediated AgNPs under sonication

4.6.5 Energy Dispersive X-Ray (EDX) Analysis

The EDX spectrum shown in Fig 4.23 had overlaying peaks at 3.0 Kev confirming the synthesized nanoparticles were of silver.
Fig. 4.23: EDX spectrum of AgNPs synthesized using *B. micrantha* under sonication

4.6.6 Fourier Transform Infra-Red (FTIR) analysis

For AgNPs synthesized using *B. micrantha*, FTIR analysis showed bands at 3263.35 cm\(^{-1}\) represent O-H bond stretching probably for phenols and at 1635 cm\(^{-1}\) corresponding to C=C bonds of aromatic ring (Fig. 4.24).
Fig. 4.24: FTIR Spectrum of *B. micrantha* mediated synthesized AgNPs

4.6.7 Antibacterial activity of *B. micrantha* mediated synthesized AgNPs

AgNPs synthesized using *B. micrantha* showed antibacterial activity on both Gram-negative and Gram-positive bacteria. The zones of inhibition measured after 24 hours of inoculation were recorded on table 4.5

**Table 4.5: Inhibition zones of AgNPs on *E. coli* and *S. aureus***

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>B. micrantha</em> bark AgNPs</td>
<td>Stationary</td>
<td>17.37±0.047d</td>
</tr>
<tr>
<td><em>B. micrantha</em> bark AgNPs</td>
<td>Sonication</td>
<td>16.13±0.098e</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>33.4±0.542a</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6h</td>
</tr>
<tr>
<td>AgNO₃ solution</td>
<td></td>
<td>11.26±0.205f</td>
</tr>
<tr>
<td><em>B. micrantha</em> stem bark extract</td>
<td>N/A</td>
<td>10.5±0.012g</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test *P*< 0.05) *p* values
On *S. aureus* the AgNPs synthesized via stationary method had inhibition zones of 18.50±0.082 mm while those synthesized by sonication had inhibition zone of 19.05±0.0481. *S. aureus* was susceptible to the AgNPs by comparing the zone of inhibition to the standard which had an inhibition of 22.5±0.988 mm. The minimum inhibitory concentration of synthesized AgNPs on *S. aureus* was 0.125 mM. On Gram-negative bacteria (*E. coli*), AgNPs formed via stationary and under sonication had 17.37±0.047 mm and 16.13±0.098 mm, inhibition zones respectively compared to the standard which had inhibition zone of 33.4±0.542 mm. The MIC for *E. coli* was 0.125 mM. The *B. micrantha* extract showed inhibition zones of 11.0±0.002 mm on *S. aureus* and 10.5±0.012 mm on *E. coli*. The high inhibition zone on *S. aureus* can be attributed to the synergistic effect of the *B. micrantha* extract and Ag NPs.

**4.7 Biosynthesis of AgNPs using *W. ugandensis* bark extract**

**4.7.1 Stationary biosynthesis of AgNPs using *W. ugandensis* bark extract**

A mixture of 15 ml of the plant extract and 135ml of 0.001M AgNO₃ solution was left stationary/ standing on the bench. The reaction medium changed from yellow to dark brown within 120 minutes indicating formation of AgNPs (Plate 4.26). The colour change was due to silver surface Plasmon resonance of AgNPs (Eustis and El-Sayed, 2006).
Plate 4.26: Mixture of *Warburgia ugandensis* bark extract and AgNO₃ solution at (i) 0 time (ii) after 120 minutes

The UV-VIS spectrum of AgNPs formed using *W. ugandensis* bark and AgNO₃ showed peaks at $\lambda_{\text{max}}$ 435nm to 439nm. The absorbance increased steadily with time indicating an increase in the concentration of AgNPs synthesized (Fig 4.25). The maximum absorbance was obtained after 180 minutes. The peaks were broad representing polydisperse AgNPs. This is the first time AgNPs synthesized from *W. ugandensis* are being reported.

Fig. 4.25: UV-VIS spectra of AgNPs using *W. ugandensis* bark extract on stationary method
4.7.2 Ultrasound enhanced synthesis of AgNPs using *W. ugandensis* bark extract

The *W. ugandensis* bark extract and silver nitrate solution changed from yellow to dark brown in 60 minutes during sonication (Plate 4.27). After 60 minutes no further colour change was observed. Due to excitation of surface Plasmon vibration in metal nanoparticles, AgNPs show brown colour in water (Ahmad *et al.*, 2010).

**Plate 4.27: Colour change of *W. ugandensis* bark extract and AgNO$_3$ mixture**

(i) 0 minutes (ii) 60 minutes on sonication

Formation of the AgNPs was monitored by UV-VIS spectrometer at regular intervals and had absorption peaks at $\lambda_{\text{max}}$ 448 nm which shifted slightly to 442 nm during sonication (Fig. 4.26). The slight shift of the maximum absorbance is an indication that there was no significant difference in the size of the synthesized nanoparticles with time as previously observed on the study of synthesis of uniform AgNPs with a controllable size (Dadosh, 2009). The blue shift is an indication of decrease in size of AgNPs under sonication. From the result, the reduction of the Ag$^+$ to form Ag$^0$ took 90 minutes to completion. Peaks observed on sonication were shaper than on stationary, an indication that the size range for AgNPs synthesized via sonication is narrow than the size range of those formed via stationary.
Fig. 4.26: UV-VIS spectra of *W. ugandensis* extract synthesized AgNPs on sonication

4.7.3 High Resolution Transmission Electron Microscope (HRTEM) analysis

The HRTEM analysis showed the synthesized nanoparticles via sonication were quasi-spherical (Plate 4.28 image A-E). They were evenly distributed and their sizes were in the range of 10 to 34 nm. The quasi spherical AgNPs had smooth surface as portrayed in Plate 4.28 image F.
Plate 4.28: HRTEM images of AgNPs synthesized using *W. ugandensis*

The sizes of the synthesized nanoparticles via sonication ranged from 10 to 34 nm with modal class being in the range of 20 to 24 nm (Fig 4.27). This indicated that, the nanoparticles are monodispersed. The average size of the nanoparticles synthesized using *W. ugandensis* by Stationary method was $22.5\pm4.74$ nm.

Fig. 4.27: Size distribution of AgNPs synthesized using *W. ugandensis*
4.7.4 Scanning Area Electron diffraction (SAED) analysis

Scanning Area Electron diffraction (SAED) showing shiny discrete spots (Plate 4.29) affirmed crystallinity of the AgNPs (Pandey and Ramontja, 2016).

Plate 4.29: SAED image of AgNPs synthesized using W. ugandensis extract

4.7.5 The Energy Dispersive X-ray (EDX) analysis

The EDX spectrum (Fig. 4.28) confirmed the presence of AgNPs with peaks at 3.0 KeV characteristic of AgNPs.
Fig. 4.28: EDX spectrum of AgNPs

4.7.6 Fourier Transform Infra-Red (FTIR) analysis

FTIR analysis for AgNPs synthesized using *W. ugandensis* bark extract (Fig 4.29) showed strong peaks at 3280 cm\(^{-1}\) due to O-H stretch and at 1636 cm\(^{-1}\) corresponding to C=C stretching. Polyphenolic compounds could be involved in capping of the nanoparticles.
Fig. 4.29: FTIR Spectrum of *W. ugandensis* mediated synthesized AgNPs

4.7.7 Antibacterial activity of *W. ugandensis* mediated synthesized AgNPs

Clear hollows were observed indicating the synthesized AgNPs inhibited the growth of the micro-organisms. On *E. coli*, *W. ugandensis* mediated AgNPs synthesized via stationary and sonication showed inhibition zones of 18.87±0.544 mm and 16.03±0.125 mm respectively. The inhibition zones were significantly different at 95% confidence level. The positive control (ciprofloxacin) had inhibition zone of 33.4±0.443 mm. On the Gram-positive bacteria, inhibition zones were 18.13±0.047 and 15.0±0.081 mm for AgNPs synthesized via stationary and sonication method respectively. The positive control had inhibition zone of 22.5±0.988 mm. *S. aureus* was susceptible to the AgNPs. Zones of inhibition were recorded in table 4.6
Table 4.6: Inhibition zones on *E. coli* and *S. aureus*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Zones of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>E. coli</em></td>
</tr>
<tr>
<td><em>W. ugandensis</em> bark AgNPs</td>
<td>Stationary</td>
<td>18.87±0.544&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>W. ugandensis</em> bark AgNPs</td>
<td>Sonication</td>
<td>16.03±0.125&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>33.4±0.443&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>N/A</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6.000&lt;sup&gt;i&lt;/sup&gt;</td>
</tr>
<tr>
<td>AgNO₃ solution</td>
<td></td>
<td>11.26±0.205&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>W. ugandensis</em> bark extract</td>
<td></td>
<td>9.00±0.000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

The minimum Inhibitory concentration determined for *E. coli* was – 0.0625mM AgNPs while for *S. aureus* was 0.125 mM.

4.8. Biosynthesis of AgNPs using *Urtica dioica* roots extract

4.8.1 Ultrasound enhanced biosynthesis of AgNPs using *U. dioica*

The *U. dioica* and silver nitrate solution changed from light yellow to shinny brown in 30 minutes and finally to brown after one hour (Plate 4.30). The colour change was an indication of formation of AgNPs.
Plate 4.30: Colour development of *U. dioica* roots extract and AgNO₃ mixture at (i) 0 minute (ii) 30 minutes (iii) 60 minutes on sonication

AgNPs UV-VIS spectra (Fig 4.30) showed absorption peaks at $\lambda_{\text{max}}$ 421 nm with a red shift to 426 nm during progression of the reaction. The red shift indicated slight increase in size due to agglomeration of the nanoparticles. A similar trend was observed by Von *et al.*, (2012) on synthesis of AgNPs using *Allium sativum* (garlic) as a reducing agent. The size of spectra increased for one hour then broadened. Broadening of the peak and the low absorbance indicated agglomeration of the nanoparticles as also shown by HRSEM images.
Fig. 4.30: UV-VIS spectra of U. dioica roots extract synthesized AgNPs on sonication

4.8.2 High Resolution Scanning Electron Microscope (HRSEM) Analysis

High Resolution Scanning Electron Microscope showed that, AgNPs formed aggregated to form a cluster (Plate 4.31). This is an indication that. U. dioica phytochemicals were able to reduce Ag\(^+\) to form Ag\(^0\) but the capping agents were an able to prevent agglomeration.

Plate 4.31: HRSEM micrograph of U. dioica leaves mediated AgNPs
4.8.3 Fourier Transform Infra-Red (FTIR) analysis

The FTIR spectrum of *U. dioica* mediated synthesized AgNPs showed peaks at 3426 cm⁻¹, 1637 cm⁻¹ and 1384 cm⁻¹ (Fig.4.31) These peaks represent O-H bonds, C=C bonds of aromatic rings and C-N stretching vibrations of primary amine respectively.

![FTIR spectrum of U. dioica mediated synthesized AgNPs](image)

**Fig. 4.31: FTIR Spectrum of *U. dioica* mediated synthesized AgNPs**

4.8.4 Antibacterial activity of AgNPs synthesized using *U. dioica*

The antibacterial activity of *U. dioica* mediated AgNPs done against pathogenic bacteria *E. coli* and *S. aureus* showed clear hollows an indication that, the synthesized AgNPs inhibited the growth of the micro-organisms (Plate 4.32). The nanoparticles showed inhibition zones for *S. aureus* of 19.8±0.163 mm, compared to the standard which had inhibition zone of 22.5±0.988 mm. The *S. aureus* was susceptible to the AgNPs. For *E. coli*, an inhibition zone of 14.8±0.206 mm was recorded while the standard had 33.4±0.443 mm. The big difference in the inhibition zone of the standard and AgNPs means *E. coli* was intermediate to the AgNPs. The root extracts showed inhibition zones of 8.2±0.126 and 9.1±0.241 mm on *E. coli* and *S. aureus* respectively, while the pure silver nitrate
showed an inhibition zone of an average 12mm. This confirms that the AgNPs were responsible for the high inhibition zones. The inhibition zones were recorded in table 4.7.

**Table 4.7: Inhibition zones of AgNPs synthesized using *U. dioica* roots extract on *E. coli* and *S. aureus***

<table>
<thead>
<tr>
<th>Sample</th>
<th>Method</th>
<th>Zones of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. dioica</em> roots AgNPs</td>
<td>Sonication</td>
<td>14.8±0.206d, 19.8±0.163e</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td>33.4±0.443a, N/A</td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td>22.5±0.988b</td>
</tr>
<tr>
<td>Negative control</td>
<td></td>
<td>6j, 6j</td>
</tr>
<tr>
<td>AgNO₃ solution</td>
<td></td>
<td>11.26±0.205e, 10.13±0.206f</td>
</tr>
<tr>
<td><em>U. dioica</em> roots extract</td>
<td></td>
<td>8.2±0.126h, 9.1±0.241g</td>
</tr>
</tbody>
</table>

NB: N/A- not applicable

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

The Minimum inhibitory concentration (MIC) for *E. coli* was found to be 0.125mM for *U. dioica* AgNPs while MIC for *S. aureus* was 0.25 mM. In Plate 4.32, the clear hollows on the petri dish show inhibition zones caused by the activity of the AgNPs on *S. aureus*. The observation was in accordance with prior observation that AgNPs inhibits growth of bacterial (Kim *et al.*, 2011).
4.9 Synthesis of AgNPs using *U. dioica* leaves

On synthesis of AgNPs using *U. dioica* leaves, there was no observable colour change of the reaction medium even after keeping it for a week. Upon performing the UV-VIS scan, there was no observable peak shown in the UV-VIS spectra in the range of 350 nm to 450 nm, an indication that AgNPs were not formed (Fig 4.32). However, Kumari *et al.*, (2015) had reported the synthesis of AgNPs using *U. dioica* leaves in the size range of 20 to 30 nm and showed absorption peaks at $\lambda_{\text{max}}$ 414 nm, but in this study AgNPs were not formed.
4.10 Comparison on the sizes and morphology of the AgNPs

AgNPs synthesized using *A. digitata* leaves synthesized via sonication method had the smallest size of 13.2±2.6 nm, followed by *B. micrantha* 16.07±3.172 nm. The largest AgNPs were obtained using *P. africana* bark via stationary method of average size 36.75±3.6 nm.

Table 4.8 shows the various sizes, shapes and morphology of the AgNPs synthesized using different plants.

**Table 4.8: Sizes, shapes and morphology of the AgNPs**

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part</th>
<th>Method</th>
<th>Shape</th>
<th>Morphology</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. digitata</em></td>
<td>Leaves</td>
<td>Sonication</td>
<td>Spherical</td>
<td>Non-uniform</td>
<td>13.2±2.6&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. digitata</em></td>
<td>Fruit pulp</td>
<td>Stationary</td>
<td>Spherical</td>
<td>Non-uniform</td>
<td>19.8±5.93&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. africana</em></td>
<td>Stem bark</td>
<td>Stationary</td>
<td>Spherical</td>
<td>Non-uniform</td>
<td>36.75±3.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. africana</em></td>
<td>Stem bark</td>
<td>Sonication</td>
<td>Spherical</td>
<td>Non-uniform</td>
<td>23±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>P. africana</em></td>
<td>Leaves</td>
<td>Stationary</td>
<td>Spherical</td>
<td>Uniform</td>
<td>22.33±3.078&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>B. micrantha</em></td>
<td>Stem bark</td>
<td>Sonication</td>
<td>Quasi-spherical</td>
<td>Uniform</td>
<td>16.07±3.172&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>W. ugandensis</em></td>
<td>Stem bark</td>
<td>Sonication</td>
<td>Quasi-spherical</td>
<td>Uniform</td>
<td>22.5±4.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.
ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values.

The results showed that the sizes of the AgNPs synthesized using different plants were significantly different. This concurs with literature that different plants produce nanoparticles of different sizes (Okafor et al., 2013). AgNPs synthesized using A. digitata leaves and fruit pulp had average size of 13.2±2.6 nm and 19.8±5.93 nm respectively which is significantly different at 95% confidence level. This shows that different plant parts can also produce AgNPs of significant different sizes.

4.11 Comparison on the antibacterial activity of AgNPs

The antibacterial activity of the AgNPs on E. coli was compared to determine if there was any significant difference and the results are tabulated in table 4.9.

Table 4.9 Inhibition zones of the synthesized AgNPs on E. coli

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part</th>
<th>Method</th>
<th>Inhibition zones (mm) E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. digitata</td>
<td>Leaves</td>
<td>Sonication</td>
<td>17.1±0.130^c</td>
</tr>
<tr>
<td></td>
<td>Fruit pulp</td>
<td>Stationary</td>
<td>17.9±0.081^b</td>
</tr>
<tr>
<td>P. africana</td>
<td>Stem bark</td>
<td>Stationary</td>
<td>11.9±0.251^f</td>
</tr>
<tr>
<td></td>
<td>Stem bark</td>
<td>Sonication</td>
<td>14.2±0.208^e</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Stationary</td>
<td>19.06±1.00^a</td>
</tr>
<tr>
<td>B. micrantha</td>
<td>Stem bark</td>
<td>Sonication</td>
<td>16.03±0.125^d</td>
</tr>
<tr>
<td>W. ugandensis</td>
<td>Stem bark</td>
<td>Sonication</td>
<td>16.13±0.098^d</td>
</tr>
<tr>
<td>U. dioica</td>
<td>Roots</td>
<td>Sonication</td>
<td>14.8±0.206^e</td>
</tr>
</tbody>
</table>

i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values.

The results of the study showed that, antibacterial activity of AgNPs synthesized using different plants was significantly different. AgNPs synthesized P. africana leaves had the best antibacterial activity on E. coli of 19.06±1.00mm. The lowest inhibition was shown by those synthesized using P. africana bark on stationary method (11.9±0.251mm). This
observation can be attributed to the difference in size of the AgNPs synthesized. The inhibition zones of all the AgNPs were significantly different except for B. micrantha (16.03±0.125mm) and W. ugandensis (16.13±0.098mm) which had no significant difference.

Antibacterial activity of AgNPs on S. aureus was also compared to determine if there was any significant difference in the inhibition zones. The results were tabulated in table 4.10

Table 4.10: Inhibition zones of the synthesized AgNPs on S. aureus

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part</th>
<th>Method</th>
<th>Inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. digitata</td>
<td>Leaves</td>
<td>Sonication</td>
<td>12.9±0.082f</td>
</tr>
<tr>
<td></td>
<td>Fruit pulp</td>
<td>Stationary</td>
<td>13.3±0.45f</td>
</tr>
<tr>
<td>P. africana</td>
<td>Stem bark</td>
<td>Stationary</td>
<td>16.97±0.205c</td>
</tr>
<tr>
<td></td>
<td>Stem bark</td>
<td>Sonication</td>
<td>16.03±0.204d</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>Stationary</td>
<td>17.0±0.408c</td>
</tr>
<tr>
<td>B. micrantha</td>
<td>Stem bark</td>
<td>Sonication</td>
<td>15.0±0.081c</td>
</tr>
<tr>
<td>W. ugandensis</td>
<td>Stem bark</td>
<td>Sonication</td>
<td>19.05±0.048b</td>
</tr>
<tr>
<td>U. dioica</td>
<td>Roots</td>
<td>Sonication</td>
<td>19.8±0.163a</td>
</tr>
</tbody>
</table>

NB:
i. Means in the above table followed by same superscripted letter are not significantly different at 95% confidence level.

ii. Means in the above table followed by different superscripted letter are significantly different (Tukey’s test P< 0.05) p values

AgNPs synthesized using U. doica showed the highest activity against S. aureus followed by those synthesized W. ugandensis with inhibition zones of 19.8±0.163mm and 19.05±0.048mm respectively. The inhibition zones of AgNPs synthesized using different plant extracts were significantly different.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i. Biosynthesis of AgNPs was achieved through rapid, cheap and eco-friendly methods using locally available Kenyan medicinal plants. The synthesized nanoparticles were of spherical shape and quasi spherical. They were crystalline as revealed by EDX analysis and their sizes ranged from 5 nm to 45 nm with no agglomeration as portrayed in HRTEM analysis. UV- VIS spectrophotometer was used to monitor the formation of the AgNPs and this technique proved that, their absorbance ranged from 400 to 450 nm. The variation of the wavelength of maximum absorbance was due to difference in size or morphology of the nanoparticles, where *Prunus africana* bark had absorption at 448 with a size of size was 36.75±3.6 nm while *Adansonia digitata* leaves absorption at 425 nm, their size was 13.2±2.6 nm.

ii. The study confirms that, the different medicinal plant extracts have the ability to mediate synthesis of AgNPs of significantly different sizes. The time taken for synthesis of AgNPs at the same conditions vary from one plant to the other. *Adansonia digitata* fruit pulp AgNPs synthesis took the shortest time of 30 minutes while *Prunus africana* leaves AgNPs took the longest time (300 minutes).

iii. The study also shows that the rate of synthesis was faster via sonication method than stationary method. *W. ugandensis* AgNPs synthesized via stationary method took 3 hours while via sonication it took 90 minutes. FTIR spectra showed that O-H stretch and –C=C- functional groups were responsible for the reduction and
capping the AgNPs. Moreover, the results showed that *Escherichia coli* and *staphylococcus aureus* bacteria were susceptible to the synthesized AgNPs. There were higher inhibition zones with the synthesized AgNPs than the plant extracts, example; *A. digitata* AgNPs showed an inhibition zones of 17.9±0.081mm on *E. coli* while the *A. digitata* fruit extract had an inhibition zone of 6mm.

iv. The study proves that, this non-toxic AgNPs prepared by simple, cheap and ecofriendly methods with ability to be scaled up can be used to formulate new drugs for medical use.

5.2 Recommendations

i. Biosynthesized AgNPs should be used to formulate new bacteriacidal drugs.

ii. Local Kenyan medicinal plants can be used in the synthesis of AgNPs.

5.2.2 Areas for further research

i. The exact mechanism for the action of AgNPs on bacteria should be studied.

ii. Studies should be done on anti-oxidant properties of the AgNPs.

iii. The specific biomolecules that contributed towards reduction and capping of AgNPs should be studied and characterized

iv. Antifungal activity of the AgNPs to be studied.

v. The action of AgNPs on killer viruses like HIV should also be studied.
REFERENCES


Rajoiya, P. (2017). Green synthesis of AgNPs, their characterization and antimicrobial potential. Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, Sam Higginbottom University of Agriculture, Technology & Sciences Allahabad-211007, UP (India).


APPENDIX

Samples

A. digitata fruit pulp  
B. micrantha leaves  

Prunus africana leaves  
W. ugandensis leaves