GREEN SYNTHESIS OF SILVER NANOPARTICLES USING
*Citrullus lanatus* RIND EXTRACT AS A REDUCTANT FOR
SELECTED ANTIBACTERIAL APPLICATIONS

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the Degree of Master of Science (Applied Analytical Chemistry) in the School of
Pure and Applied Sciences of Kenyatta University

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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To my wife, Elizabeth Mbatha, your support, encouragement and understanding made it possible.
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<tr>
<td>AgNPs</td>
<td>Silver nanoparticles</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>CGA</td>
<td>Chlorogenic Acid</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic Voltammetry</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>MBC</td>
<td>Minimal Bactericidal Concentration</td>
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<tr>
<td>MIC</td>
<td>Minimal Inhibitory Concentration</td>
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<td>TEM</td>
<td>Transmission Electron Microscopy</td>
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<td>WMRE</td>
<td>Watermelon Rind Extract</td>
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ABSTRACT

Silver nanoparticles (AgNPs) are the most studied metal nanoparticles due to their ever growing range of applications in areas such as chemical sensing, nanomedicine and electronics which has led to their increased demand. The AgNPs synthesis involves the use of hazardous reagents and toxic solvents. There is need to develop methods AgNPs that use environmentally benign reagents and solvents. This research work reports a green method where AgNPs were synthesized using silver nitrate as a precursor with aqueous extract of *Citrullus lanatus* fruit rind as the reductant as well as capping agent. The optimized conditions for AgNPs synthesis were a temperature of 80 °C, pH 10, 0.001 M AgNO₃, 250 g/L watermelon rind extract (WMRE), and a reactant ratio of 4:5 (AgNO₃:WMRE). The resultant AgNPs were characterized by Ultra violet–Visible (UV/Vis) Spectroscopy, Cyclic Voltammetry (CV) and Transmission Electron Microscopy (TEM). UV/Vis results showed a $\lambda_{\text{max}}$ at 404 nm which is consistent with the spectra of spherical AgNPs within the wavelength range of 380-450 nm. CV results showed a distinct oxidation peak at +291 mV while the standard reference AgNPs (20 nm diameter) oxidation peak occurred at +290 mV and TEM micrographs revealed spherical shaped AgNPs. The AgNPs were found to have an average diameter of 17.96 nm. Their antimicrobial activity against clinical isolates of *Escherichia coli* (E.coli) and *Salmonella typhi* (S.typhi) were evaluated using the disc-diffusion method. The Minimum Inhibition Concentration of the nanoparticles was found to be 45.00 ± 0.01 µg/mL for S.typhi and 38.50 ± 0.00 µg/ml for E.coli while the Minimum Bactericidal Concentration was found to be 60.00 ± 0.05 µg/ml for S.typhi and 50.00 ± 0.00 µg/ml for E.coli.
CHAPTER ONE

INTRODUCTION

1.1 Background Information

Nanotechnology is a field with main focus on materials synthesis at nanometre ($10^{-9}$ m) scale. Major advancement of mankind has usually been preceded by the development of technological innovation. Nanotechnology promises to be the next frontier of mankind’s technological advancement. For nearly half a century since the use of the word “Nanotechnology” by Richard Feynman in his lecture dubbed, “There is plenty of room at the bottom” on December 29, 1959, there has been increased research interest in nanoparticles (Jeremy, 2009).

Nanotechnology is bound to have incredible impact on mankind by helping to solve most major challenges facing humanity and in particular in health and energy. This is ascribed to the practical applications of metal nanoparticles in various areas such as medicine (Kairemo et al., 2008), chemical sensing, catalysis and electronics (Hall et al., 2011). Nanotechnology is the design, characterization, production and application of materials, devices and systems by controlling the shape and size of materials at the nanometre ($10^{-9}$ m) scale (Jeremy, 2009).

Nanoparticles are particles that have a size of between 1 to 100 nm in at least one dimension and possess unique physical and chemical properties due to their large surface area to volume ratio and smaller size (Kato, 2011). There are two basic approaches used in nanoparticle synthesis; the top-down (comminution and dispersion) approach and the
bottom-up (nucleation and growth) approach (Jeremy, 2009). The decision on which method to adopt depends on the approach that can deliver the specified properties and on cost (Jeremy, 2009). The three main methods of nanoparticle synthesis are physical, chemical and biological.

The physical method involves high energy milling of bulk material to nanoscale size or physical vapour deposition in which a bulk material is vaporized by heat source followed by rapid condensation to form nano-sized clusters that settle in the form of powder (Reza et al., 2011). The chemical methods employed include sonochemical process, photochemical reduction and sol-gel process. Plants and microbes such as bacteria, yeast and fungi are used in the biological method of nanoparticle synthesis (Li et al., 2011).

The physical and chemical methods of nanoparticle synthesis involve the use of toxic solvents and hazardous reagents (Parashar et al., 2009). Elaborate laboratory preparation of microbial cultures, complex extraction and purification process of the synthesized nanoparticles make the microbial technique of nanoparticle synthesis expensive (Charusheela et al., 2013). Therefore, there is a need to develop new methods of synthesizing nanoparticles that are less costly, energy efficient and use non-toxic, environment-friendly renewable resources such as phytochemicals extracted from plants. This would definitely mean applying the ‘Green chemistry’ principles as stated by Anastas and Warner, 1998:

1. **Prevention**: It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy:** Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.

3. **Less Hazardous Chemical Syntheses:** Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.

4. **Designing Safer Chemicals:** Chemical products should be designed to achieve their desired function while minimizing their toxicity.

5. **Safer Solvents and Auxiliaries:** The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.

6. **Design for Energy Efficiency:** Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.

7. **Use of Renewable Feedstocks:** A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.

8. **Reduce Derivatives:** Unnecessary derivatization (use of blocking groups, protection/deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. **Catalysis:** Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for Degradation:** Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.

11. **Real-time analysis for Pollution Prevention:** Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.

12. **Inherently Safer Chemistry for Accident Prevention:** Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

Silver nanoparticles (AgNPs) have continued to greatly draw the interest of many researchers because of their unique physical and chemical properties that make them find a wide range of applications. It has been reported that AgNPs are active against HIV-1 (Elechiguerra *et al.*, 2005) and inhibit HIV-1 replication (Sun *et al.*, 2005). In addition, studies have shown that AgNPs have potent antimicrobial activity (Sharma *et al.*, 2009). The strong antimicrobial activity of AgNPs make them potentially capable of being applied in water treatment against waterborne pathogens such as *Vibrio cholerae*, *Escherichia coli* and *Salmonella typhi* especially at the point-of-use in homes (Pradeep, 2009). This would be a better alternative to the widely used drinking water disinfection and microbial control approach which employ the use of chlorine or chlorine based compounds such as sodium hypochlorite. Harmful disinfection by-products such as the potentially carcinogenic trihalomethanes are usually formed when free chlorine reacts with natural organic matter in the water (Sapone *et al.*, 2007).
Various plant extracts have been successfully used in the synthesis of silver nanoparticles. Examples of plant extracts used include \textit{Camellia sinensis} (Green tea) leaf extract (Vilchis-Nestor \textit{et al.}, 2008), \textit{Cinnamomum camphora} leaf extract (Huang \textit{et al.}, 2007), \textit{Aloe vera} extract (Chandran, 2006), \textit{Allium sativum} (Garlic) extract (Gregory \textit{et al.}, 2012) and \textit{Capsicum annum} (pepper) leaf extract (Li \textit{et al.}, 2007). Although many plants have been used to synthesize silver nanoparticles, most of them have other competing applications for example; in beverages (Vilchis-Nestor \textit{et al.}, 2008) and spices (Huang \textit{et al.}, 2007). The cheapest and environment-friendly source of silver nanoparticles would ideally use biomass that has no other useful competing applications.

Agricultural wastes such as watermelon rind would offer such a source. Extensive review of available literature reveals that very few studies have been done to investigate the potential of agricultural wastes in synthesizing silver nanoparticles. The watermelon rind may potentially be used to synthesize AgNPs. The \textit{Citrullus lanatus} (watermelon) plant is an herbaceous creeping plant that originated from the Kalahari desert of southern Africa that produces 3 to 5 fruits weighing 3 to 8 kilogrammes each. The fruit can be round, oval or oblong having a light green to dark green rind with or without stripes.

The seeds contain significant amounts of trace elements such as zinc, magnesium, and calcium (Mirjana \textit{et al.}, 2005). The rind though edible, is usually discarded as an agricultural waste due to its unpleasant flavour.

Watermelon rind is a good candidate for this work because studies have shown that the \textit{Citrullus lanatus} (watermelon) rind extract contains polyphenols, tannins, alkaloids, flavonoids, and saponins (Johnson \textit{et al.}, 2012). Furthermore, Begum \textit{et al.} (2009)
reported that polyphenols and flavonoids may be responsible for the synthesis of silver nanoparticles using solutions of black tea extracts.

1.2 Statement of the Problem and Justification

The conventional physical and chemical methods of synthesizing metal nanoparticles involve the use of high energy amounts, toxic solvents and hazardous chemicals. Therefore, there is a need to develop other methods of metal nanoparticle synthesis that are energy efficient and which make use of non-toxic, environment-friendly renewable resources. Phytochemicals extracted from discarded agricultural wastes, such as the watermelon rind can be used in nanoparticle synthesis. Studies have shown that watermelon rind contains polyphenols and it has been demonstrated that polyphenols are involved in the synthesis of AgNPs. An extensive review of literature reveals that very few studies have been done to investigate the potential of discarded agricultural waste in synthesizing AgNPs. The purpose of this study was to investigate the potential of *Citrullus lanatus* rind extract in the synthesis of AgNPs and determine the antimicrobial activity of the AgNPs against *S.typhi* and *E.coli*. The findings of this study will add to the existing body of scientific knowledge on the synthesis of AgNPs using environment-friendly plant derived phytochemicals.

1.3 Hypotheses

i. Phenolic compounds in *Citrullus lanatus* rind extract can reduce silver ions to silver nanoparticles.

ii. Silver nanoparticles synthesized using *Citrullus lanatus* rind extract are active against *E.coli* and *S.typhi*. 
1.4 Objectives

1.4.1 General Objective
To synthesize AgNPs using *Citrullus lanatus* rind extract as a reductant and apply them against *E.coli* and *S.typhi*.

1.4.2 Specific Objectives
i. To probe optimal synthetic parameters for AgNPs using *Citrullus lanatus* rind extract as a reductant and capping agent.

ii. To determine the physical and chemical characteristics of the synthesized AgNPs using UV-Vis Spectroscopy, Cyclic Voltammetry (CV) and Transmission Electron Microscopy (TEM).

iii. To carry out bioassay of the AgNPs against *S.typhi* and *E.coli* using agar disc diffusion technique.

1.5 Significance of the Study

The outcome of this study will have added to the scientific knowledge on plant mediated synthesis of silver nanoparticles. The findings of this study may potentially provide cheap silver nanoparticles for use in water treatment against selected bacteria and a relatively cleaner environment due to the collection of water melon rinds for synthesis of silver nanoparticles. Moreover, the synthesis method applied in this study involved the application of the green chemistry principles which emphasize the reduction or elimination of the use of toxic solvents and reagents so as to protect the environment. The bioassay results obtained demonstrated that the WMRE-AgNPs can be used against *S.typhi* and *E.coli* which are prevalent in water.
1.6 Scope and Limitations of the Study

There are three main types of watermelon fruits: the red pulp variety, the white pulp variety and the orange pulp variety however, this study only used rind extract from the red-fleshed variety of *Citrullus lanatus* fruit because it is the most widely available watermelon fruit variety in Kenya. Similarly, the study only used the mature and ripe *Citrullus lanatus* fruits sold in major retail agricultural produce markets within Nairobi County. The limitation of this study was the source of the watermelon fruits since we purchased them from the retail markets supplied by traders from the entire country.
CHAPTER TWO

LITERATURE REVIEW

2.1 Nanotechnology

The control of the structure of matter at the nanometre scale to produce novel materials and devices that have useful and unique properties is referred to as nanotechnology (Jeremy, 2009). Nanoparticles are particles that have a size of 1-100 nm in at least one dimension. These particles have unique properties that are entirely different from those of the bulk due to their extremely small size and large surface area to volume ratio.

The unique physical and electronic properties make the nanoparticles suitable for use in various applications. Metallic nanoparticles include, Iron, gold and silver. Metallic nanoparticles are of great interest because of the modification of properties observed due to size effects, modifying the catalytic, electronic and optical properties (Thomas and Kamat, 2003). Nanoparticles come in a range of different types. Their size and shape can be controlled during synthesis to make them possess highly desired and specific properties suitable for various applications.

2.1.1 Quantum dots

These are ultra-fine semi-conductor particles that have at least one dimension between 1 nm and 10 nm and a narrow size distribution range (Fahlman, 2007). Quantum dots exhibit discrete energy levels and their band gap can be precisely modulated by varying the size. These quantum dots absorb white light and then re-emit a specific colour a few nanoseconds later depending on the band gap of the material (Bakalova et al., 2004) as shown in figure 2.1. The variation in colour of each solution illustrates the particle size
dependence of the optical absorption for each sample. The smaller particles are in the blue solution (absorbs blue), and that the larger ones are in the red (absorbs red). The potential application of quantum dots include areas such as biomarker detection in various cancers, imaging and sensing of infectious diseases as well as in fabrication of more efficient solar cells in the energy field (Valideh et al., 2012).

![Figure 2.1: Solutions of quantum dots of varying size. (Source: www.physicsopenlab.org accessed 31/08/2017)](image)

2.1.2 Carbon Nanotubes
These are hollow, carbon-based, cage-like nanostructures. The two carbon-based nanostructures that have drawn great attention in recent years are nanotubes and fullerenes. Single-Wall Nanotubes (SWNTs), Multi-Wall Nanotubes (MWNTs) and C_{60} fullerenes. SWNTs and C_{60} fullerenes have diameters in the order of 1 nm. The MWNTs have diameters ranging from several nanometers to tens of nanometers depending on the number of walls in the structure. Carbon Nanotubes and fullerenes are typically made using electric arc discharge, chemical vapour deposition or combustion processes (Abhilash, 2010).
Figure 2.2: Schematic diagram of Single-walled and multi-walled carbon nanotubes (Source: Slideplayer.fr accessed 19/11/2015).

2.1.3 Metallic nanoparticles

Metal nanoparticles have been synthesized using various methods including physical methods, chemical methods and biological methods that involve use of microbes such as Bacteria, Yeast and Fungi (Li et al., 2011) and plant extracts (Bar et al., 2009). The physical and chemical methods of metal nanoparticle synthesis use huge amounts of energy, toxic solvents and hazardous chemicals (Thakkar et al., 2010). The biological method of using microbes in metal nanoparticle synthesis is eco-friendly but is expensive due to the elaborate laboratory process of preparing and maintaining the microbial cultures, complex extraction and purification processes (Charusheela et al., 2013). Plant
extracts have been used to synthesize metal nanoparticles successfully and provide a cost
effective and an environment-friendly means of synthesizing metal nanoparticles.

2.2 Potential Applications of Metal Nanoparticles

Metal nanoparticles have been shown to possess unique properties that make them
suitable candidates for applications in targeted drug delivery, catalysis, sensors and
antibiotics (Doria et al., 2012).

2.2.1 Targeted Drug Delivery Applications

Hollow metal nano-shells are being investigated for drug delivery applications (Doria et
al., 2012). The fabrication methods involve templating of the thin metal shell around a
core material such as a silica nanoparticle. Typical metals used include gold, silver,
platinum and palladium. The metal nanoparticles are usually linked to or embedded
within polymeric drug carriers. Therefore, the metal nanoparticles can be used as thermal
release triggers when irradiated with infrared light or excited by an alternating magnetic
field (Gareth, 2005).

2.2.2 Application in Catalysis

Catalysts play a very vital role in many industrial processes. Many of these catalysts are
expensive and maybe readily made inefficient by impurities. The use of metal
nanoparticles can overcome these two challenges. The advantages of metal nanoparticles
as catalysts include:

i. The metal nanoparticles can readily be dispersed through the use of inorganic
   supports such as silica gel, alumina and activated charcoal.
ii. The resulting dispersed metal nanoparticles exhibit a much larger total surface area per unit weight than the bulk metal. This enables more effective utilization of the expensive transition metal as a result of dispersion.

iii. A polymer-protected metal nanoparticle has an additional benefit in that; the protecting polymer can interact attractively or repulsively with substrates, which results in high selectivity (Toshima, 2004).

### 2.2.3 Biosensing Applications

Noble metal nanoparticles have been employed in the development of new biosensors as well as the enhancement of existing biosensing techniques. The unique physical and chemical properties of these metals at the nanoscale have led to the development of a wide range of biosensors especially in the field of point of care diagnostics. Gold and silver nanoparticles have been used extensively in the development of new biosensors due to their unique Surface Plasmon Resonance and surface properties (Saerens et al., 2008).

These properties enable them to be functionalized with different biosensing molecules such as antibodies, enzymes and proteins (Doria et al., 2012). Mirkin and co-workers developed a biosensor for cancer protein biomarkers using gold nanoparticles functionalized with antibodies (Mirkin et al., 2009). Highly sensitive and specific biosensors based on metal nanoparticles open up new possibilities for early stage detection of various diseases (Doria et al., 2012).
2.2.4 Antibiotic Applications

Emergence of antibiotic resistant pathogens has become a serious health issue and thus, numerous studies have been reported to improve the current antimicrobial therapies. It is known that over 70% of bacterial infections are resistant to one or more of the antibiotics that are generally used to eradicate the infection (Allahverdiyev et al., 2011). Development of new and effective antimicrobial agents seems to be of paramount importance. The antimicrobial activity of metals such as silver (Ag), copper (Cu), gold (Au), titanium (Ti), and zinc (Zn), each having various properties, potencies and spectra of activity, has been known and applied for centuries (Malarkodi et al., 2014).

In vitro studies revealed that metal nanoparticles inhibited several microbial species. The kind of the materials used for preparing the nanoparticles as well as the particle size were two important parameters that affected the resultant antimicrobial effectiveness (Seil and Webster, 2012). Generally nanoparticles have different properties compared to the same material with the larger particles owing to the fact that the surface/volume ratio of the nanoparticles increases considerably with decrease in the particle size (Buzea et al., 2007).

The exact mechanisms for antibacterial effect of nanoparticles are still being investigated, but there are two more popular proposed possibilities in this regard: (a), free metal ion toxicity arising from dissolution of the metals from surface of the nanoparticles and (b), oxidative stress via the generation of reactive oxygen species (ROS) on surfaces of the nanoparticles (Besinis et al., 2014). Furthermore, morphological and physicochemical
characteristics of the nanoparticles have been proven to exert an effect on their antimicrobial activities (Mohammadi et al., 2010). It is known that the small nanoparticles have the strongest bactericidal effect (Fellahi et al., 2013).

The positive surface charge of the metal nanoparticles facilitates their binding to the negatively charged surface of the bacteria which may result in an enhancement of the bactericidal effect (Seil and Webster, 2012). The shape of the nanoparticles also influences their antimicrobial effects (Pal et al., 2007). Lee et al. (2003) demonstrated the antibacterial effect of nanosized silver colloidal solution on textile fabric. Studies by Shrivastava et al., 2007 showed that silver nanoparticles had antibacterial activity against E. coli. They reported that the effect was dose dependent and that the major mechanism through which AgNPs manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall. The selected bacteria in this study were E. coli and S. typhi which are prevalent in water.

2.3 Silver Nanoparticles

Silver nanoparticles are particles of silver having a size of 1 nm to 100 nm in at least one dimension. They can be synthesized through the reduction of silver ions by reducing agents under controlled conditions of temperature and pH. The reduction of Ag$^+$ ion to Ag$^0$ is represented by the chemical equation (1);

$$\text{Ag}^+_{(aq)} + e^- \rightarrow \text{Ag}^0_{(s)}\text{ equation (1)}$$

Studies have demonstrated that silver nanoparticles can be synthesized using sun dried leaves extract of Cinnamomum camphora (Huang et al., 2007). The polyol components
and water soluble heterocyclic components present in the extract were reported to be responsible for the reduction of silver ions and the stabilization of the silver nanoparticles formed. *Capsicum annum* (pepper) leaf extract has been used in the synthesis of silver nanoparticles. It was reported that phenolic acids in the extract were involved in reduction of silver ions and stabilization of the silver nanoparticles formed (Li *et al.*, 2007). *Camellia sinensis* (Green tea) leaf extract has been used to synthesize silver nanoparticles. It has been reported that phenolic acid-type molecules present in the extract were presumed to be responsible for the reduction of silver ions and stabilization of nanoparticles formed (Vilchis-Nestor *et al.*, 2008).

The post-harvest waste is almost 80% of the biomass on the agricultural fields and is often burnt in fields resulting in large amount of green gas emissions, also problems like smog arises, causing serious health impact. Although composting the material for manure production and few for bio-fuels production are being practiced, this agro-waste can also be utilized for the synthesis of valuable nanoparticles. The waste management represents an important challenge in the agro-food based industries and demands an integrated approach in the context of recycling, reuse and recovery (Krishnaswamy *et al.*, 2014). Banana peel extracts were reported by Bankar *et al.* (2010) to contain amino and carboxy groups that were involved in the reduction of silver ions to silver nanoparticles. Flavonoids contained in the orange peel extract were reported by Manal *et al.* (2014) to be involved in the reduction of silver ions to silver nanoparticles. Nisha *et al.* (2015) demonstrated that amino acids in the pomegranate fruit peel extract were responsible for the reduction of silver ions to silver nanoparticles.
Studies have shown that an enzyme in the latex of *Jatropha curcas* called curcain was responsible for the stabilization of the silver nanoparticles that were prepared (Bar *et al*., 2009). Banana and orange peel are composed of mainly complex carbohydrates like hemicelluloses, cellulose and pectin (Emaga *et al*., 2007). The functional groups in these carbohydrates and also the associated proteins may be involved in reducing the Ag\(^+\) to Ag\(^0\). These phytochemicals interact with the metal salts through their functional groups and facilitate their reduction to particles of nanoscale (Anil *et al*., 2015).

In another study, leaf extract of *Citrus limon* was prepared by boiling leaf pieces in water for 5 to 10 min. The filtrate obtained was treated with silver nitrate at room temperature in dark. The synthesis of AgNPs was observed after one hour. Synthesis of heterogeneous, well dispersed AgNPs stabilized by phytochemicals like terpenoids, polysaccharides and proteins was reported (Padma *et al*., 2012). The fruit extract of *Carica papaya* (Pawpaw) has been used to synthesize silver nanoparticles. It was reported that the presence of proteins and other ligands were responsible for the reduction of silver ions to silver nanoparticles (Jain *et al*., 2009). *Allium sativum* (Garlic) extract has been reported to reduce silver ions to silver nanoparticles (Gregory *et al*., 2012). The cited studies demonstrate that agro-waste can be potentially employed in synthesis of nanoparticles, but a lot of research is required in order to standardize the protocols and methods of synthesis. Utilization of agro-waste in prospect of nanoparticle production would lead to sustainable development with respect to environment and economy development.
2.4 Characterization Methods of Silver Nanoparticles

Methods of nanoparticle characterization are as vital as synthetic routes/recipes. A set of complementary analytical techniques are used to fully characterize materials at nanometre scale. Some of the methods employed in characterizing AgNPs include UV-Visible spectroscopy, Cyclic Voltammetry and Transmission Electron Microscopy.

2.4.1 Ultraviolet-Visible Spectroscopy

UV-Visible Spectroscopy is an analytical technique that involves the measurement of the attenuation of a beam of light after it passes through a sample or after reflection from sample surface. Absorption measurements can be made at a single wavelength or over an extended spectral range. Figure 2.3 shows a schematic diagram of a UV-Vis Spectrophotometer. The Ultraviolet and Visible light radiations are energetic enough to promote ground state electrons to higher energy levels and noble metal nanoparticles have characteristic optical absorption spectra in the UV-Visible region of the electromagnetic spectrum due to their unique localized Surface Plasmon Resonance (Kulkarni, 2009). Studies by Guzman et al. (2009) demonstrated that the UV-Vis spectra of silver nanoparticles synthesized using hydrazine and sodium citrate exhibited a maximum absorbance peak at 418 nm. The UV-Vis spectroscopy was used to give qualitative information about the synthesized AgNPs by comparing the spectra with that of the Standard reference AgNPs.
2.4.2 Cyclic Voltammetry (CV) Measurements

Cyclic voltammetry is an electrochemical technique used for acquiring qualitative information about electrochemical reactions. It is based on varying the applied potential at a working electrode in forward and reverse directions (at some scan rate) while monitoring the current based on the procedure reported by Frank (1997).

This technique takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When cyclic voltammetry reaches a set potential, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution (Ying et al., 2013). AgNPs have zero valence and cyclic voltammetry can be used to confirm whether a given sample has AgNPs in it by determining the oxidation peak and comparing it with that of a reference standard.
Dobre et al. (2014) used cyclic voltammetry to investigate the voltammetric profile of silver nanoparticles on platinum, gold and glassy carbon electrodes. Studies by Pumera and Giovanni (2012) which applied the use of cyclic voltammetry showed that different sized silver nanoparticles have different voltammetric profiles. Figure 2.4 shows a schematic diagram of a 3-electrode cell system.

![Schematic diagram of a 3-electrode cell system](www.researchgate.net)

**Figure 2.4:** A schematic diagram of a 3-electrode cell system. (Source: www.researchgate.net accessed 31/08/2017).

### 2.4.3 Transmission Electron Microscopy (TEM)

TEM is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen and interacts with the sample as it passes through the sample. Micrographs are formed from the electrons transmitted through the sample, magnified and focused by an objective lens and appear on an imaging screen (Joshi et al., 2009). This technique is able to give size as well as the morphology of the silver nanoparticles. Gregory et al. (2012) determined the size and the shape of silver nanoparticles using
Transmission electron microscopy. Figure 2.5 shows a schematic diagram of a transmission electron microscope.

**Figure 2.5:** A schematic diagram of a Transmission Electron Microscope.

### 2.5 Antimicrobial Activity of AgNPs

Studies have demonstrated that silver nanoparticles synthesized using the leaf extract of *Acalypha indica* have a high antimicrobial activity (Krishnaraj *et al.*, 2010). Silver nanoparticles synthesized using leaf extract of *Moringa oleifera* were reported to have potent antimicrobial activity (Anamika *et al.*, 2012).

According to literature AgNPs are the most widely applied inorganic nanoparticles used as antimicrobial agents (Zinjarde, 2012). The antimicrobial application of AgNPs is widely used in the various injection molded plastic products, textiles and coatings (Egger *et al.*, 2009). AgNPs also possess a range of biomedical applications (Malarkodi *et al.*, 2014). It has been revealed that, AgNPs show a high antimicrobial activity comparable
with its ionic form (Jo et al., 2009). It has also been demonstrated that AgNPs are potential antimicrobial agents against drug resistant bacteria (Allahverdiyev et al., 2011). According to literature, antibacterial action of AgNPs results from damage of the bacterial outer membrane (Lok et al., 2006). Some researchers suppose that, AgNPs can induce structural disintegration in the bacterial membrane and then fragment the cell (Yun et al., 2013). It has also been demonstrated that Ag ions interact with disulfide or sulf-hydryl groups of enzymes that lead to disruption of metabolic processes which in turn cause cell death (Egger et al., 2009).

The work of Jo et al. (2009) investigated the effect of size reduction on the antimicrobial effect of AgNPs. They used AgNPs to control Bipolaris-sor okiniana and Magnaporthe grisea. Similarly, they also evaluated the efficacy of AgNPs on different types of pathogens such as soil-borne fungi which rarely produce spores. According to their results, AgNPs (20 to 30 nm) could better penetrate and colonize within the plant tissue. They suggested that, AgNPs had a great potential for use in controlling spore-producing fungal plant pathogens. They suggested that these nanoparticles might be less toxic than synthetic fungicides.

A study by Mie et al. (2014) tested the antibacterial activity of their synthesized AgNPs (19 nm) against eight micro-organisms using the disk diffusion method. Their results revealed that the AgNPs showed potential antibacterial activity against Gram-negative bacteria. Thus, the authors suggested that the synthesized AgNPs could be applied in the pharmaceutical and biomedical industries.
Hernández-Sierra et al. (2008) reported comparative investigation of the bactericidal activity of AgNPs, ZnO, and Au on *Streptococcus mutans* (*S. mutans*). Their results indicated that AgNPs exhibited the most activity for controlling *S. mutans*. The authors suggested that AgNPs could be used in dental caries since it is commonly caused by *S. mutans*. Likewise, Besinis et al. (2014) investigated the antibacterial effect of AgNPs on *S. mutans*. Their results showed that the antibacterial effect of AgNPs against *S. mutans* was more superior to that of chlorhexidine.

The work of Zarei et al. (2014) evaluated antibacterial effect of AgNPs against four food borne pathogens namely *Listeria monocytogenes*, *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*) and *Vibrio parahaemolyticus*. According to their results, AgNPs had great antibacterial effect on the mentioned pathogens. They concluded that AgNPs could be a good alternative for cleaning and disinfection of equipment and surfaces in the food-related environments. Beside the particle size reduction, shape-dependent properties of nanoparticles have also been investigated by researchers. Pal et al. (2007) reported the shape dependent antibacterial activity of AgNPs (in three different forms: spherical, rod-shaped and truncated triangular). According to their findings, truncated triangular nanoparticles were more reactive due to their high-atom-density surfaces, and therefore showed higher antimicrobial activity.

A study by Bera et al. (2014) investigated the size and shape-dependent antimicrobial activity of fluorescent AgNPs (1–5 nm) against Gram-positive (*Staphylococcus epidermidis* and *Bacillus megaterium*) and Gram-negative bacteria (*Pseudomonas*
aeruginosa). They emphasized that the shape and size of the particles controlled their activity. According to these investigations, the smaller particles easily penetrated the cell wall and showed the enhanced antimicrobial activity. The authors suggested that these AgNPs could be used for different purposes such as clinical wound dressing, bioadhesives, biofilms and the coating of biomedical materials.

Bahrami et al. (2014) prepared Ag–Au alloy nanoparticles to evaluate their antimicrobial effect against Staphylococcus aureus (S. aureus). The antibacterial activity of Ag-Au alloy nanoparticles was intensified when they combined with penicillin G and piperacillin. The authors suggested that Ag-Au alloy nanoparticles could be used as an adjuvant in combination therapy of antibiotics.

Although numerous plant extracts have been used in the synthesis of silver nanoparticles, very few studies have been done to investigate the potential of discarded agricultural wastes to synthesize silver nanoparticles. The Citrullus lanatus (Watermelon) rind is usually discarded as an agricultural waste. It has been reported that Citrullus lanatus rind contains phytochemicals such as polyphenols, tannins, alkaloids, flavonoids and saponins (Johnson et al., 2012; Oseni et al., 2013). Watermelon rind contains chlorogenic acid (CGA) which is a phenolic acid as demonstrated by Al-Sayed and Ahmed (2013).
CHAPTER THREE

METHODOLOGY

3.1 Sampling of Watermelon (*Citrullus lanatus*) Fruits

Nine ripe watermelon fruits of four kilogrammes each were randomly selected and purchased from nine fruit vendors at Wakulima Market, Muthurwa Market and Githurai Market within Nairobi County. Three watermelon fruits were selected from each market, one fruit from each of the three sampling sites per market and labeled. All the watermelon fruits were then transported to Kenyatta University Chemistry research laboratory.

The watermelon variety purchased was the Charleston grey variety which is the most common sweet watermelon variety sold within Nairobi County shown in figure 3.1 (A).

![Figure 3.1: A schematic diagram of the WMRE extraction process](image)

3.2 Preparation of Watermelon Rind Extract

One ripe watermelon fruit was thoroughly washed, rinsed with distilled water and then cut into four-quarter portions using a clean sterilized knife. The red coloured pulp in the interior of each watermelon portion was removed to obtain the watermelon rind as
shown in figure 3.1(B). The water melon rinds were cut into small pieces (5mm by 10 mm) using a sterile knife and placed inside a blender (Sunny blender) for blending. Exactly, 100 g of the crushed water melon rind material were carefully weighed using an analytical balance and transferred into a clean 1000 ml conical flask and diluted with 400 ml of distilled water. The above procedure was repeated with the other eight watermelon fruits in subsequent experiments.

The 1000 ml conical flask was then placed in a shaking water-bath and heated at a temperature of 80 °C for ten minutes in order to increase the yield of the water soluble polyphenols in the water melon rind extract. After the ten minutes, the mixture was removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The cold water melon rind material was then filtered using Whatman No.1 filter paper. A light-green coloured filtrate was obtained from WMRE as shown in figure. 3.1 (C)

3.3 Preparation of 0.001 M Silver Nitrate Solution

The Analytical grade Silver nitrate (99.5% Purity) was purchased from Sigma-Aldrich (U.S.A) and used as purchased. To prepare a 0.001 M solution of AgNO₃, 0.1699 grams of the analytical grade AgNO₃ was carefully weighed using an Analytical balance and then transferred into a 1000 ml volumetric flask that contained 400 ml of distilled water. Stirring was done to ensure that all the solid AgNO₃ dissolved and more distilled water was added to make up to the mark.
3.4 Synthesis of Silver Nanoparticles

3.4.1 Method used in AgNPs Synthesis

AgNPs were prepared by reacting 0.001 M silver nitrate solution with the WMRE (250.0 g/L) prepared as described in section 3.3 as the reductant and stabilizing agent. The control method employed Sileikaite et al. (2006) method which is known and optimized using trisodium citrate with a soluble silver salt. This involved the reaction of 0.001 M silver nitrate solution with 1% trisodium citrate solution as the reductant and stabilizing agent. The second method was used for comparison purposes against the green method of using WMRE in silver nanoparticle synthesis.

3.4.2 Synthesis of AgNPs using WMRE as a Reductant

The WMRE- Ag NPs were synthesized by reacting 50 mL of 0.001 M AgNO₃ with 50 ml of 250 g/L WMRE, at temperature of 80 °C, pH 10 (the pH of the reaction mixture was 5.92 and was adjusted to pH 10 using 0.1 M NaOH solution) and heating the reaction mixture in a shaking water-bath for 35 minutes in order to increase the yield of water-soluble polyphenols from the WMRE.

3.4.3 Method Optimization

To obtain silver nanoparticles that are monodispersed with tunable particle size and morphology, various reaction parameters were controlled and optimized as described in section 3.4.3.1 to achieve the desired results. These reaction parameters include the reaction temperature, pH of reaction mixture, concentration of reactants and the ratio of the reactants (Tripathy et al., 2010).
3.4.3.1 Optimization of the AgNO₃ to WMRE Ratio

To determine the optimal ratio of reactants (by volume), different ratio of reactants were used. Six WMRE-AgNPs samples were prepared in order to determine the optimal ratio (by volume) of the reactants. These samples were labelled as A1WM1, A1WM4, A1WM5, A2WM3, A3WM5, and A4WM5 (where A is silver nanoparticles and WM is watermelon extracts). The ratio (by volume) of the reactants: 1mM AgNO₃ to WMRE used to prepare the samples were as shown and described in Table 3.1. The UV-Vis Spectra of the samples prepared was then obtained using CECIL CE 2041 2000 Series Spectrophotometer in the wavelength range of 300-700 nm. UV-Vis absorption spectra have been proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibit intense absorption peak due to the Surface Plasmon excitation (Sileikaite et al., 2006).

Table 3.1: Effect of Ratio of Reactants on WMRE AgNPs Synthesis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ratio of Reactants</th>
<th>Reaction time (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1WM1</td>
<td>1:1</td>
<td>35</td>
</tr>
<tr>
<td>A1WM4</td>
<td>1:4</td>
<td>29</td>
</tr>
<tr>
<td>A1WM5</td>
<td>1:5</td>
<td>27</td>
</tr>
<tr>
<td>A2WM3</td>
<td>2:3</td>
<td>26</td>
</tr>
<tr>
<td>A3WM5</td>
<td>3:5</td>
<td>25</td>
</tr>
<tr>
<td>A4WM5</td>
<td>4:5</td>
<td>23</td>
</tr>
</tbody>
</table>

Where A is silver nanoparticles and WM is watermelon extract.
Sample A1WM1 was prepared using a volume ratio of 1:1 by adding 50 mL of 0.001 M AgNO₃ solution to 50 mL of 250 g/L water melon rind extract in a 250 mL conical flask. The pH of the reaction mixture was 5.92 and was adjusted to pH 10 using 0.1 M NaOH solution. This was done to promote the formation of AgNPs as an alkaline pH is known to favour formation of AgNPs as reported by Darroudi et al. (2010).

The 250 mL conical flask containing the reaction mixture was then placed in a shaking water-bath at 80 °C as higher temperatures used in preliminary experiments had shown an effect of increasing the maximum absorbance peak and the reaction rate. The 250 mL conical flask containing the sample was then removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The AgNPs sample separation was done by centrifugation using Omega 6 Centrifuge at 5000 rpm for 30 minutes. The centrifugation of the sample was performed three times. The WMRE-AgNPs supernatant obtained was placed in clean glass vials. Samples A1WM4, A1WM5, A4WM5, were prepared similarly using a volume ratio of 1:4, 1:5 and 4:5 of 0.001 M AgNO₃ solution to 250 g/L of WMRE.

3.4.4 Variation of Reaction Parameters on the WMRE-AgNPs Synthesis using Optimized Ratio of 4:5

3.4.4.1 Effect of Variation of Temperature on the WMRE-AgNPs Synthesis

Sample A4WM5 was placed in a conical flask and the pH of the reaction mixture was adjusted from 5.92 to 10 using 0.1 M NaOH solution. The 250 mL conical flask was then placed in a shaking water-bath at temperature of 40 °C. The temperature of the reaction mixture was maintained at 40 °C for 23 minutes which was the optimal reaction time in a
shaking water-bath. The reaction mixture was removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The AgNPs were size separated by centrifugation using Omega 6 Centrifuge at 5000 rpm for 30 minutes. The centrifugation of the synthesized WMRE-AgNPs sample was performed three times. The supernatant of the WMRE-AgNPs obtained was placed in clean glass vials. The above procedure was repeated for temperature values of 50 °C, 60 °C, 70 °C and 80 °C. The UV-Vis Spectra of the samples prepared using the different temperature values of 40 °C, 50 °C, 60 °C, 70 °C and 80 °C was obtained as described in section 3.5.1.

### 3.4.4.2 Effect of Variation of pH on the WMRE-AgNPs Synthesis

Sample A4WM5 was placed in a conical flask and the pH of the reaction mixture was adjusted from 5.92 to 6 using 0.1 M NaOH solution. The 250 mL conical flask was then placed in a shaking water-bath at temperature of 80 °C. The temperature of the reaction mixture was maintained at 80 °C for 23 minutes which was the optimal reaction time in a shaking water-bath. The reaction mixture was removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The AgNPs were size separated by centrifugation using Omega 6 Centrifuge at 5000 rpm for 30 minutes. The centrifugation of the synthesized WMRE-AgNPs sample was performed three times. The supernatant of the WMRE-AgNPs obtained was placed in clean glass vials. The above procedure was repeated for pH values of 8, 9 and 10. The UV-Vis Spectra of the samples prepared using different pH values 6, 8, 9 and 10 was obtained as described in section 3.5.1.
3.4.4.3 Effect of Variation of Concentration of AgNO₃ on the WMRE-AgNPs

Synthesis

Sample A4WM5 was placed in a conical flask and the pH of the reaction mixture was adjusted from 5.92 to 10 using 0.1 M NaOH solution. The 250 mL conical flask was then placed in a shaking water-bath at temperature of 80 °C. The temperature of the reaction mixture was maintained at 80 °C for 23 minutes which was the optimal reaction time in a shaking water-bath. The reaction mixture was removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The AgNPs were size separated by centrifugation using Omega 6 Centrifuge at 5000 rpm for 30 minutes. The centrifugation of the synthesized WMRE-AgNPs sample was performed three times. The supernatant of the WMRE-AgNPs obtained was placed in clean glass vials. The procedure was repeated for AgNO₃ concentration values of 0.0001 M, 0.0002 M, 0.0004 M, 0.0008 M and 0.001 M. The UV-Vis Spectra of the samples prepared using different AgNO₃ concentration values of 0.0001 M, 0.0002 M, 0.0004 M, 0.0008 M and 0.001 M was obtained as described in section 3.5.1.

3.4.4.4 Effect of Variation of WMRE Concentration on the WMRE-AgNPs

Synthesis

20 mL of 0.001 M AgNO₃ were added to 25 mL of 100 g/ L WMRE in a 250 mL conical flask and the pH of the reaction mixture was adjusted from 5.92 to 10 using 0.1 M NaOH solution. The 250 mL conical flask was then placed in a shaking water-bath at temperature of 80 °C. The temperature of the reaction mixture was maintained at 80 °C for 23 minutes in a shaking water-bath. The reaction mixture was removed from the shaking water-bath and allowed to cool to room temperature (25 °C). The AgNPs were
size separated by centrifugation using Omega 6 Centrifuge at 5000 rpm for 30 minutes. The centrifugation of the synthesized WMRE-AgNPs sample was performed three times. The supernatant of the WMRE- AgNPs obtained was placed in clean glass vials. The above procedure was repeated for WMRE concentrations of 150 g/L, 200 g/L and 250 g/L. The UV-Vis Spectra of the samples prepared using different concentration values of WMRE concentrations of 150 g/L, 200 g/L and 250 g/L was obtained as described in section 3.5.1.

3.4.5 Synthesis of Citrate-AgNPs

3.4.5.1 Preparation of 1% Trisodium Citrate

Analytical grade trisodium citrate dihydrate (C₆H₅O₇Na₃.2H₂O, Purity >99%) was purchased from Sigma-Aldrich (U.S.A). About, 2.941 grams of trisodium citrate (294.1 g/mol) was carefully weighed using an analytical weighing balance and then transferred into a 1000 mL volumetric flask that contained 400.0 mL of distilled water. Stirring was done to ensure that all the solid trisodium citrate dissolved and more distilled water was added to make up to the mark.

3.4.5.2 Citrate-AgNPs Sample Preparation

The AgNPs were prepared using an adapted method described by Sileikaite (Sileikaite et al., 2006). Exactly, 50.0 mL of 0.001 M AgNO₃ was heated to boiling. To this solution, 5.0 mL of 1% trisodium citrate was added drop wise. During the process, the solutions were mixed vigorously and heated until the colour change from colourless to yellow was evident in 10 minutes. Then the reaction mixture was removed from the heating element.
and stirred until cooled to room temperature (25 °C). The citrate-AgNPs sample SD₁ was prepared using the above procedure.

3.5 Characterization of synthesized AgNPs

The synthesized AgNPs were characterized using UV-Visible spectroscopy, Cyclic Voltammetry and Transmission Electron Microscopy.

3.5.1 UV-Visible Spectroscopy

Exactly, 4 mL of the supernatant of the WMRE-AgNPs sample was placed in a quartz cuvette with a 1cm path length and inserted in a UV-Vis Spectrophotometer (CECIL CE 2041 2000 SERIES) and the spectrum recorded in the wavelength range of 300-700 nm.

3.5.2 Cyclic Voltammetry

Cyclic voltammetry experiments were performed in an analytical system using a BASi EC Epsilon model Potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy carbon electrode (GCE; area 0.07 cm²). Accurately weighed, 0.05 M Phosphate Buffer (PB) was used as the supporting electrolyte. In the experiments, all the potentials were reported versus the Ag/AgCl reference electrode. All the experiments were performed at room temperature (25°C).
3.5.2.1 Preparation of 0.05 M Phosphate Buffer Solution (pH 7.4)

About 1.7522 g of monosodium phosphate dihydrate (NaH$_2$PO$_4$.2H$_2$O) and 13.7402 g of disodium hydrogen phosphate dodecahydrate (Na$_2$HPO$_4$.12H$_2$O) were added to 600 mL of distilled water in a 1000 mL volumetric flask and stirred to ensure all the solid dissolved and then topped up to the mark using distilled water. The pH of the solution was adjusted to 7.4 using concentrated phosphoric acid and 10 M NaOH solution.

3.5.2.2 Cyclic Voltammetry Experiment Procedure

Accurately measured 10 mL of 0.05M Phosphate buffer (pH 7.4) was placed in a voltammetric glass cell as the supporting electrolyte. The reference electrode was Ag/AgCl, the working electrode used was Glassy Carbon electrode and the counter electrode used was a Platinum wire. The experimental parameters used for the bare Glassy Carbon electrode were as follows: Initial potential applied was -1000 mV, the switching potential was +1100 mV and the final potential was -1000 mV. The scan rate used was 100 mV/s. The CV experiment was then performed with bare Glassy Carbon electrode using the above stated experimental parameters.

3.5.2.3 Cyclic Voltammetry of Standard AgNPs (0.02 mg/mL, 20 nm AgNPs Colloidal Dispersion)

The Glassy Carbon electrode surface was cleaned using 0.05 µm alumina and then rinsed with distilled water. AgNPs (0.02 mg/mL, 20 nm colloidal dispersion purchased from Sigma Aldrich, U.S.A) were immobilized on the Glassy Carbon electrode surface by directly depositing 4 µL of the standard AgNPs reference sample (0.02 mg/mL, 20 nm
AgNPs dispersion) on the electrode surface and the solvent (water) allowed to evaporate at room temperature (25 °C).

A 10 mL of 0.05 M Phosphate Buffer (pH 7.4) solution was placed in a voltammetric glass cell as the supporting electrolyte. The reference electrode used was Ag/AgCl, the working electrode was the Glassy Carbon electrode and the counter electrode was a Platinum wire. The experimental parameters used were: Initial potential -1000 mV, switching potential +1100 mV and final potential of -1000 mV. The scan rates used ranged from 20 mV/s to 100 mV/s.

3.5.2.4 Cyclic Voltammetry of AgNPs prepared using WMRE

The Piranha solution used for cleaning Glassy Carbon electrode surface was prepared by mixing 6 mL of concentrated sulphuric (VI) acid and 2 mL of 30 % H₂O₂ in a 3:1 v/v ratio. The Glassy Carbon electrode was rinsed with ethanol and then with distilled water. The electrode was further cleaned by polishing the surface with 0.05 µm alumina as per the polishing procedure in the polishing kit provided. Both the reference electrode (Ag/AgCl) and the auxiliary/counter electrode were rinsed with distilled water, wiped and kept ready for the experiment.

AgNPs sample prepared using watermelon rind extract were immobilized on the Glassy Carbon electrode surface by directly depositing 4 µL of the AgNPs sample on the electrode surface and the solvent (water) allowed to evaporate at room temperature (25 °C). 10 mL of 0.05 M Phosphate Buffer (pH 7.4) solution was placed in a voltammetric glass cell as the supporting electrolyte. The reference electrode was Ag/AgCl, the working electrode was the Glassy Carbon electrode and the counter electrode was a
Platinum wire. The experimental parameters used were: Initial potential -1000 mV, switching potential +1100 mV and final potential of -1000 mV. The scan rates used ranged from 20 mV/s to 100 mV/s.

3.6 Transmission Electron Microscopy (TEM)

Samples of the WMRE-AgNPs, citrate-AgNPs and standard reference-AgNPs were drop-coated on carbon-coated copper grids which had been placed on blotting paper and then allowed to dry in air for five minutes. The electron microscopy of the AgNPs was performed using Zeiss Libra 120 TEM (120 KV).

3.7 Antimicrobial Activity of WMRE-AgNPs and Std-AgNPs

3.7.1 Antimicrobial Activity Experiments

The antimicrobial sensitivity tests were done using the disc-diffusion method. Mueller Hinton Agar was purchased from Sigma Aldrich (U.S.A). Briefly, 100 µL of suspension containing 10^6 colony forming units per mL of bacteria was inoculated on the agar plates. 6.0 mm diameter sterilized paper discs were impregnated with 10 µL of the WMRE-AgNPs colloidal dispersion and placed on the agar plates streaked with clinical isolates of *E.coli* and *S.typhi*. This procedure was repeated using standard reference-AgNPs (20 nm diameter). Negative controls were prepared using the same reactants (0.001 M silver nitrate and WMRE 250 g/L) and the solvent (water) used to synthesize the WMRE-AgNPs. These controls were evaluated against each clinical isolate of *E.coli* and *S.typhi*. The standard antibiotics in the study were Ciprofloxacin (10 µg per disc) and
Vancomycin (10 μg per disc) and were used as positive reference standards to determine the sensitivity of one strain per isolate for each microbial species tested.

The streaked plates were incubated in an oven at 37 °C for 24 hours. The antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition for the test microbes.

3.7.2 Chlorogenic Acid Experiments

Watermelon rind extract contains Chlorogenic acid and these experiments were carried out to determine whether the acid was involved in the reduction of Ag⁺ to Ag⁰ as described in section 3.7.2.1 and 3.7.2.2.

3.7.2.1 Preparation of Chlorogenic Acid Standard Solution

Chlorogenic acid 99% purity was purchased from Sigma Aldrich (U.S.A). Accurately measured 0.354 g of Chlorogenic acid was placed in a 1000 mL volumetric flask containing 400 mL of distilled water at 70 °C and more distilled water at the same temperature was added to top up to the mark. Stirring was done to ensure that the entire solid dissolved. The concentration of the Chlorogenic acid standard prepared was 0.001 M.

3.7.2.2 UV-Visible Spectroscopy of Chlorogenic Acid Standard Solution, WMRE and WMRE-AgNPs

Exactly, 4 mL of chlorogenic acid standard solution (0.001 M) was placed in a quartz cuvette with a 1cm path length and inserted in a UV-Vis Spectrophotometer (CECIL CE 2041 2000 SERIES) in the wavelength range of 200-400 nm to obtain the UV-Visible
spectra of the solution. The above procedure was repeated to obtain the UV-Visible spectra of WMRE and WMRE-AgNPs in the wavelength of 300-700 nm.

3.8 Data Analysis

The experiments carried out in this study were done in triplicates and the data obtained was analyzed using one way-ANOVA and Student Newman Keul-test.
CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 UV-Vis Spectroscopy Characterization of AgNPs

UV-Visible spectroscopy of the AgNPs was performed to investigate the formation of AgNPs with changes in reaction parameters or suitable conditions to produce monodispersed AgNPs. These parameters include reactant ratio, temperature, pH and concentration of reactants. The results obtained are discussed in the subsequent sections.

4.1.1 Effect of Temperature on WMRE-AgNPs Synthesis

The temperature of the reaction mixture was varied to determine the optimal temperature value. To investigate the effect of temperature on WMRE-AgNPs, different values of temperature were used in the synthesis of the AgNPs. The temperature range applied was 40 °C to 80 °C as described in section 3.4.4.1.

A broad peak was observed at 447 nm as shown in Figure 4.1, for the colloidal suspension obtained after heating the reaction mixture at 40 °C. As the temperature was increased from 40 °C to 80 °C, a blue shift (hypsochromic shift) occurs due to reduction in the size of particles. The bands become narrow. Broadening and a red shift are attributed to agglomeration or increase in size of the particles (Anamika et al., 2012). The colloidal yellowish-brown solution, obtained after 23 minutes at 80 °C, exhibited an absorption peak at 404 nm which was consistent with the formation of AgNPs (Reza et al., 2011).
The optimum temperature required for the completion of reaction was found to be 80 °C and the reaction time was 23 minutes. It was observed that reduction rate of silver ions increased by the increasing of the temperature to 80 °C. However at temperatures above 80 °C the agglomeration of the AgNPs occurred very fast within 2 minutes and no AgNPs were formed. This could be attributed to a higher rate of growth of nuclei compared to the rate of formation of nuclei. Muhammad et al. (2012) reported similar results and attributed the trend to the increased solubility of the water soluble phenolic at higher temperatures. The position (wavelength) of the absorbance peak depends on the size of the synthesized nanoparticle (Daizy, 2010).

**Figure 4.1:** UV-Vis absorption spectra of WMRE-AgNPs sample A4WM5 showing the effect of variation of temperature on WMRE-AgNPs synthesis.
4.1.2 Effect of pH on WMRE-AgNPs Synthesis

The synthesis of AgNPs by WMRE was performed over a pH range of 6–10. Figure 4.2 shows the UV-Vis spectra of WMRE-AgNPs showing the effect of variation of pH on WMRE-AgNPs synthesis as described in section 3.5.2.

At pH 6, the UV-Vis absorption peak is very broad as shown in Figure 4.2; this result might be due to the increase in size of the AgNPs. The increase in size of AgNPs at low pH to form large nanoparticles is favoured over the nucleation (Sathishkumar et al., 2009).

There was a blue shift (hypsochromic shift) as the pH increased from pH 8 to pH 10 due to reduction in size of particles. A narrowing of the peak was also observed. Broadening and red shift are attributed to agglomerization or increase in size of the particles. This indicated that the AgNPs size became smaller with increase in the pH. As the pH level increases, the bulk concentration of H+ ions decreases, resulting in a higher surface charge on the particle (Barisik et al., 2014).

At higher pH, the large number of phenolic functional groups available for silver binding facilitated a higher number of Ag+ ions to bind and subsequently form a large number of nanoparticles with smaller diameters. There was no formation of AgNPs at pH<5, this phenomenon could be due to the instability of the nanoparticles at acidic pH (Sadowski et al., 2008). At pH values above 10, the reaction occurred very fast within 2 minutes and agglomeration was observed and no AgNPs were formed. This indicated that the rate of nuclei growth was faster than that of nuclei formation. This result confirmed the vital role
played by pH in controlling the size of the AgNPs. The optimum pH chosen for the synthesis was pH 10.

![UV-Vis absorption spectra of WMRE-AgNPs sample A4WM5 showing the effect of variation of pH on WMRE-AgNPs synthesis.](image)

**Figure 4.2:** UV-Vis absorption spectra of WMRE-AgNPs sample A4WM5 showing the effect of variation of pH on WMRE-AgNPs synthesis.

### 4.1.3 Optimization of Reactants Ratio on WMRE-AgNPs Synthesis

The nature of AgNPs formed under different reactant ratios was investigated. The ratio of reactants was used to determine the optimal reactant concentration. From Figure 4.3, it can be observed that there is a red shift and the broad absorption peaks suggest that the AgNPs in samples A1WM1, A1WM4 and A1WM5 are agglomerated AgNPs. The intense narrow absorption peaks of samples A2WM3, A3WM5 and A4WM5 as shown in Figure 4.3 indicates that the AgNPs in the samples are of relatively smaller sized AgNPs.

The sample that exhibited a sharp, narrow intense peak of maximum absorption was A4WM5 as shown in figure 4.3. This was indicative of the formation of relatively smaller sized AgNPs because the sharpness in absorbance peak depends on the size of the
synthesized nanoparticle (Daizy, 2010). Sample A4WM5 was the best sample obtained after optimizing the reaction conditions and was therefore chosen for all subsequent investigations of the reaction parameters. The Appendix section shows tables 4.4, 4.5, 4.6, and 4.7 which show the effect of the variation of the reaction parameters on WMRE-AgNPs synthesis.

These ratios of reactants represented different concentrations of the reactants. The ratio that gave the optimal reactant concentration was 4:5. The ideal concentration of the reactants was $4.44 \times 10^{-4}$ M AgNO$_3$ and 138.89 g/L of WMRE.

![UV spectra of WMRE-AgNPs samples under optimized conditions of temperature (80 °C) and pH 10.](image)

**Figure 4.3:** UV spectra of WMRE-AgNPs samples under optimized conditions of temperature (80 °C) and pH 10.
An overlay of the UV-Vis Spectra of watermelon rind extract (WMRE), AgNO₃ (0.001 M) solution, sample A4WM5 and sample SD₁ (Citrate-AgNPs) in figure 4.4 shows that the peaks within the spectral range of 400-450 nm which represent the maximum absorption peaks of samples A4WM5 and SD₁ (Citrate-AgNPs) are not due to unreacted watermelon rind extract and unreacted AgNO₃ 0.001 M solution. Spherical AgNPs are known to show an intense absorption peak within the wavelength range of 380-450 nm (Pandey et al., 2012). This suggested that the AgNPs in sample A4WM5 were of a smaller size than those in sample SD₁ (Citrate-AgNPs) because small size AgNPs absorb and scatter electromagnetic radiation at shorter wavelengths than larger size AgNPs (Alzoubi and Bidier, 2013). Figure 4.4 shows that the maximum absorption peaks of samples A4WM5 and SD₁ (Citrate-AgNPs) are not due to unreacted watermelon rind extract and unreacted AgNO₃ 0.001 M solution.

**Figure 4.4:** UV-Vis Spectra of watermelon rind extract (WMRE), AgNO₃ (0.001 M) solution, sample A4WM5 and sample SD₁ (Citrate-AgNPs).
4.1.4 Effect of AgNO₃ Concentration on WMRE-AgNPs Synthesis

The absorbance peak of the WMRE-AgNPs at low concentration of AgNO₃ (0.0001 M) is broad and less intense as shown in figure 4.5. This indicates that the AgNPs are agglomerated. However, as the AgNO₃ concentration increases gradually from 0.0001 M to 0.001M, the absorbance peak becomes sharper and intense and a blue shift occurs. A blue shift is consistent with a reduction in the size of the nanoparticles. This suggests that number of the WMRE-AgNPs increase and also get relatively smaller as the AgNO₃ solution concentration increases to 0.001 M. This can be attributed to the increased number of silver ions that are available for reduction by the reductant to AgNPs as the AgNO₃ concentration increases. These results compare well with those obtained by Begum et al. (2009).

Figure 4.5: UV-Vis spectra of WMRE-Ag NPs showing the effect of variation of AgNO₃ concentration on WMRE-AgNPs synthesis.
4.1.5 Effect of WMRE Concentration on AgNPs Synthesis

The absorbance peak of the WMRE-AgNPs when the WMRE concentration was 100 g/L is broad and less intense as shown in figure 4.6 and occurs at 456 nm. However, as the WMRE concentration increases gradually from 100 g/ L to 250 g/L, the absorbance peak becomes more narrow and intense. There is a gradual blue shift as the WMRE concentration increases from 100 g/ L to250 g/L (Figure 4.6).

A blue shift coupled with sharp and intense absorbance peak is associated with a reduction in the size of AgNPs. Since at lower extract concentration a smaller number of nucleation sites would be present so more reduction would take place at one nuclei leading to formation of a bigger particle. It is also possible that at higher concentrations the polyphenols in the WMRE had effectively reduced the Ag\(^+\) ions to Ag\(^0\) and provided enough capping agent for the stabilization of the synthesized nanoparticles through steric hindrance thus preventing their aggregation, which probably leads to the formation of smaller particles at a higher extract concentration (Rastogi and Arunachalam, 2013). These results are in agreement with those obtained by Subramanian et al. (2013).

![Figure 4.6: UV-Vis spectra of WMRE-AgNPs showing the effect of variation of WMRE concentration on WMRE-AgNPs synthesis.](image-url)
4.2 Electrochemical Characterization of AgNPs Samples

4.2.1 Cyclic Voltammetry

Cyclic voltammetry or CV is a type of potentio-dynamic electrochemical measurement. CV takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When cyclic voltammetry reaches a set potential, the working electrode's potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram trace. Cyclic voltammetry is generally used to study the electrochemical properties of an analyte in solution (Ying et al., 2013).

The electrochemical detection of metal nanoparticles can be carried out in two ways: immobilizing the nanoparticles on the electrode surfaces or direct detection of the nanoparticles hitting the surface of the electrode (Pumera and Giovanni, 2012).

4.2.2 Cyclic Voltammetry Experiments

The C.V experiments were performed with bare Glassy Carbon electrode using the procedure described in section 3.5.2.2. No peaks were observed in the resulting voltammogram as presented in Figure 4.7. This was expected and indicative of the absence of an electro-active species. Similar results were reported by Pumera and Giovanni (2012).
Figure 4.7: Cyclic voltammogram of bare Glassy Carbon electrode.

4.2.3 Cyclic Voltammetry of standard-AgNPs (0.02 mg/mL, 20 nm AgNPs colloidal dispersion)

The cyclic voltammogram obtained exhibited a distinct anodic peak at 291 mV at a scan rate of 100 mV/s as shown in Figure 4.8. The electrochemical experiments were carried out at room temperature (25 °C).
4.2.4 Cyclic Voltammetry of AgNPs prepared using Watermelon Rind Extract

The cyclic voltammogram obtained exhibited a distinct anodic peak at 290 mV at a scan rate of 100 mV/s as shown in figure 4.9. The electrochemical experiments were carried out at room temperature (25 °C).

Cyclic Voltammetry experiments on the standard Ag NPs (0.02 mg/mL, 20 nm AgNPs colloidal dispersion) and AgNPs samples prepared using watermelon rind extract as the reductant and stabilizing agent were carried out to confirm whether AgNPs were present in the samples prepared using the green chemistry method.
The cyclic voltammogram of standard Ag NPs exhibited distinct oxidation and reduction peaks at +290 mV and +100 mV respectively while the peak current increases with increase in the scan rate (Figure 4.8 section 4.2.3).

The cyclic voltammogram of AgNPs sample prepared using watermelon rind extract as the reductant shows a distinct oxidation peak at +291 mV and the peak current increases with increase in the scan rate (Figure 4.9).

Different sized AgNPs exhibit different voltammetric profiles (Pumera and Giovanni, 2012). The slight difference in the position of oxidation peak potential of the standard AgNPs (290 mV) and Watermelon rind extract-AgNPs (291 mV) as shown in figure 4.10 could be due to a difference in the sizes of AgNPs.

**Figure 4.9:** Cyclic voltammogram of WMRE-AgNPs sample at different scan rates.
Figure 4.10: Overlay of cyclic voltammograms of standard reference AgNPs (0.02 mg/mL, 20 nm AgNPs colloidal dispersion) and WMRE-AgNPs.
4.3 Transmission Electron Microscopy (TEM)

The WMRE-AgNPs formed were spherical in shape with an average hydrodynamic diameter of 17.96 nm while the citrate-AgNPs were spherical with an average hydrodynamic diameter of 36.96 nm. The size distribution was uniform. Thus, the watermelon rind aqueous extract as a reductant and capping agent yielded smaller and more stable AgNPs than those obtained using trisodium citrate as the reductant and capping agent as shown in figure 4.11 (a), (b) and (c).

![TEM Micrographs of WMRE-AgNPs](image)

**Figure 4.11:** TEM Micrographs of WMRE-AgNPs (a) average diameter 17.96 nm, Citrate-AgNPs (b) average diameter 36.96 nm, (c) std-reference AgNPs average diameter 20.0 nm.
4.4 Antimicrobial Activity Experiments

Agar disc plates showing inhibition zones of *E.coli* and *S.typhi* as shown in Figure 4.12 (a), (b),(c), 4.13 (d),(e), (f) and (g). The Std-AgNPs and WMRE-AgNPs inhibited the growth of gram-negative *E.coli* and *S.typhi* clinical isolates that were used in the study. The actual bactericidal mechanism of silver nanoparticles is not clear. Several studies have investigated the interaction of the AgNPs with bacteria. Sondi and Salopék (2004) revealed that the AgNPs were localized on the membranes of treated *E. coli* cells. Lok *et al.* (2006) reported that the antibacterial action of AgNPs results from damage of the bacterial outer membrane and that the biological mode of action is similar to that of silver nitrate. AgNPs can induce structural disintegration in the bacterial membrane and then fragment the cell (Yun *et al.*, 2013). It has also been demonstrated that Ag ions interact with disulfide or thiol groups of enzymes that lead to disruption of metabolic processes which in turn cause cell death (Egger *et al.*, 2009).

![Image of agar disc plates showing inhibition zones](image)

**Figure 4.12:** Inhibition zone against *E. coli* and *S. typhi* (a) silver nitrate (0.001 M), (b) WMRE (250 g/L)
Figure 4.12: (c): Inoculated plates showing the zone of inhibition due to standard antibiotics (Ciprofloxacin (10 µg per disc) and Vancomycin (10 µg per disc) against S.typhi and E.coli.

Figure 4.13: (d) Std-AgNPs against S.typhi  (e) Std-AgNPs against E.coli, (f) WMRE-AgNPs against E.coli and (g) WMRE-AgNPs against S.typhi showing the zones of inhibition.
4.4.1 Antibacterial Activity of WMRE-AgNPs

Antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition for the test organisms. Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA, SNK-test). Table 4.1, shows that inhibition of bacterial growth is concentration dependent and that the change in concentration of the test antibiotics was statistically significant (p<0.05).

Table 4.1: Effect of WMRE-AgNPs Concentration on E.coli and S.typhi

<table>
<thead>
<tr>
<th>WMRE–Ag NPs Concentration ( %)</th>
<th>E.coli Zone of Inhibition Diameter (mm) Mean±SE</th>
<th>S.typhi Zone of Inhibition Diameter (mm) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.125</td>
<td>13.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.25</td>
<td>15.33±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.33±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>12.5</td>
<td>17.33±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.33±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>19.33±0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.00±0.00&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>22.67±0.33&lt;sup&gt;e&lt;/sup&gt;</td>
<td>18.67±0.33&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>25.00±0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20.33±0.33&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>29.90±0.00&lt;sup&gt;g&lt;/sup&gt;</td>
<td>23.50±0.00&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small letter (a, b, c, d, e, f and g) within the same column are not significantly different from one another (one way ANOVA, SNK-test).
4.4.2 Std-AgNPs Antibacterial Activity

Antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition for the test organisms. Mean values followed by same small letter within the same column are not significantly different from one another (one way ANOVA, SNK-test). Table 4.2 shows that inhibition of bacterial growth is concentration dependent and that the change in concentration of test antibiotics is statistically significant (p<0.05).

Table 4.2: Effect of Std-AgNPs Concentration on *E.coli* and *S.typhi*

<table>
<thead>
<tr>
<th>std–Ag NPs Concentration ( %)</th>
<th><em>E.coli</em> Zone of Inhibition Diameter (mm) Mean±SE</th>
<th><em>S.typhi</em> Zone of Inhibition Diameter (mm) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.125</td>
<td>13.50±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.67±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>6.25</td>
<td>14.33±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.33±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12.5</td>
<td>18.33±0.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.00±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>20.33±0.33&lt;sup&gt;d&lt;/sup&gt;</td>
<td>17.17±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>23.50±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>19.00±0.58&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>75</td>
<td>24.50±0.00&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20.83±0.17&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>29.50±0.00&lt;sup&gt;g&lt;/sup&gt;</td>
<td>23.00±0.00&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small letter (a, b, c, d, e, f and g) within the same column are not significantly different from one another (one way ANOVA).
4.4.3 Antibacterial Activity of Standard Antibiotics and Control

Antimicrobial activity was evaluated in triplicate by measuring the zone of inhibition for the test organisms. Mean values followed by same small letter within the same column are not significantly different from one another while Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA).

The results shown in tables 4.1, 4.2 and 4.3 (a) and 4.3 (b) reveal that both clinical isolates of *E.coli* and *S.typhi* were susceptible to the antimicrobial activity of WMRE–Ag NPs, standard antibiotics and the standard-Ag NPs since the inhibition zone diameter was > 21 mm. The MIC value of the nanoparticles was found to be 45.00 ± 0.01 µg/ml for *S.typhi* and 38.50 ± 0.00 µg/ml for *E.coli* while the MBC value was found to be 60.00 ± 0.05 µg/ml for *S.typhi* and 50.00 ± 0.00 µg/ml for *E.coli*. These results are in close agreement to those demonstrated by Ruparelia *et al.* (2008) that applied ANOVA and reported a MIC of 40 µg/ml and MBC of 60 µg/ml for *E.coli*. 
Table 4.3 (a): Zones of Inhibition Diameter (mm) of Antibiotics Against *E. coli* and *S. typhi*

<table>
<thead>
<tr>
<th>Antibiotics and control</th>
<th><em>E. coli</em> Zone of Inhibition Diameter (mm) Mean±SE</th>
<th><em>S. typhi</em> Zone of Inhibition Diameter (mm) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001M AgNO3</td>
<td>13.83±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.17±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>250g/L WMRE</td>
<td>10.17±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.50±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>WMRE-Ag NPs 40 µg/mL</td>
<td>29.90±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.50±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>std- Ag NPs 40 µg/mL</td>
<td>29.50±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
<td>23.00±0.00&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>10µg Ciproflaxacin</td>
<td>35.17±0.17&lt;sup&gt;f&lt;/sup&gt;</td>
<td>33.17±0.17&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>10µg Vancomycin</td>
<td>24.17±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.17±0.17&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sterile distilled water</td>
<td>6.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.00±0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small letter (a, b, c, d, e, f and g) within the same column are not significantly different from one another (one way ANOVA).
Table 4.3 (b): Zones of Inhibition Diameter (mm): Standard Table of Antibiotics

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Disk Concentration</th>
<th>Diameter of Inhibition Zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10 μg</td>
<td>≤13</td>
</tr>
<tr>
<td>Carbenicillin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100 μg</td>
<td>≤13</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30 μg</td>
<td>≤14</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30 μg</td>
<td>≤14</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2 μg</td>
<td>≤14</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 μg</td>
<td>≤15</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 μg</td>
<td>≤13</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 μg</td>
<td>≤12</td>
</tr>
<tr>
<td>Methicillin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5 μg</td>
<td>≤9</td>
</tr>
<tr>
<td>Penicillin G&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10 units</td>
<td>≤28</td>
</tr>
<tr>
<td>Penicillin G&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10 units</td>
<td>≤14</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>250 or 300 μg</td>
<td>≤12</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30 μg</td>
<td>≤14</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30 μg</td>
<td>≥15</td>
</tr>
<tr>
<td>Vancomycin&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30 μg</td>
<td>≤14</td>
</tr>
</tbody>
</table>

R: Resistant, I: Intermediate, S: Susceptible
4.4.4 Chlorogenic Acid Experiments

4.4.4.1 The UV-Visible Spectra of Chlorogenic Acid Standard Solution
The UV-Visible spectra of chlorogenic acid showed two maximum absorption peaks, the first peak at 217 nm with a shoulder at 240 nm while the second peak was the highest and occurred at 325 nm with a shoulder at 295 nm. Figure 4.14 shows an overlay of the UV-Visible spectra of Chlorogenic acid (CGA), WMRE and WMRE-AgNPs colloidal dispersion.

Watermelon rind contains Chlorogenic acid as demonstrated by Al-Sayed and Ahmed (2013). Figure 4.14 (b) and table 4.8 in the appendix shows there is a difference (statistically significant, p<0.05) in the amount of Chlorogenic acid in the WMRE and WMRE-AgNPs suspension. This could be attributed to the possible role of the Chlorogenic acid acting as a reductant in reducing Ag\(^+\) to Ag\(^0\).

![Figure 4.14 (a): Structure of Chlorogenic Acid.](image)

Chlorogenic acid as shown in figure 4.14 (a) has phenolic hydroxyl groups which are capable of donating a hydrogen atom and in the process forming a stable phenolate anion which is resonance stabilized.
Figure 4.14 (b): Overlay of the UV-Visible spectra of Chlorogenic acid (CGA), WMRE and WMRE-Ag NPs colloidal dispersion.
CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The green method synthesis of AgNPs was successful with preparation of AgNPs. The method utilizes *Citrullus lanatus* fruit rind as a reductant and a capping agent in the synthesis of AgNPs through Green Chemistry. This study has revealed that Chlorogenic acid, a water soluble phenolic acid contained in the watermelon rind maybe involved in the reduction of Ag$^+$ ion to Ag$^0$. This method yields stable, spherical silver nanoparticles with an average hydrodynamic diameter of 17.96 nm that is comparable with the conventional method that uses sodium borohydride as the reductant.

The ultraviolet-visible spectrum of the green synthesized AgNPs showed a maximum absorption peak at 404 nm which is consistent with the spectrum of spherical AgNPs. These results are in close agreement with those reported by Muhammad *et al*, 2012. The Cyclic Voltammetry of the WMRE-AgNPs exhibited peak at a potential of 290 mV while the standard reference AgNPs showed a distinct peak at a potential of 291 mV. This compares well with the work of Pumera and Giovanni, 2012. The antimicrobial activity of the WMRE-AgNPs against clinical isolates of *E.coli* and *S.typhi* was evaluated using the disc-diffusion method. The MIC of the nanoparticles was found to be 45.00 ± 0.01 μg/ml for *S.typhi* and 38.50 ± 0.00 μg/ml for *E.coli* while the MBC was found to be 60.00 ± 0.05 μg/ml for *S.typhi* and 50.00 ± 0.00 μg/ml for *E.coli*.

The study has also demonstrated that the WMRE-AgNPs synthesized were effective against *E.coli* and *S.typhi*. 
5.2 Recommendations

i. The green method developed in this study should be adopted for synthesis of AgNPs.

ii. This work specifically dealt with fresh watermelon rinds and based on the results of our study we can recommend that the potential of dry watermelon rind in AgNPs synthesis be determined.

iii. This study demonstrated that WMRE-AgNPs were effective against *E.coli* and *S.typhi* which are gram-negative bacteria. Therefore, we can recommend the determination of the antimicrobial activity of the WMRE-AgNPs against gram-positive bacteria.
REFERENCES


www.researchgate.net figure/47613038 (accessed 31/08/2017)


APPENDIX I: TABLES

Mean values followed by same small letter (a, b, c, d, e, f and g) within the same column are not significantly different from one another (one way ANOVA). Mean values followed by different small letter within the same column are significantly different from one another (one way ANOVA).

Table 4.4: Effect of Variation of Temperature on WMRE-AgNPs Synthesis

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>Absorbance (WMRE-Ag NPs) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>1.037±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>1.087±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>1.247±0.003&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>70</td>
<td>1.513±0.013&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>80</td>
<td>1.740±0.000&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 4.5: Effect of Variation of pH on WMRE-AgNPs Synthesis.

<table>
<thead>
<tr>
<th>pH</th>
<th>Absorbance (WMRE-AgNPs) Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 6</td>
<td>0.936±0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 8</td>
<td>1.147±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 9</td>
<td>1.363±0.003&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH 10</td>
<td>1.680±0.000&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small letter (a, b, c and d) within the same column are not significantly different from one another (one way ANOVA).
Table 4.6: Effect of Variation of AgNO₃ Concentration on WMRE-AgNPs Synthesis

<table>
<thead>
<tr>
<th>AgNO₃ Concentration</th>
<th>Absorbance (WMRE-Ag NPs) Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0001M</td>
<td>0.914±0.000^a</td>
</tr>
<tr>
<td>0.0002M</td>
<td>1.147±0.003^b</td>
</tr>
<tr>
<td>0.0004</td>
<td>1.357±0.003^c</td>
</tr>
<tr>
<td>0.0008M</td>
<td>1.467±0.003^d</td>
</tr>
<tr>
<td>0.001M</td>
<td>1.707±0.003^e</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small (a, b, c, d and e) letter within the same column are not significantly different from one another (one way ANOVA).

Table 4.7: Effect of WMRE Concentration on WMRE-AgNPs Synthesis

<table>
<thead>
<tr>
<th>WMRE concentration</th>
<th>Absorbance (WMRE-AgNPs) Mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 g/L</td>
<td>0.80±0.00^a</td>
</tr>
<tr>
<td>150 g/L</td>
<td>1.17±0.03^b</td>
</tr>
<tr>
<td>200 g/L</td>
<td>1.50±0.00^c</td>
</tr>
<tr>
<td>250 g/L</td>
<td>1.76±0.00^d</td>
</tr>
<tr>
<td>p-value</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

There is a significant effect of dose on the absorbance of particles (P<0.05, one-way ANOVA, α=0.05).
Table 4.8: Absorbance of Chlorogenic Acid Std Solution, WMRE and WMRE-AgNPs Colloidal Dispersion

<table>
<thead>
<tr>
<th>λ nm</th>
<th>Absorbance of Chlorogenic Acid std</th>
<th>Absorbance of WMRE</th>
<th>Absorbance of WMRE-AgNPs</th>
</tr>
</thead>
<tbody>
<tr>
<td>217 nm</td>
<td>0.782±0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.346±0.000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.210±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>240 nm</td>
<td>0.530±0.000&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.200±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.130±0.000&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>295 nm</td>
<td>0.864±0.001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.461±0.000&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.240±0.003&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>325 nm</td>
<td>0.985±0.001&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.730±0.002&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.361±0.000&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>p value</td>
<td>&lt; 0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Mean values followed by same small letter (a, b, c and d) within the same column are not significantly different from one another (one way ANOVA).
APPENDIX II: LIST OF PUBLICATIONS
APPENDIX III: LIST OF CONFERENCE PRIZE AWARDS
