



***ALLIUM ASCALONICUM* PLANT MEDIATED SYNTHESIS OF  
SILVER NANOPARTICLES AND EVALUATION OF THEIR  
ANTIMICROBIAL ACTIVITY**

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## **DEDICATION**

This is dedicated to my mother Mavis, my brother Matthew and my sister Mary who have always been there for me.

## **ACKNOWLEDGEMENTS**

First of all, I would like to thank the Lord Almighty who has blessed and protected me my entire life. Sincere thanks to my academic supervisors Dr F. Chigondo and Miss T. Murinzi who guided me every step of the way. I would also like to extend my gratitude to the entire Midlands State University and Chemical Technology Department staff for their assistance in this research.

I would like to thank my family, especially my mother who has always had faith in me and supported me every bit of the way.

## ABSTRACT

The increasing demand for silver nanoparticles has led to the need for new superior methods of synthesis to be developed. In this study, the *Allium Ascalonicum* plant was used in the plant-mediated synthesis of silver nanoparticles. The phytochemicals of the plant were extracted using seven different solvents with a range of polarities and the methanol/water <sup>50</sup>/<sub>50</sub> solvent had the highest extraction yield of 63 %. The phytochemical tests showed that the plant is rich in phytochemicals such as flavonoids, alkaloids, steroids and glycosides which possess great potential for reducing and capping silver nanoparticles. The optimum conditions for the synthesis of silver nanoparticles were determined to be 6 mL of the plant extract, 8 mM silver nitrate solution at a 50 °C temperature and a reaction time of 3 hours. The synthesised silver nanoparticles showed great inhibition against the *Escherichia coli* and the *Staphylococcus aureus* bacteria, exhibiting maximum inhibition zones of 23 mm and 24 mm respectively. In conclusion, the *Allium Ascalonicum* showed great potential as an alternative for the synthesis of silver nanoparticles and the plant-mediated silver nanoparticles showed great potential as alternatives to antibiotics.

## **DECLARATION**

I, David Kudakwashe Nyaguze do hereby declare that I am the sole author of this dissertation and I authorize the Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research.

Signature.....

Date.....

## **APPROVAL**

This dissertation entitled “*ALLIUM ASCALONICUM* PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES AND EVALUATION OF THEIR ANTIMICROBIAL ACTIVITY” by David Kudakwashe Nyaguze meets the regulations governing the award of the degree of Chemical Technology of the Midlands State University and is approved for its contribution to knowledge and literal presentation.

Supervisor .....

Date .....

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## LIST OF ABBREVIATIONS

AgNPs	- Silver nanoparticles
AST	- Antimicrobial Susceptibility Testing
<sup>13</sup> CNMR	- <sup>13</sup> Carbon Nuclear Magnetic Resonance
DNA	- Deoxyribonucleic Acid
EHEC	- <i>Enterohemorrhagic Escherichia coli</i>
FTIR	- Fourier Transform Infrared Spectrometry
HPLC	- High Pressure Liquid Chromatography
HRMR	- High Resolution Mass Spectroscopy
MH	- Mueller-Hinton
NCCLS	- National Committee for Clinical Laboratory Standards
PVP	- Poly vinyl pyrrolidone
PWD	- Pulsed wire discharge
SEM	- Scanning Electron Microscopy
SERS	- Surface-Enhanced Raman Scattering
SPR	- Surface Plasmon Resonance
TEM	- Transmission Electron Microscopy
TMT	- Trimethoprim
UV-VIS	- Ultra Violet-Visible Spectrometry

WHO - World Health Organisation

XRD - X-ray Diffraction

## CHAPTER ONE

### 1.0 Introduction

This chapter gives an insight on the background, aim and objectives of the research. It also provides an overview of the problem statement and the justification of this particular research.

### 1.1 Background

Nanoscale materials are structures ranging from 1 to 100 nm, as defined in the chemistry context, which have contributed to the development of nanoscience and nanotechnology at an exponential rate in recent years [1]. Over the last decades, silver nanoparticles have found applications in catalysis, optics, electronics and other areas due to their unique size-dependent optical, electrical and magnetic properties [2]. Nanoparticles have also been introduced to the medical, pharmaceutical and engineering field in recent years. Samsung has created and marketed a material called Silver nano that includes silver nanoparticles on the surfaces of household appliances [2]. Various metals have been used for the synthesis of stable dispersions of nanoparticles which are useful in the areas of photography, catalysis, biological labelling, photonics, optoelectronics and Surface-Enhanced Raman Scattering (SERS) detection [3].

Nanomaterials show unique and considerable changed physical, chemical and biological properties compared to their macro-scaled counterparts [3]. The transition from micro to nano particles can lead to a number of changes in the physical properties. Two of the major factors in this are the increase in the ratio of surface to area volume, and the size of the particle moving into the realm of quantum effects predominate. The increase in the surface-area-to-volume ratio, which is a gradual progression as the particle gets smaller, leads to an increasing dominance of the behaviour of atoms on the surface of the particle over that of those in the interior of the particle. This affects the properties of the particle in isolation and its interaction with other materials [4].

Silver nanoparticles (AgNPs) have emerged as one of the most intensively studied areas in the field of nanotechnology due to their well-known effectiveness in biomedical, electronic, catalytic and optical applications. Silver is the single most used material in all of nanotechnology and the desirable antimicrobial property activity of Ag is drastically increased at the nanoscale [5]. Silver is one of the most commercialized nanomaterials with five hundred tons of silver nanoparticles production per year and is estimated to increase in next few years. Including its profound role in the field of high sensitivity bio-molecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities [6]. The use of silver begun in historic times as it has been used due to its disinfecting effect in the macro scale, it has been used from traditional medicines to conventional equipment used in medicine to culinary utensils.

Antimicrobial properties of silver nanoparticles and silver ions have been known for decades. Silver in both forms not only has suppressive effects on bacterial growth but also efficiently destroys existing bacteria. Recent reports have indicated that AgNPs exhibit toxic effects on the most pathogenic bacteria posing a health risk during wound healing, and postoperative recovery [7]. Silver has long been recognized as having an inhibitory effect on microbes present in medical and industrial processes. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds. Further, these biologically synthesized nanoparticles were found highly toxic to different multi-drug resistant human pathogens [8].

Nanoparticles can be synthesized by two main approaches which encapsulate the various specific synthesis methods. In the bottom-up approach, the structure of the nanoparticles is constructed by atoms, molecules or clusters. In the top-down approaches, a bulk piece of a required material is

reduced to nanosized dimensions using cutting, grinding and etching techniques, i.e., nanomaterials are prepared from larger entities without atomic-level control [9]. Chemical reduction, microemulsion (colloidal) techniques, sonochemical reduction, electrochemical, microwave-assisted, and hydrothermal synthesis are the main techniques for the synthesis of nanoparticles through the chemical approach. Biological or biosynthesis techniques are also considered as bottom-up or chemical processes. Physical methods for nanoparticles synthesis are laser (pulse) ablation, vacuum vapour deposition, pulsed wire discharge (PWD) and mechanical milling [9].

The conventional methods of nanoparticles synthesis have resulted in several long-term problems and environmental challenges and this has led to the extended interest in the plant-mediated synthesis method of nanoparticle production. Green synthesis techniques in nanotechnology refer to the use of moderately pollutant free chemicals in the synthesis of nanomaterials. This kind of synthesis seeks to reduce pollution at the source by using benign solvents such as water and plant extract. With increasing focus on green chemistry, natural compounds like glucose, chitosan, soluble starch and some microorganisms, have attracted considerable research interest as safer alternatives, reducing and stabilizing agents to synthesize nanoparticles. Green synthesis is a preferred alternative of synthesis since is safer for the biological systems, environmentally friendly and physical and chemical characteristics of nanoparticles are still suitable for biomedical use [10].

The green synthesis approach has been aided by the use several plants being used and these include plants such as *Crataegus douglasii* [11], *Vitis vinifera* [12], *Aloe vera* [13], *Allium sativum* [14] and *Solanum trilobatum* [15]. Phytochemicals are the chemicals that present naturally in plants and these are responsible for the plant's medicinal properties. These phytochemicals have become

more popular due to their countless medicinal uses in both traditional and modern medicines. Phytochemicals play a vital role against a number of diseases such as asthma, arthritis and cancer. and unlike pharmaceutical chemicals, these phytochemicals do not have any side effects [16].

*Allium ascalonicum*, commonly known as shallots were used in the plant synthesis of silver nanoparticles in this current research. *Allium ascalonicum* (Shallot) is an annual herbaceous plant which is from the *Allium* genus which includes common species such as *Allium sativum* (garlic), *Allium cepa* (onions), *Allium schoenoprasum* (chives). *Allium* species have been subjected to thorough phytochemical analysis and the phytochemical analysis has revealed that the ratios and amounts of the characteristic sulphur compounds vary with the part of the plant sampled (bulb, stem, leaves, flowers), with the growth stage, if the plant has been harvested, and with the storage conditions [14]. This research explores the use of *Allium ascalonicum* extracts in the synthesis of AgNPs and the evaluation of their antibacterial activity.

## **1.2 AIM**

Plant-mediated synthesis of silver nanoparticles using *Allium Ascalonicum* plant extract and evaluation of their antimicrobial activity.

## **1.3 OBJECTIVES**

- To analyse *Allium Ascalonicum* powder using FTIR
- To extract phytochemicals from the bulbs of the *Allium Ascalonicum* plant using different solvents
- To carry out qualitative phytochemical tests of the plants extracts
- To separate the plants extracts using column chromatography
- To optimize the conditions necessary for the synthesis of silver nanoparticles (temperature,

the concentration of silver nitrate, reaction time and volume of plant extract) using UV-VIS spectroscopy

- To analyse the synthesised silver nanoparticles using FTIR and UV-VIS
- To investigate the stability of the synthesised nanoparticles
- To investigate the antimicrobial activity of the plant extract and the silver nanoparticles using the Kirby-Bauer disc diffusion method.

#### **1.4 Problem statement**

Many of the current nanoparticle synthesis or production methods involve the use of hazardous chemicals, low material conversions, high energy requirements, difficult and wasteful purifications [17] thus new methods with less hazardous and costly methods are needed. There is a need to design an economic, commercially feasible as well environmentally sustainable route of synthesis of silver nanoparticles in order to meet its growing demand in diverse sectors [18]. Majority of the currently prevailing synthetic methods are usually dependent on the use of organic solvents because of hydrophobicity of the capping agents used [18]. Chemical-based synthesis techniques are discouraged as they involve the use of noxious reducing and/or stabilizing agents like sodium borohydride and N,N-dimethylformamide and toxic solvents [19]. Development of new, effective and low-cost antimicrobial agents has been an object of research activity of many groups due to the build-up of resistance of microbial organisms to traditional antibiotics [20]. Antibiotic resistance is considered as one of the greatest health threats by the World Health Organization (WHO). The first case of bacterial resistance could be traced back to 1967 in which penicillin-resistant *Streptococcus pneumoniae* (*S. pneumonia*) was reported in Australia [5]. Plants constitute of various important phytochemicals which have not been extensively studied in their natural form. It is now firmly established that gastric and duodenal ulcers are generally caused by

*H. pylori* which survives and grows in acidic environments and although an eradication rate of more than 80 % has been reported by the use of relevant therapy, different side-effects including the emergence of antibiotic-resistant in *H. pylori* due to overuse of antibiotics are still to be addressed [19]. The ability of such microbial caused diseases and infections to resist the currently used antibiotics has led to the need for new agents that possess pharmacological effects to be discovered. The antimicrobial effect of natural products and the comparison between results is often difficult, because of the use of different non-standardized approaches, inoculum size, growth medium, incubation conditions and end-points determination [21]. *Staphylococcus aureus* (*S. aureus*) is an opportunistic pathogen in human and other different animal species. The pathogen is mainly related to food poisoning and is the third largest cause of food-related illness throughout the world. *S. aureus* can cause a number of infectious diseases such as dermatitis, pneumonia, meningitis, osteomyelitis in human, bovine mastitis in cattle and bumblefoot disease in poultry. *Methicillin* resistance in this bacterial species are very alarming for human health, as it has shown potential for zoonotic transmission [22]. *E. coli* are a large and diverse group of bacteria. Although most strains of *E. coli* are harmless, others can make people sick. Some kinds of *E. coli* cause disease by making a toxin called Shiga toxin. The bacteria live in the intestines of many animals and are usually transmitted to people when they eat foods contaminated with the bacteria[23]. Alternative methods of fighting against such microbial are needed whilst considering their drug resistance to antibiotics that are overused. Mutations in most disease and infection-causing bacteria have led to some drugs being rendered ineffective against certain diseases and infections due to the loss of the effective pharmacophore structure relative to the receptor site.

## **1.5 Justification**

In recent times, plant-mediated synthesis of nanoparticles has garnered wide interest owing to its

inherent features such as rapidity, simplicity, eco-friendliness and cheaper costs [24]. Synthesis of nanoparticles using various plant materials opens a new scope for the phytochemist and discourages the use of toxic chemicals [25]. Silver has long been recognized as having an inhibitory effect on microbes present in the medical and industrial process [8]. The synthesis of silver nanoparticles using plant extracts is the best eco-friendly alternative to available traditional chemical and physical methods. This method is mainly used for reducing the toxicity and also for the development of green chemistry [12]. Silver nanoparticles have been given more attention due to their numerous applications in catalysis, bio-molecular detection and diagnostic, therapeutic, micro-electronics fields and sensing [25]. Currently, the applications of nanomaterials are becoming increasingly important in order to address the problems associated with material sciences, including solar energy conversion, photonics, catalysis, microelectronics, antimicrobial functionalities, and water treatment [19].

Plants are recognized in the pharmaceutical industry for their broad structural diversity as well as their wide range of pharmacological activities. Phytochemicals have become more popular due to their countless medicinal uses. Phytochemicals play a vital role against a number of diseases such as asthma, arthritis and cancer. Unlike pharmaceutical chemicals, these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as “man-friendly medicines” [16]. *Allium* species are characterized by their rich content in sulphur compounds that are responsible for the organoleptic parameters and contribute to their antioxidant and antimicrobial activities [26]. The antioxidant activity of *Allium* species is due to several of sulphur-containing compounds and their precursors, but it is also related to other bioactive compounds such as polyphenols, dietary fibre and microelements [26]. The various phytochemicals present are responsible for the reduction and

capping of silver nanoparticles that are synthesized using the biological method thus it is crucial to identify these phytochemicals using methods such as column chromatography and FTIR analysis. There is an increasing interest in silver nanoparticles on account of the antimicrobial properties that they display. They are even being projected as future generation antimicrobial agents. Silver nanoparticles are important materials that have been studied extensively, such nanoparticles possess unique electrical, optical as well as biological properties and are thus applied in catalysis, biosensing, imaging, drug delivery, nanodevices fabrication and in medicine [27].

Silver nanoparticles exhibit the highest efficiency of Plasmon excitation and they are becoming an increasingly important material in many technologies. Silver nanoparticles are the only material whose Plasmon resonance can be of any wavelength in the visible spectrum [28]. Silver and most silver compounds have an oligodynamic effect and are toxic to bacteria, algae, and fungi. Among the elements that have this effect, silver is the least toxic for humans [28]. This characteristic is of very significant importance because more extensive research can now be carried out in order to discover how best silver nanoparticles can be used as substitutes for the common antibiotic. It may also be an important discovery when it comes to human health by reducing the frequent intake of antibiotics that are becoming less effective and cause various side effects.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.0 Introduction**

This chapter serves to give an introduction to nanotechnology, comparison of the various methods that are used in the synthesis of silver nanoparticles and the methods used for the characterisation of silver nanoparticles which include FTIR spectroscopy, UV-VIS spectroscopy, SEM, TEM and XRD. It also describes the plant of choice in this particular research and its constituents and their properties. It provides an insight into the potential pharmacological benefits of both the plant and the silver nanoparticles to the world.

#### **2.1 Nanoscience**

Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nanosized objects [29]. In the past decade, synthesis of nanomaterials using various plant materials is an emerging field in nanoscience, with emphasis to avoid the use of toxic chemicals and supports the development of ecofriendly technique [25].

##### **2.1.1 Nanotechnology**

Nanotechnology refers to the branch of science and engineering dedicated to materials, having dimensions in the order of 100th of nm or less. The term is new, but has been widely used for the development of more efficient technology. In recent years, nanotechnology has been embraced by industrial sectors due to its applications in the field of electronic storage systems, biotechnology, magnetic separation and pre-concentration of target analytes, targeted drug delivery and vehicles for gene and drug delivery [28]. The transition from microparticles to nanoparticles has led to a

number of changes in physical properties. Two of the major factors in this is the increase in the ratio of surface area to volume, and the size of the particle moving into the realm of quantum effects predominate. The increase in the surface-area-to-volume ratio, which is a gradual progression as the particle gets smaller, leads to an increasing dominance of the behaviour of atoms on the surface of the particle over that of those in the interior of the particle, this affects both the properties of the particle in isolation and its interaction with other material [4]. In recent years, the synthesis and utilization of novel antimicrobial metal nanoparticles have increased due to the gradual increase of drug resistance among microorganisms. For this reason, silver compounds have also been employed as antimicrobial and antifungal agents. By using nanotechnology, which enhances the antimicrobial activity of silver metal by manipulating it to the nanoparticles, silver nanoparticles have been widely used to control fungal crop diseases as well as acting as a disinfectant on farms [30]. Nanoparticles possess wondrous optical, electronic, magnetic, and catalytic properties than the bulk material owing to their high surface area to volume ratio. Metal nanoparticles like silver and gold show different colours due to their Surface Plasmon Resonance (SPR) phenomenon. It is a collective oscillation of free electrons of the metal nanoparticles in resonance with the frequency of the light wave interactions causing the SPR band to appear in the visible and infrared region [29].

### **2.1.2 Methods used in the synthesis of silver nanoparticles**

The methods used for the synthesis of silver nanoparticles are mainly categorised into two groups.

Illustrations of the two groups are shown in Figure 2.1.

#### **(a) Top-down approach**

The principle behind the top-down approach is to take a bulk piece of the material and then modify

it into the wanted nanostructure and subsequent stabilization of the resulting nanosized metal nanoparticles by the addition of colloidal protecting agents. Cutting, grinding and etching are typical fabrication techniques, which have been developed to work on the nanoscale. The sizes of the nanostructures which can be produced with top-down techniques are between 10 to 100 nm [4].

#### Advantages

1. Preparation of colloidal suspension free of contaminants and pollutions stemming from chemical compounds used during the synthesis [31].
2. Wide size distribution and diverse particle shape or geometry.

#### Disadvantages

1. Usually involve the usage of high energy sources and expensive equipment, for example in laser ablation synthesis techniques.
2. Imperfect surface structure and significant crystallographic damage to the processed patterns [32].

#### (b) Bottom-up approach

Bottom-up self-assembly refers to the construction of a structure atom by atom, molecule-by-molecule or cluster-by-cluster. Colloidal dispersion used in the synthesis of nanoparticles is a good example of a bottom-up approach. An advantage of the bottom-up approach is the better possibilities to obtain nanostructures with fewer defects and more homogeneous chemical compositions [4].

#### Advantages

1. Explore aggregation and self-organization processes which create uniform particles [31].

2. Relatively simpler, lower cost and produce higher yields.
3. Highly favourable in the synthesis of silver and other noble metal nanoparticles [33].

#### Disadvantages

1. Involve the usage of hazardous chemicals which are difficult to dispose of such as dimethyl formamide [9].
2. The nanoparticles produced from methods such as chemical reduction are unable to stabilize themselves therefore external stabilizing agents are needed such as sodium dodecyl sulphate [28].

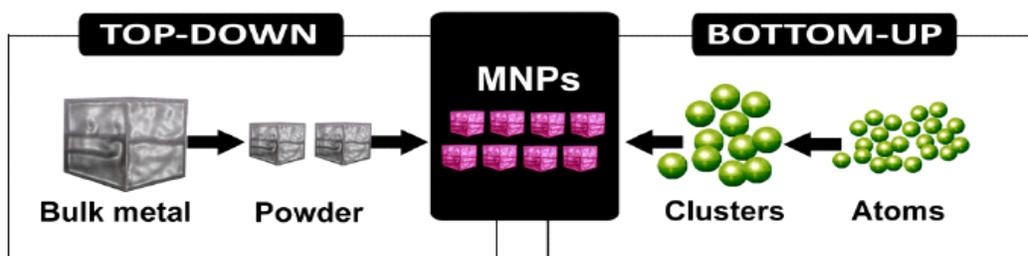


Figure 2.1: Schematic diagram of Top-down and Bottom-up synthesis methods

The particular methods used for the synthesis of silver nanoparticles all fall into two these categories. These methods are further distinguished as either physical synthesis methods, chemical reduction methods or biological synthesis methods.

#### 2.1.3 Physical synthesis methods

In physical processes, metal nanoparticles are generally synthesized by evaporation-condensation, which could be carried out using a tube furnace at atmospheric pressure. The source material within a boat centred at the furnace is vaporized into a carrier gas. Nanoparticles of various materials, such as Ag, Au, PbS and fullerene, have previously been produced using the evaporation/condensation technique. However, the generation of silver nanoparticles using a tube

furnace has several drawbacks, because a tube furnace occupies a large space, consumes a great deal of energy while raising the environmental temperature around the source material, and requires a lot of time to achieve thermal stability [4]. In order to fabricate nanoparticles, the vaporization method has been frequently used, in which the target materials are vaporized by the heat source and then rapidly condensed. The vaporization process can be subdivided into physical and chemical methods depending on whether the reaction is present [28].

#### **2.1.4 Chemical synthesis methods**

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles as stable, colloidal dispersions in water or organic solvents. Commonly used reducing agents are borohydride, citrate, and elemental hydrogen. The reduction of silver ions ( $\text{Ag}^+$ ) in aqueous solution generally yields colloidal silver with particle diameters of several nanometers. Initially, the reduction of various complexes with  $\text{Ag}^+$  ions leads to the formation of silver atoms, which is followed by agglomeration into oligomeric clusters [18]. These clusters eventually lead to the formation of colloidal silver particles. When the colloidal particles are much smaller than the wavelength of visible light, the solutions have a yellow colour with an intense band in the 380–400 nm range and other less intense or smaller bands at a longer wavelength in the absorption spectrum [4]. This band is attributed to collective excitation of the electron gas in the particles, with a periodic change in electron density at the surface (surface plasmon absorption). Controlled synthesis of Silver nanoparticles is based on a two-step reduction process. In this technique, a strong reducing agent is used to produce small Silver particles, which are enlarged in a secondary step by further reduction with a weaker reducing agent. Chemical reduction of metal salts using various reducing agents in the presence of stabilizer is currently of interest for the preparation of metal nanoparticles. Reducing agents such as sodium Borohydride ( $\text{NaBH}_4$ ),

hydrazine (N<sub>2</sub>H<sub>4</sub>) (Equation 1), formaldehyde, etc. can be used to reduce a silver-containing salt to produce nanosilver particles [4].



Some of the chemical reducing reactions can be carried out at room temperature but however, most of them need elevated temperatures for a higher reaction rate. Thermal methods such as reduction of Ag<sup>+</sup> by dextrose and/or hydrazine as a reduction agent and the well-known Tollen's reduction with a reducing agent of m-hydroxy benzaldehyde are also chemical reduction methods [28].

### **2.1.5 Biological synthesis methods**

Biosynthetic methods employing either biological microorganisms or plant extracts have emerged as a simple and viable alternative to chemical synthetic procedures and physical methods [15]. The rewards of using plants and plant metabolites over other biological methods for nanoparticle synthesis have fascinated researchers to investigate mechanisms of metal ions uptake and bio-reduction by plants [29]. Another natural source for feasible bio-reduction of metal ions to metal nanoparticles is by the use of flavonoids. A whole class of flavonoids are found in abundance in a variety of plants and derived plant products. Quercetin (molecular formula C<sub>15</sub>H<sub>10</sub>O<sub>7</sub>) is a polyphenolic flavonoid found in many fruits, vegetables, leaves, and grains. It can be used as an ingredient in supplements, beverages, and foods, and is an excellent antioxidant [34]. Biosynthesis of nanoparticles is a kind of bottom-up approach where the main reaction occurring is reduction [3]. So, in the search for cheaper pathways for nanoparticles synthesis, scientists have used microbial enzymes and plant extracts (phytochemicals). With their antioxidant or reducing properties, they are usually responsible for the reduction of metal compounds into their respective nanoparticles [3]. Although rapid synthesis of silver nanoparticles within 5 min was recently reported using culture supernatants of *Enterobacteria*, the silver nanoparticles synthesized were



*ellipticum* plant was done by maceration for 48 hours using hexane and ethanol as solvents. The ethanol showed great superiority by extracting phytochemicals such as polyphenols, flavonoids, alkaloids, terpenoids and carbohydrates whilst the hexane was only able to extract the alkaloids, polyphenols and terpenoids [13]. Silver nanoparticles with spherical shapes, with an average size in the range between 0.51 to 0.73  $\mu\text{m}$  were then synthesised using the ethanol extract. Hazarika conclude that the ethanol extract of *Rhynchosyris ellipticum* leaf was capable of producing stable spherical silver nanoparticles which indicated its potential in the production of other valuable nanostructures in the future [13].

In a study carried out by Azizinezhad, the camomile plants phytochemicals were extracted by soxhlet extraction using water, ethanol, hexane and dimethyl sulphoxide as the extraction solvents. Silver nanoparticles with an SPR peak wavelength at 415 nm were synthesised with the dimethyl sulphoxide extract showing the greatest absorbance, followed by the ethanol whilst the water and the hexane extracts did not achieve optimum extraction. The synthesized nanoparticles exhibited good inhibitory activity against *E.coli* [39]. Banne successfully synthesised silver nanoparticles by the chemical reduction technique using sodium borohydride. The silver nanoparticles showed a well-defined plasmon band between the 380 and 395 nm wavelengths with an average particle size between 30-100 nm. It was then conclude that the agglomeration of silver nano particles can be avoided by addition of small amount of poly-vinyl-pyrrolidone (PVP) solution [32].

AgNPs synthesized with mangrove plant *Avicennia marina* extracts exhibited the highest inhibition activity against *E. coli* ( $18.40 \pm 0.97$  mM) and lowest against *S. aureus* ( $10.87 \pm 1.33$  mM). [40]. AgNPs, which are filled with polyphenolic compounds, disrupt the cell walls of bacteria, which make gram- negative bacteria specifically sensitive. Polyphenolic compounds generate free radicals and other oxygen- based reactive species, which can induce considerable

damage and toxicity [40]. The silver nanoparticles synthesised using banana peel extracts were crystalline, uniform, spherical and monodispersed with average particle size of 23.7 nm and exhibited antimicrobial activity against *S. aureus* and *E. coli* with inhibition zones of 23 mm and 21 mm respectively [27].

All these plants exhibited significant abilities to reduce and stabilise/cap the silver nanoparticles despite their varying nature and this was due to the presence of naturally occurring phytochemicals present in the plants. The biosurfactant molecules present inside plant extracts play a vital role together as stabilizing and reducing agents, even though the correct mechanism for the formation of nanoparticles is not yet clear. However, one can employ techniques such as Fourier Transform Infrared (FTIR) Spectroscopy, Gas Chromatography-Mass Spectroscopy (GCMS), High-resolution Mass Spectroscopy (HRMS) and  $^{13}\text{C}$  NMR studies to detect or identify the presence of different biomolecules especially biosurfactants in the plant extract [41].

## **2.2 Characterization**

Various instrumentation techniques have been developed and these have the ability to offer an extensive insight on the chemical and physical properties of silver nanoparticles. These methods include UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM).

### **2.2.1 Ultraviolet-visible spectroscopy (UV-Vis)**

The usage of UV-VIS spectrometers has risen exponentially in the last few decades, making them one of the most important analytical tools in the modern day laboratory. In many applications other techniques could be employed but none rival UV-Visible spectrometry for its simplicity,

versatility, speed, accuracy and cost-effectiveness [42]. A schematic illustration of the UV-VIS spectrophotometer and how it functions is shown in Figure 2.3.

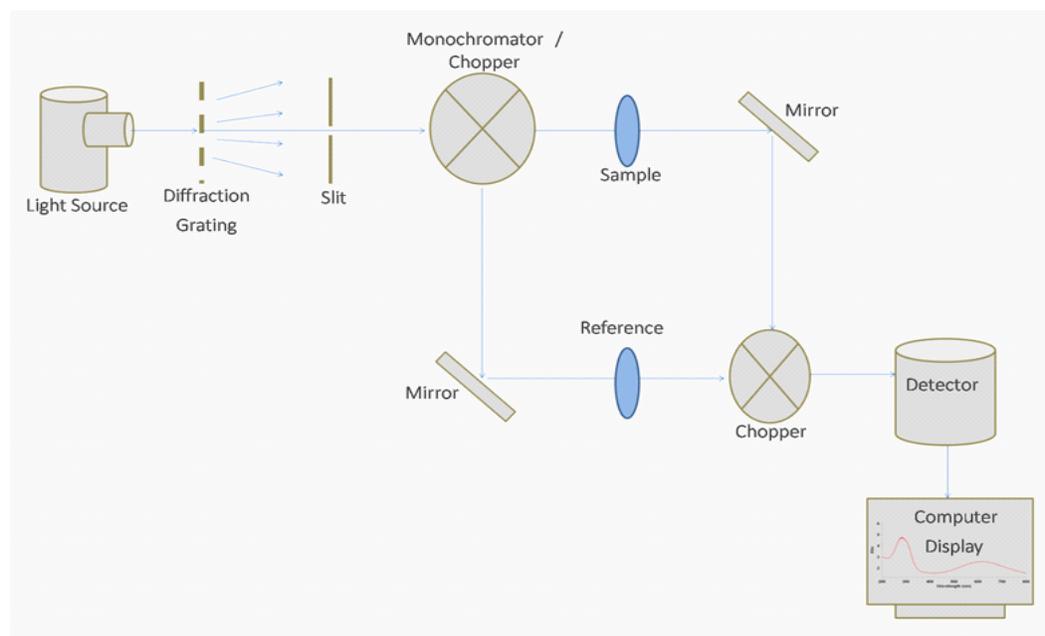


Figure 2.3: Schematic diagram of the UV-VIS spectrophotometer

In principle, the UV-VIS spectrophotometer applies the Beer-Lambert law. The law is used to calculate the concentration of a sample using the absorbance obtained. From this law absorbance is directly proportional to concentration as shown by the Beer-Lambert law (Equation 2).

$$A = \epsilon l c \dots \dots \dots (2)$$

where  $A$  is the absorbance,  $\epsilon$  is the molar absorptivity coefficient,  $c$  is the sample concentration and  $l$  is the length of the cuvette [43].

A close relationship exists between the colour of a substance and its electronic structure. A molecule or ion will exhibit absorption in the visible or ultraviolet region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the ultraviolet or visible region is accompanied by a change in the electronic state of the molecules in the sample.

The energy supplied by the light will promote electrons from their ground state orbitals to higher energy, excited state orbitals or antibonding orbitals [44]. Every metal surface has a plasmon resonance corresponding to a specific wavelength in the visible region [45]. The position of a resonance band depends on various factors, such as the size and shape of the particles, the type of capping or binding agents, and the reflective index of the surrounding medium [45].

Surface plasmon resonance (SPR) is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light. SPR is the basis of many standard tools for measuring adsorption of material onto planar metal (typically gold or silver) surfaces or onto the surface of metal nanoparticles. Silver nanoparticles exhibit a dark yellowish-brown colour in aqueous solution due to the surface plasmon resonance phenomenon [8].

### **2.2.2 Fourier Transform Infrared (FTIR) spectroscopy**

FTIR (Fourier Transform Infrared Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterise some inorganics. FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency range is measured as wave numbers typically over the range  $4000 - 400\text{cm}^{-1}$ .

When a sample is placed in the FTIR spectrophotometer, infrared radiation is passed through the sample then the absorbance/transmittance of the radiation is measured against the incident radiation. This principle is then interpreted in a spectrum the different peaks present in the spectrum provide information on which bonds/functional groups are present in the sample. Different bond exhibit different vibrations when struck by infrared radiation thus it provides a

molecular fingerprint of the sample. Figure 2.4 is a schematic diagram of the FTIR spectrophotometer.

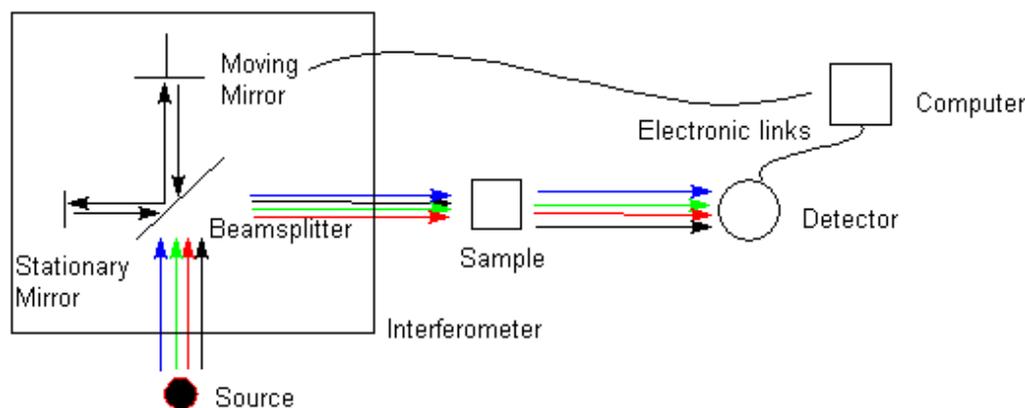


Figure 2.4: Schematic diagram of an FTIR spectrophotometer

Fourier transform infrared spectroscopic (FTIR) analysis plays a significant role in the identification of binding molecules or functional groups of the surface of nanoparticles synthesized in a biological matrix. Biomolecules in the matrix can absorb infrared radiation corresponding to a specific wavelength ranging from  $4000\text{-}400\text{ cm}^{-1}$  [45]. This technique is very useful in the characterisation and identification of the functional groups present in the plant or which functional groups are present in the phytochemicals. This information is essential in providing an overview of which phytochemicals are responsible for the reduction and capping of  $\text{Ag}^+$  to  $\text{Ag}^0$ . The spectra are given off before and after the synthesis of the silver nanoparticles also provides information on which of the functional groups participated in the reduction and which of the constituents enhanced the stability of the nanoparticles by capping. FTIR spectroscopy is useful in probing the chemical composition of the surface of the silver nanoparticles and the local molecular environment of the capping agents on the nanoparticles [46].

### 2.2.3 X-ray Diffraction (XRD)

X-ray diffraction is used to obtain structural information about crystalline solids. Its application in

the biochemistry field has become very important due to its ability to provide 3D images of bio-molecules [47]. X-ray diffraction (XRD) is a non-destructive analytical method capable of analysing several kinds of matter, ranging from fluids, powders to crystals. The x-ray diffraction pattern of a pure substance is, therefore, like a fingerprint of the substance. The powder diffraction method is thus ideally suited for characterization and identification of polycrystalline phases.

An electron in an alternating electromagnetic field will oscillate with the same frequency as the field and when an x-ray beam hits an atom, the electrons around the atom start to oscillate with the same frequency as the incoming beam. The combining of the wave which is particularly out of phase will occur, this is known as destructive interference and there will be no resultant energy leaving the solid sample [47]. Constructive interference will only occur in a few directions because crystals are arranged in a regular pattern. The waves will be in phase and this will result in well-defined X-ray beams leaving the sample at various directions. Therefore, a diffracted beam may be described as a beam composed of a large number of scattered rays which are constantly reinforcing one another [47]. The condition for constructive interference to occur is that the rays reflected from adjacent lines should differ in path length by an integral number,  $n$ , of wavelengths, then easily leading to the Bragg equation [48]. The schematic diagram of XRD is shown in Figure 2.5.

The use of XRD in the analysis of silver nanoparticles synthesised using the various synthesis routes present is very vital. Silver nanoparticles adopt a certain crystallinity which is mainly defined by the synthesis route used for example face centred cubic silver nanoparticles. This information, in turn, is used to further calculate the average particle size of the silver nanoparticles [10].

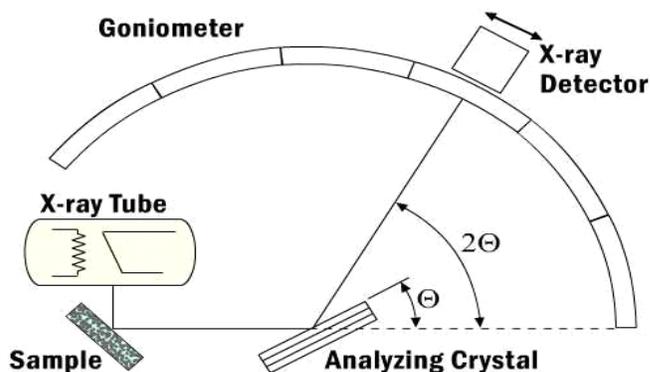


Figure 2.5: Schematic diagram of X-ray Diffraction

A typical diffraction spectrum consists of a plot of reflected intensities versus the detector angle  $2\Theta$  or  $\Theta$ , which is characterised by the goniometer configuration. The  $2\Theta$  values for the peak depending on the wavelength of the anode material of the X-ray tube. It is therefore customary to reduce a peak position to the inter-planar spacing  $d$  that corresponds to the  $h, k, l$  planes that caused the reflection. The value of the  $d$ -spacing solely depends on the shape of the unit cell. We get the  $d$ -spacing as a function of  $2\Theta$  from Bragg's law (Equation 3) [49].

$$d = \lambda / 2 \sin \theta \dots\dots\dots (3)$$

Each reflection is fully defined when the following are known: the  $d$ -spacing, the intensity (area under the peak) and the indices  $h, k, l$ . If we know the  $d$ -spacing and the corresponding indices  $h, k, l$  we can calculate the dimension of the unit cell [49].

### 2.2.4 Transmission Electron Microscopy (TEM)

Transmission electron microscopy is a microscopy technique in which a beam of electrons is transmitted through a specimen to form an image. Transmission electron microscopes (Figure 2.6) are capable of imaging at a significantly higher resolution than light microscopes, owing to the smaller de Broglie wavelength of electrons. This enables the instrument to capture fine detail even

as small as a single column of atoms, which is thousands of times smaller than a resolvable object seen in a light microscope thus it is a very important analytical technique in the biochemistry field [50]. The average size and morphology of silver nanoparticles can be determined by transmission electron microscopy.

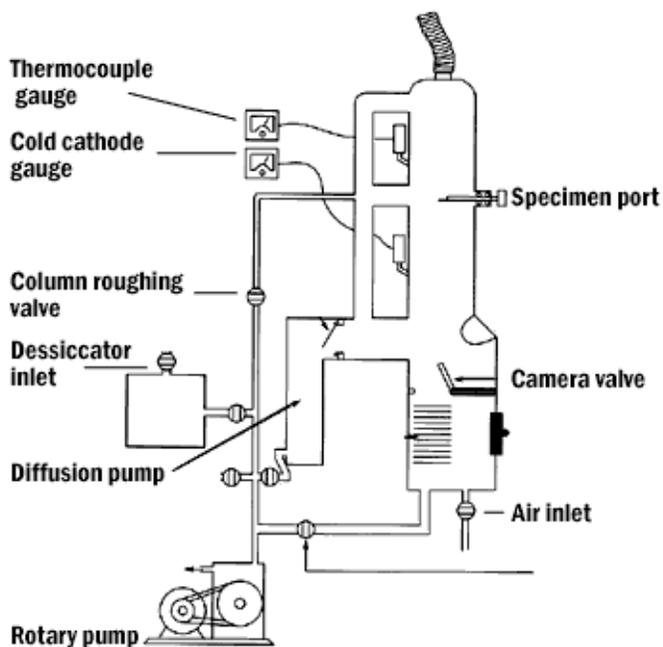


Figure 2.6: Schematic diagram of Transmission Electron Microscopy

### 2.2.5 Scanning Electron Microscopy (SEM)

A scanning electron microscope is a type of electron microscope that produces images of a sample by scanning the surface with a focused beam of electrons. The electrons interact with atoms in the sample, producing various signals that contain information about the samples surface topography and composition. A schematic diagram of the SEM is shown in Figure 2.7.

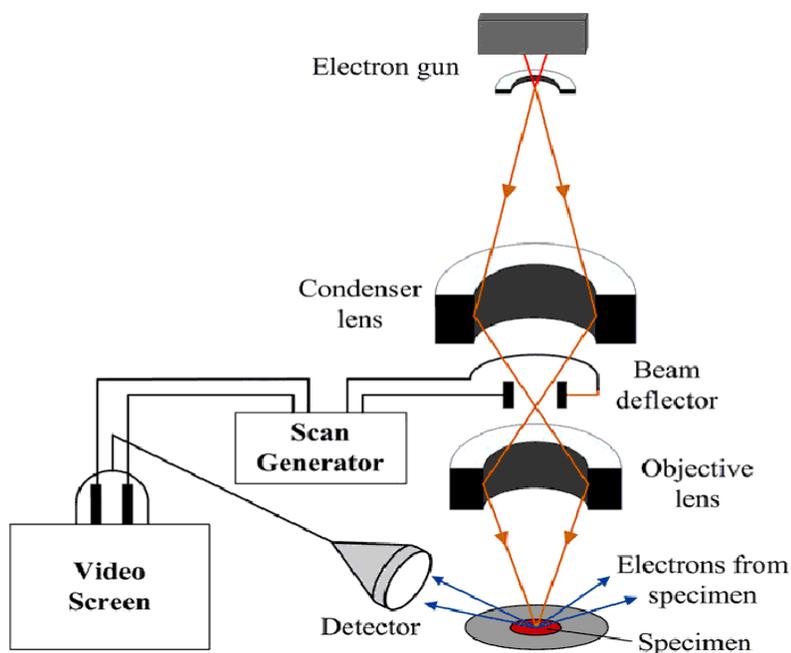


Figure 2.7: Schematic diagram of Scanning Electron Microscope

The electron gun forms a source of electrons which are accelerated towards the anode which is held at earth potential relative to the few volts, few kilovolts or tens of kilovolts on the gun cathode and control grid [51]. These electrons pass through one or more electron lenses and the image of the electron source is formed in the plane of the specimen, after successive reductions, with a diameter of a few tenths to a few tens of nanometers [51]. The SEM can give information on the morphology and size of silver nanoparticles.

### 2.3 *Allium Ascalonicum*

*Allium Ascalonicum* (Figure 2.8), commonly known as shallots are bulbous plants which from the *Allium* genus which includes commonly known plants such as onions, garlic, chives and leeks. These plants are sometimes called small onions due to their onion-like physical structure.



Figure 2.8: *Allium Ascalonicum* Plant

According to previous research, shallots have been proven to contain more flavonoids and phenols than other members of the *Allium* genus[52]. Shallots are known to have strong anti-cancer properties and immune enhancing effects. They not only add a sweet and pungent flavour to recipes, but they also come loaded with antioxidants, vitamin A, vitamin C, and some important minerals too. Two sets of compounds that make up the majority of shallots known healing properties are sulphur compounds such as allyl propyl disulphide and flavonoids such as quercetin. Studies have shown that quercetin protects against cardiovascular disease, and cancer [26]. The high amounts of sulphoxides possessed in these plants oils are responsible for their antidiabetic, antibiotic, hypo-cholesteolaemic and fibrinolytic properties [53]. This means they can help control blood sugar levels, help prevent insulin resistance, fight bacterial and viral infections, help lower cholesterol levels naturally and prevent blood clots from forming. Ascalon, a new anti-fungal peptide from shallot bulbs that can inhibit mycelial growth in *Botrytis cinerea* has been reported [54].

*Allium Ascalonicum* is one of the important *Allium* species commonly used in many diets and in folk medicine since ancient time. Although it is widely consumed, reports concerning the biological effects of shallot are scarce compared to other *Allium* species such as garlic and onion.

Shallot is normally recognized for its hypocholesterolemic and antimicrobial effect. Recently, shallot has been reported to exhibit antioxidative and free radical scavenging abilities. These properties appear to be related to the high contents of flavone, sulphur containing compounds, and polyphenolic derivatives in the bulb of shallot. More importantly, it is shown that the antioxidant potential of shallot is superior to several onion varieties and some garlic preparations. Thus, it is likely that shallot may be useful to alleviate or protect against oxidant-induced various disease conditions, including nephrotoxicity caused by Cyclosporine A [34].

## **2.4 Phytochemicals**

Phytochemicals are the chemicals that are present naturally in plants. Nowadays these phytochemicals have become more popular due to their countless medicinal uses. Phytochemicals play a vital role against a number of diseases such as asthma, arthritis and cancer. Unlike pharmaceutical chemicals, these phytochemicals do not have any side effects [16]. Phytochemicals mainly constitute of organic functional groups and are present in different plants depending on the nature of the plant, the location in which the plant grows and the moisture content of the plant. Some of these phytochemicals have properties such as a pungent smell so as to defend the plant from insects whilst some possess antimicrobial activity to protect plants from microorganisms such as fungi which may feed on the plant. Phytochemicals are produced *via* primary or secondary plant metabolisms and originate in various kinds of fruits, vegetables, grains, and herbs, endowing them with the colour, taste, smell, and other organoleptic properties of the plants. They are produced to help plants thrive or to thwart competitors, predators, or pathogens. During the last two decades, dietary phytochemicals have been found to be strongly associated with human health and diseases through their biological functions [55]. When phytochemicals are ingested by humans and other animals, they are recognized as xenobiotics and as a result, they

stimulate the genes of a series of antioxidant and detoxifying enzymes.

Phytochemicals are the most potent materials for biological activities and are known as important natural resources for the synthesis of metallic nanoparticles. Recent studies have shown that plants which constitute of phytochemicals such as alkaloids, carbohydrates, glycosides, phenolic compounds, proteins, amino acids, flavonoids and terpenoids exhibit excellent potential and efficiency in the synthesis of silver nanoparticles [56]. This is mainly attributed to their ability to reduce and cap the  $\text{Ag}^+$  to  $\text{Ag}^0$  without the addition of any other chemicals. Below are the structures of some of the phytochemicals that naturally occur in various plants.

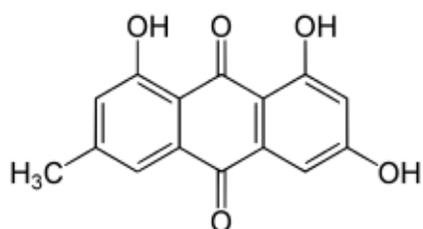


Figure 2.9: Emodin

Emodin (Figure 2.9) is a naturally occurring anthraquinone derivative and currently, a number of researchers are focusing on the pharmacological effects of this compound. Previous reviews have summarized the anti-cancer and anti-inflammatory properties of emodin. In the last three years, there have been many reports on the anti-tumour and anti-inflammatory effects of emodin [57].

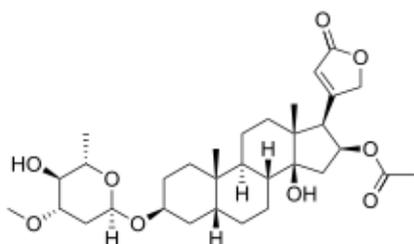


Figure 2.10: Oleandrin, a glycoside

Cardiac glycosides are used in the treatment of congestive heart failure and cardiac arrhythmia.

These glycosides are found as secondary metabolites in several plants and in some animals, such as the milkweed butterflies. Oleandrin (Figure 2.10), a toxic cardiac glycoside inhibits the activity of nuclear factor kappa-light-chain-enhancer of activated B chain (NF- $\kappa$ B) in various cultured cell lines as well as it induces programmed cell death in PC3 cell line culture [58].

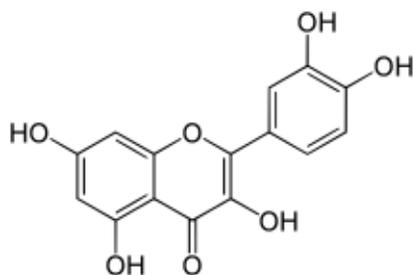


Figure 2.11: Quercetin, a flavanoid

Quercetin (Figure 2.11), a flavonoid found in fruits and vegetables, has unique biological properties that may improve mental/physical performance and reduce infection risk. These properties form the basis for potential benefits to overall health and disease resistance, including anti-carcinogenic, anti-inflammatory, antiviral, antioxidant, and psycho-stimulant activities, as well as the ability to inhibit lipid peroxidation, platelet aggregation and capillary permeability, and to stimulate mitochondrial biogenesis [59].

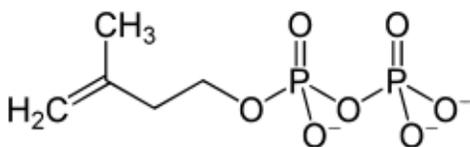


Figure 2.12: Isopentenyl pyrophosphate, a terpenoid

Rheumatoid arthritis, is a systemic, inflammatory, auto-immune disorder affecting about 0.5–1 % of the adult population worldwide. It is characterized by inflammation in joints causing both bone and cartilage destruction. Low concentrations of Isopentenyl pyrophosphate (Figure 2.12) can reduce the cartilage destruction caused by rheumatoid arthritis by enhancing the expansion of

synovial fluid cells from patients with inflammatory arthritis [60].

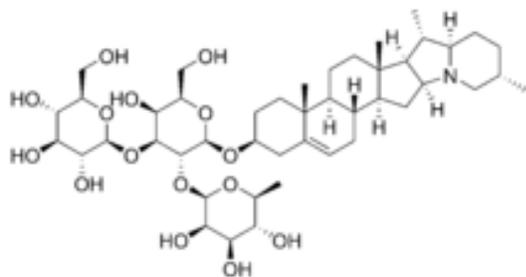


Figure 2.13: Solanine, a saponin

Historically, Solanine (Figure 2.13) was used in the treatment of epilepsy and asthma, in controlled doses. This practice is no longer common, as there are safer and more effective ways to treat these conditions. Solanine also has fungicidal and pesticidal qualities, but extraction and processing of this toxin is so time consuming that the substance is rarely used for these purposes [61].

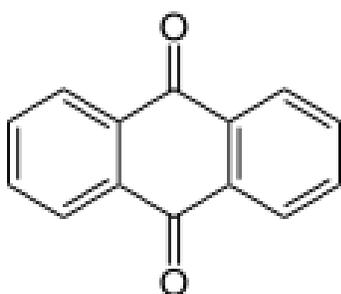


Figure 2.14: Anthraquinone, a quinone

Anthraquinone (Figure 2.14) occurs naturally in some plants, fungi, lichens, and insects, where it serves as a basic skeleton for their pigments. Anthraquinone compounds are used as laxatives mainly from their glycosidic derivatives and also used in the treatment of fungal skin diseases. Anthraquinone and its derivatives are frequently found in slimming agents and have been valued for their cathartic and presumed detoxifying action [62].

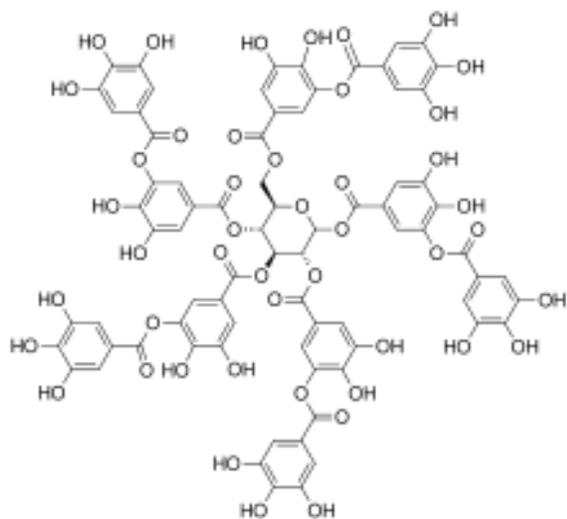


Figure 2.15: Tannic acid, a polyphenol

Tannic acid (Figure 2.15) is rated as a “GRAS” (generally recognized as safe) viand additive and finds application in food and beverages as a flavoring substance with an astringent taste. In addition, it is used in medicine, in particular as an ingredient of dermatological ointments for local treatment of burn wounds, skin infections, and chronic diseases such as eczema [63].

From the structures of the phytochemicals illustrated, it can be clearly noted that most phytochemicals constitute of various polar functional groups such as the OH<sup>-</sup> and these groups are generally reducing agents. In recent years, many plant-derived substances that are classified as polyphenols are becoming increasingly known for their various biological effects, particularly antioxidant and free radical scavenging activities. The polyphenols have been reported to act as chain-breaking antioxidants by their ability to donate hydrogen atoms, to inhibit free radical formation by chelating transition metal ions, to act as co-antioxidant by facilitating the antioxidant activity of other compounds, and to modulate signal transduction pathways and gene expression through their reducing properties [34]. Water-soluble phytochemicals including organic acids, quinones and flavones are responsible for the instantaneous reduction of the silver ions in the

reaction mixture. It has been reported that xerophytes contain Anthraquinone and Emodin that undergoes tautomerization, leads to the formation of silver nanoparticles. In the majority cases reducing agent from the plant extract also acts as the capping and stabilising agent, this facilitates us to no requirement for the addition of capping and stabilising agents from the outside [64]. It has been revealed that reduction of silver ions depends upon the type of plant extract used as reducing agent [64].

Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, the yellow colour in sunflowers, and the red colour in tomatoes [65]. Glycosides are sugars which are bound to another functional group via glycosidic bonds. Glycosides are very important to the existence living organisms such as in plants where important chemicals are stored as inactive glycosides, some insects obtain these glycosides from other plants in order to use the compounds in their defence against predators and glycosides assist in the detoxification process in the human body as they bind to the toxins and enable their metabolism and excretion. The majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons [26].

Phenols are similar to alcohols but they have higher acidities due to the aromatic ring which has a tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is commonly intermediate between that of aliphatic alcohols and carboxylic acids. Polyphenols such as lignans are widely distributed in the plant kingdom as natural defence substances. They are bioactive as phytoestrogens because of their structural and functional similarity to 17 $\beta$ -estradiol. Lignans have been suggested to induce a wide range of biological effects, such as antioxidant, antitumor, antiviral, antibacterial, insecticidal,

fungistatic, estrogenic, and antiestrogenic activities, and protect against coronary heart disease [66].

Saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. Many plants possess natural or biosurfactant molecules, which are competent enough of wetting, foaming and grease dispersion. The plant-based biosurfactants are saponins or foaming glycosides (triterpenoid glycosides), which are non-ionic in nature. The hydrophilic part of biosurfactant mainly consists of sugars like *D*-glucose, *D*-xylose, *L*-arabinose, *L*-rhamnose, and glucuronic acid, while the hydrophobic part consists of sapindic acid and oleanolic acid. Plant-based biosurfactants are biocompatible, non-toxic and non-ionic, therefore providing an eye-catching alternative to synthetic surfactants, which usually fall short to fulfil with the environmental safety standards [41].

## **2.5 Column chromatography**

Many bioactive molecules have been isolated and purified by using paper thin-layer and column chromatographic methods. Column chromatography and thin-layer chromatography are still mostly used due to their convenience, economy, and availability in various stationary phases. Silica, alumina, cellulose, and polyamide exhibit the most value for separating the phytochemicals. Plant materials include high amounts of complex phytochemicals, which make a good separation difficult [67]. Column chromatography is similar to thin-layer chromatography in that a mixture of compounds can be separated by being carried by a mobile phase through a stationary phase. However, instead of just giving us information about the mixture, column

chromatography is used to actually separate larger amounts of the compounds. The stationary phase in column chromatography is a glass column filled with alumina or silica gel. The mobile phase is a solvent (or a mixture of two solvents) which is pushed down through the column, carrying compounds through at different rates. The solvent is collected as it comes out the bottom, and separate compounds can be obtained. Column chromatography can also be used as a means of purifying mixtures or solutions based on their polarities. The application of both normal and reversed phase chromatography for isolation purposes of phytochemicals has been reported to produce successful and reliable results despite the relatively similar nature of most phytochemicals [53].

Other techniques such as GC-MS and HPLC are capable of providing more reliable and efficient results than column chromatography despite their higher cost of operation and less environmentally friendly nature relative to column chromatography. These techniques have the ability to quantify and provide the qualitative analysis results of complex phytochemical extracts. The HPLC is capable of analysing samples using the gradient elution method whereby different mobile phase is capable of transforming its properties such as polarity in order to provide maximum isolation and maximum quantification of samples. HPLC is used for compounds that cannot be vapourised or that decompose under high temperatures. GC-MS is applicable to volatile compounds and makes use of the slightest structural differences that the phytochemicals possess and this enables it to be more efficient and reliable as compared to column chromatography.

## **2.6 Bacteria**

Human beings are often infected by microorganisms such as bacteria, molds, yeasts, and viruses that are present in their living environments. Use of chemical antibiotics has led to the development of resistant bacteria that account for the majority of hospital infections [68].

### 2.6.1 *Staphylococcus Aureus*

*Staphylococcus aureus* (*S. aureus*) is a major pathogen that causes nosocomial and community-acquired infections. Therapeutic options for these infections have been reduced due to increased resistance to many classes of antimicrobial agents [69]. *Staphylococcus aureus* is an opportunistic pathogen in human and other different animal species. The pathogen is mainly related to food poisoning and is the third largest cause of food-related illness throughout the world. *S. aureus* can cause a number of infectious diseases such as dermatitis, pneumonia, meningitis, osteomyelitis in human, bovine mastitis in cattle and bumblefoot disease in poultry. Methicillin resistance in this bacterial species is very alarming for human health, as it has shown potential for zoonotic transmission [22].

The increasing incidence of childhood skin infections and prescribing of the major anti-staphylococcal drug flucloxacillin, coupled with concurrent increases in childhood hospital admissions for skin, bone and joint infections caused by *S. aureus* in hospitals suggests an increase in community-onset *S. aureus* disease over the past 10 years [70]. The genus *Staphylococcus* is composed of gram-positive bacteria with diameters of 0.5-1.5  $\mu\text{m}$ , characterized by individual cocci that divide in more than one plane to form grape-like clusters. These bacteria are non-motile, non-spore forming facultative anaerobes, featuring a complex nutritional requirement for growth, a low G+C content of DNA (in the range of 30-40 mol %), a tolerance to high concentrations of salt and resistance to heat [71]. The genus *Staphylococcus* is traditionally divided in two groups based on the bacteria able to produce coagulase, an enzyme that causes blood clotting: the coagulase-positive staphylococci, which includes the most known species *Staphylococcus aureus*; and the coagulase-negative staphylococci, which are common commensals of the skin. *S. aureus* is the most pathogenic species of the genus *Staphylococcus*, being implicated in both

community-acquired and nosocomial infections [71]. It often asymptotically colonizes the skin and mucous membranes of healthy individuals, in particular, the anterior nares [71].

The epidemiology of *Staphylococcus aureus* is dynamic and has changed significantly over the years. The proven ability of *Staphylococcus aureus* to acquire resistance genes is a concern among physicians worldwide. The search for new therapeutic alternatives associated with policies to control antibiotic use and hospital-acquired infections guided by epidemiological surveillance studies should be constant habits among health professionals and hospitals as an alternative to minimize the problem [71].

### **2.6.2 *Escherichia. Coli***

*Escherichia coli*, originally called “*Bacterium coli commune*,” was first isolated from the faeces of a child in 1885 by the Austrian paediatrician. *Escherichia coli* is a common inhabitant of the gastrointestinal tract of humans and animals. There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E. coli* are divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites [72].

*Escherichia coli* are gram-negative, non-spore forming bacilli. They are approximately 0.5  $\mu\text{m}$  in diameter and 1.0–3.0  $\mu\text{m}$  in length. Virulent strains of *E. coli* are differentiated clinically from one another on the basis of epidemiology, signs and symptoms of their respective diseases, microscopic observations of their interactions with host cells, and of biotypes and unique gene markers. *Enterohemorrhagic Escherichia coli* (EHEC) is a subset of pathogenic *E. coli* that can cause diarrhoea or haemorrhagic colitis in humans [73]. Haemorrhagic colitis occasionally progresses to the haemolytic uremic syndrome, an important cause of acute renal failure in children and morbidity and mortality in adults [74].

*Escherichia coli* is one of the most dangerous pathogenic bacteria, which is also responsible for many types of diseases like abdominal pain, fatigue, septicaemia and even kidney failure. The existence of *E. coli* in drinking water and foodstuffs is a chronic worldwide problem. In the last decades, detection of *E. coli* using nanomaterials is a hot research topic in the field of biosensors [75]. The key mechanisms by which *E. coli* cause enteric diseases include attachment and colonization of the intestinal mucosa, manipulation of the host cell cytoskeleton or evading host immune defences, and production of toxins. In general, monotherapy with trimethoprim-sulfamethoxazole, aminoglycoside, cephalosporin, or a fluoroquinolone is recommended as the treatment of choice for most known infections with *E. coli*, although many broad-spectrum agents remain highly active [76].

## **2.7 Antimicrobial drugs**

An antibiotic is a drug that kills or slows the growth of bacteria. Antibiotics are one class of antimicrobials, a larger group which also includes anti-viral, anti-fungal, and anti-parasitic drugs. Extensive use of antimicrobial drugs in humans and in animal farming for the therapeutic and preventive purpose is a major cause for the prevalence of drug resistance among foodborne pathogens. Different antimicrobial agents such as penicillin, erythromycin, and tetracycline are extensively used in poultry for treating staphylococcal and other infections, which leads to the development of drug resistant strains of pathogens [22].

The resistance to the first antibiotic, penicillin, emerged in 1942, only a few years after its introduction into the clinical practice. Penicillin-resistant strains soon began to cause community infections, and by the early 1950s, they had become pandemic. Since 1960, around 80 % of all *S. aureus* strains were resistant to penicillin. These strains produce a plasmid-encoded penicillinase, which hydrolyses the  $\beta$ -lactam ring of penicillin deactivating the molecules antibacterial

properties [71]. Many pathogenic strains that are able to cause illness have become resistant to antibiotics. The rise of antibiotic resistance has motivated researchers to find antimicrobial alternatives of which probiotics have gained a growing interest.

The use of *Lactobacillus* spp. and *Bifidobacterium* spp. as probiotics to combat microbial infections and boosting human health inspired many studies. Probiotics have been associated with the treatment of gastroenteritis, antibiotic-associated diarrhoea, necrotizing enterocolitis, pouchitis, inflammatory bowel diseases, allergic disorders and others. The antimicrobial activity of a range of probiotics against pathogens including *E. coli* has been reported [74].

Bacteria have a number of ways how they become antibiotic-resistant. For example, they possess an internal mechanism of changing their structure so the antibiotic no longer works, they develop ways to inactivate or neutralize the antibiotic. Also, bacteria can transfer the genes coding for antibiotic resistance between them, making it possible for bacteria never exposed to an antibiotic to acquire resistance from those which have [77]. The problem of antibiotic resistance is worsened when antibiotics are used to treat disorders in which they have no efficacy (e.g. antibiotics are not effective against infections caused by viruses), and when they are used widely as prophylaxis rather than treatment [77].

Like all drugs, antibiotics have the potential to cause unwanted side effects. Many of these side effects are not dangerous, although they can make life miserable while the drug is being taken. In general, antibiotics rarely cause serious side effects. The most common side effects of antibiotics are diarrhoea, nausea, vomiting. Fungal infections of the mouth, digestive tract and vagina can also occur with antibiotics because they destroy the protective good bacteria in the body (which help prevent overgrowth of any one organism), as well as the bad ones, responsible for the infection being treated. Some people are allergic to antibiotics, particularly penicillin's. Allergic reactions

cause swelling of the face, itching and a skin rash and, in severe cases, breathing difficulties. Allergic reactions usually require prompt treatment to prevent any serious damage.

In this research project, Trimethoprim was used as the antibiotic standard. Trimethoprim is a type of antibiotic drug which works by killing bacteria. Trimethoprim tablets are used to treat a wide range of infections including urinary infections, respiratory tract infections and for long-term prevention of recurrent urinary tract infections. Some of the side effects that are associated with the intake of trimethoprim include headaches, skin rashes, constipation, dizziness, shortness of breath and lupus erythematosus (an auto-immune disorder) [78]. The chemical name for trimethoprim is (2,-4-diamino-5-(3,-4,-5-trimethoxybenzyl))-pyrimidine. The structure of trimethoprim is shown in Figure 2.16.

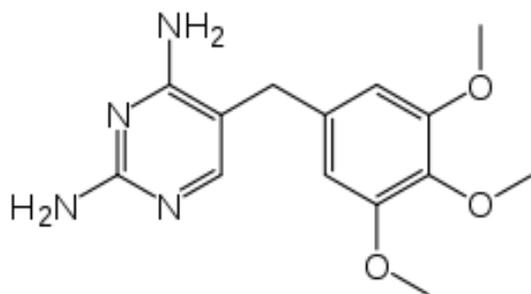


Figure 2.16: Trimethoprim

The mode of action of the (2,-4-diamino-5-(3,-4,-5-trimethoxybenzyl))-pyrimidine involves the hindering of the synthesis of tetrahydrofolic acid by interacting with the bacteria dihydrofolate reductase enzyme. Bacteria are not capable of obtaining folic acid from the environment thus they have the ability to synthesise their own thus the trimethoprim interaction with the enzyme dihydrofolate reductase results in the starvation of the bacteria and leads to its death [78]. Trimethoprim is usually formulated alongside Sulfamethoxazole and one of the popularly known tablets which constitute of these drugs is Cotrimoxazole.

### **2.7.1 Antimicrobial susceptibility testing**

Antimicrobial susceptibility testing (AST) is performed daily on bacterial isolates in clinical laboratories. It was quickly recognized by early investigators that there were many variables affecting the results of these tests. Consequently, there was a recognition (as early as the late 1950s) that standardization of these techniques was required. This need has led to many organisations producing standardized AST methodologies [79].

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include: Diffusion (Kirby-Bauer and Stokes), Dilution (Minimum Inhibitory Concentration) and Diffusion & Dilution (E-Test method). Antimicrobial susceptibility testing in the clinical laboratory is most often performed using the disc diffusion method. The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [80].

The purpose of the Kirby-Bauer disk diffusion susceptibility test is to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria to various antimicrobial compounds in order to assist a physician in selecting treatment options for their patients [81]. The pathogenic organism is grown on Mueller-Hinton agar in the presence of various antimicrobial impregnated filter paper disks. The presence or absence of growth around the disks is an indirect measure of the ability of that compound to inhibit that organism. When a 6 mm filter paper disk impregnated with a known concentration of an antimicrobial compound is placed on a Mueller-Hinton (MH) agar plate, immediately water is absorbed into the disk from the agar [81]. The antimicrobial begins to diffuse into the surrounding agar. The rate of diffusion through the agar is not as rapid as the rate of extraction of the antimicrobial out of the disk, therefore the

concentration of antimicrobial is highest closest to the disk and a logarithmic reduction in concentration occurs as the distance from the disk increases [81]. The rate of diffusion of the antimicrobial through the agar is dependent on the diffusion and solubility properties of the drug in MH agar and the molecular weight of the antimicrobial compound. Larger molecules will diffuse at a slower rate than lower molecular weight compounds. These factors, in combination, result in each antimicrobial having a unique breakpoint zone size indicating susceptibility to that antimicrobial compound [81].

A cell wall is present around the outside of the bacterial cell membrane and it is essential to the survival of bacteria. It is made from polysaccharides and peptides named *peptidoglycon* [82]. There are broadly speaking two different types of cell wall in bacteria, called gram-positive and gram-negative. The names originate from the reaction of cells to the gram stain, a test long-employed for the classification of bacterial species. Gram-positive (e.g. *Staphylococcus aureus*) bacteria possess a thick cell wall containing many layers of peptidoglycan [83]. In contrast, gram-negative (e.g. *Escherichia coli*) bacteria have a relatively thin cell wall consisting of a few layers of *peptidoglycan* [84].

## CHAPTER THREE

### METHODOLOGY

#### 3.1 Introduction

This chapter gives a description of the methods employed for the extraction of phytochemicals, the phytochemical tests, the optimization studies for the synthesis of the silver nanoparticles, the characterisation of the silver nanoparticles and the antimicrobial testing. The steps followed are illustrated in Figure 3.1.

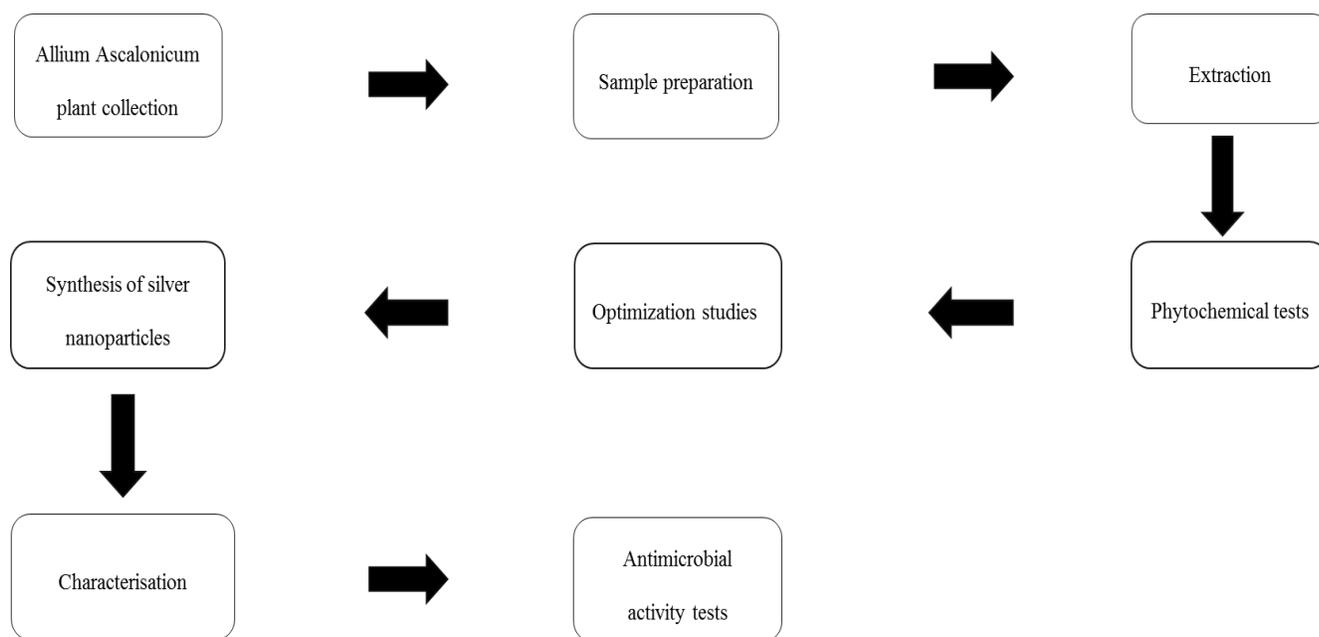


Figure 3.1: Methodology flow chart

#### 3.1.1 Reagents

All the reagents used during the course of the research project were analytical grade and no further purification was carried out. Distilled water was used throughout the course of the research.

### 3.2 Sample collection, preparation and extraction

The *Allium Ascalonicum* plant was collected from a local farm near Bulawayo, Zimbabwe. The plant was thoroughly washed using tap water to remove all the dirt then lastly, distilled water was used to wash the plant as well. The stems and the roots of the plant were cut off, leaving the only the bulbs. The bulbs were then sundried for a week and then oven-dried at 70 °C for 24 hours and then crushed into a powder using a pestle and mortar [46]. A homogeneous sample of the powder was collected by using a 150 µm mesh sieve to sieve the powder and collect particles with the maximum surface area. The extraction of the plant's phytochemicals was carried out using 7 different solvents with varying polarity index values as shown in Table 3.1.

Table 3.1: Polarity index values of solvents

	Ethanol	Cyclo-hexane	Distilled water	Hexane	Ethyl acetate	Methanol	Methanol /water 50/50
Polarity index	4.3	0.04	10.2	0.1	4.4	5.1	7.65

A mass of 5 g of plant powder was mixed with 250 mL of each solvent and maceration extraction was carried out for 48 hours [85]. The extraction suspension was then separated by vacuum filtration and the different extract solutions were stored at 4 °C in sealed flasks [86]. The residues on the filter papers were oven-dried and weighed in order to calculate the extraction yields. The formula Equation 4 was used to calculate the yield [87].

$$\text{Yield (\%)} = (W_1 \times 100) \div W_2 \dots\dots\dots (4)$$

Where  $W_1$  was the weight of extract after evaporation of solvent and  $W_2$  was the dry weight of the sample.

FTIR analysis of the plant's crude powder was carried out in the  $4000\text{ cm}^{-1}$ - $400\text{ cm}^{-1}$  range.

### **3.3 Phytochemical tests**

The presence of phytochemicals in all the 7 solvent extracts were tested using several different qualitative analysis methods. The phytochemicals tested were steroids, alkaloids, glycosides, quinones, flavonoids, phenols, anthocyanins, saponins, terpenoids, tannins, coumarines and emodins

#### **3.3.1 Test for steroids**

By using a syringe, 1 mL of plant extract was measured and dissolved in 10 mL chloroform and 10 mL concentrated sulphuric acid (98 %) in a test tube. The acid was added drop-wise. The resulting mixture was shaken vigorously. The existence of two layers indicated the presence of steroids. The top layer displays a red colour while the bottom acid layer displays a yellow colour with green fluorescence [88].

#### **3.3.2 Test for alkaloids**

Wagner's test. A fraction of the extract (1 mL) was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL distilled water) and observed for the formation of reddish-brown coloured precipitate [89].

#### **3.3.3 Test for glycosides**

Salkowski's Test was used. Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of concentrated Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of glycosides [89].

#### **3.3.4 Test for quinones**

Mayer's test. A fraction of the extract was treated with Mayer's reagent (1.36 g of mercuric

chloride and 5 g of potassium iodide in 100 mL of distilled water) and observed for the formation of cream coloured precipitate [89].

### **3.3.5 Test for flavonoids**

The aqueous sodium hydroxide test was used: A fraction of the extract (2 mL) was treated with 1M aqueous NaOH solution and observed for the formation of yellow-orange colouration [90].

### **3.3.6 Test for phenols**

The ferric chloride test was used. A fraction of the extract (2 mL) was treated with 5% FeCl<sub>3</sub> reagent and observed for the formation of deep blue-black colour [53].

### **3.3.7 Test for anthocyanins**

2 mL of 2M HCl with ammonia was added to 2 mL of the plant extracts in test tubes and observed. The appearance of a pink-red colour that turns blue-violet after a short while is the indication of the presence of anthocyanins [91].

### **3.3.8 Test for saponins**

The froth test was used in which 1 mL of the extracts was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes and formation of 1 cm layer of foam indicates the presence of saponins [92].

### **3.3.9 Test for terpenoids**

A 2 mL sample of the plant extract was placed in a test tube and 2 mL of chloroform, 2 mL of acetic anhydride and a few drops of concentrated sulphuric acid were added to the test tube and observed. The formation of a pink colour indicates the presence of terpenoids [92].

### **3.3.10 Test for tannins**

A few drops of 1 % lead acetate were added to 2 mL of the plant extract in a test tube. The presence

of tannins was to be indicated by the formation of a yellow precipitate [93].

### **3.3.11 Test for coumarines**

1 mL of 10 % sodium hydroxide was added to 1 mL of plant extract in a test tube and observed.

The presence of coumarines is indicated by the formation of yellow colour [94].

### **3.3.12 Test for emodins**

To a test tube, 2 mL of benzene and 2 mL of ammonium hydroxide were added to 2 mL of plant extract and the mixture was shaken and observed. The appearance of a red colour indicates the presence of emodins [95].

## **3.4 Column chromatography**

The solvent extract with the highest extraction yield, the Water/Methanol <sup>50</sup>/<sub>50</sub> extract was separated using column chromatography. A small wad of cotton was placed at the bottom of the burette using a long glass rod. To pack the column, a stream of silica gel was poured into the burette using a funnel whilst tapping the burette to allow it to pack evenly. The column was packed until it reached the 25 mL mark on the burette. The mobile phase was a 1:4 mixture of ethyl acetate and hexane, the mixture was poured into the column and allowed to wet the entire column before the separation of the extract began. The concentrated extract was poured into the column and then more of the mobile phase was added. The extract was allowed to form fractions and elute from the column whilst their respective retention times were noted [96]. The resultant fractions from the column chromatography procedure were tested for the different phytochemicals present in each of the fractions. The procedures described earlier were used for the phytochemical tests.

## **3.5 Optimization studies for the synthesis of silver nanoparticles**

The optimisation studies were carried out using the Water/Methanol <sup>50</sup>/<sub>50</sub> extract which had the

highest extraction yield. All the optimization studies were monitored using UV-VIS analysis and a volume of 1 mL from each of the resultant sample was diluted with 10 mL of distilled water to reduce the colour intensity of the samples for the UV-VIS spectroscopic analysis. A Water/Methanol <sup>50</sup>/<sub>50</sub> solution was used as the blank for the UV-VIS analysis which was carried out between the 250nm-600nm wavelengths. A graph of absorbance versus wavelength was plotted for each of the samples to determine the best condition for the synthesis of the silver nanoparticles [97].

### **3.5.1 Effect of the volume of plant extract**

The effect of the volume of plant extract for the synthesis of silver nanoparticles was determined by using 10 mL of 10 mM silver nitrate solution against five different volumes of the plant extract. Plant extract volumes of 2 mL, 4 mL, 6 mL, 8 mL and 10 mL were used to prepare different sample solutions. The samples were left to react for 24 hours before they were analysed using UV-VIS spectroscopy [98].

### **3.5.2 Effect of silver nitrate concentration**

The effect of the concentration of silver nitrate in the synthesis of silver nanoparticles was determined by using 10 mL of the plant extract against five 10 mL samples of silver nitrate with different concentrations. The concentrations used were 2 mM, 4 mM, 6 mM, 8 mM and 10 mM solutions of silver nitrate. A 10 mM solution of silver nitrate was prepared and then serial dilutions were carried out to reduce the concentration to the required concentrations for the procedure. The solution were all left to react for 24 hours before a UV-VIS spectrophotometer was used for their analysis [99].

### **3.5.3 Effect of temperature**

The effect of temperature on the synthesis of silver nanoparticles was studied. 10 mL samples of

the plant extract were mixed with a 10 mL samples of 10 mM silver nitrate solution and placed into five separate labelled reagent bottles with rubber stoppers. The samples were then allowed to react at different temperatures (20 °C, 30 °C, 40 °C, 50 °C and 60 °C) [8]. The samples were maintained at their respective temperatures with the aid of an ice bath, a water bath and a thermometer, depending on the temperature required. The samples were maintained at their respective temperatures for 6 hours each and then UV-VIS spectroscopy was carried out for their analysis [32].

#### **3.5.4 Effect of reaction time**

The effect of reaction time in the synthesis of silver nanoparticles was also optimised. A volume of 30 mL of the plant extract was mixed with 50 mL of the 8 mM silver nitrate solution and the UV-VIS analysis of the samples was carried out at different intervals as the reaction proceeded. A small aliquot of the reaction solution was collected and analysed at the following time intervals, 0 minutes, 5 minutes, 10minutes, 30minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours and 8 hours for the respective reaction time solution. The data obtained was used to plot the UV-VIS spectra in reference to reaction time and graphically present the effect of time [100].

#### **3.6 Stability studies**

The optimum conditions determined from the above optimisation studies were used to synthesize silver nanoparticles to be used in the determination of the stability of the synthesised silver nanoparticles. 30 mL of the plant extract was mixed with 50 mL of 8 mM silver nitrate solution and placed in a rubber stoppered reagent bottle. The sample was then placed in a water bath at a temperature of 50 °C for 24 hours. After 24 hours, the sample was analysed using UV-VIS spectroscopy at a wavelength of 400nm (the approximate SPR wavelength determined from the optimisation studies) [97]. The UV-VIS analysis was carried out at different intervals for the

following 24 days. A graph was plotted using the absorbance values determined at 400nm and the stability of the synthesised nanoparticles was noted [56].

### **3.7 Synthesis of silver nanoparticles**

The synthesis of silver nanoparticles was carried out for each of the 7 solvents used in the plant extraction procedure. The optimum conditions for the synthesis of silver nanoparticles using the water/methanol <sup>50</sup>/<sub>50</sub> solution were employed in the synthesis of silver nanoparticles using each of the solvents. The silver nitrate solution was prepared using the respective extraction solvent for the water immiscible solvent i.e. hexane, cyclohexane and ethyl acetate. To aid the rate solvation of the solid silver nitrate in the solvents in order to prepare a solution of the optimum concentration was carried out using a sonicator. A volume of 30 mL of the plant extracts of each of the solvents were mixed with 50 mL of 8 mM silver nitrate solution and then placed in a water bath at 50 °C for 24 hours. The resultant silver nanoparticles solutions were then analysed using UV-VIS spectrophotometry to determine the formation of the silver nanoparticles [12]. The UV-VIS analysis for the 8 mM silver nitrate solution was also carried out and used as a reference.

FTIR analysis of the different solvent extracts used and their respective silver nanoparticles solutions were carried out using a liquid KBr disk. A few drops of the analysis sample was placed onto the clean disk and FTIR analysis was carried out.

The synthesised silver was separated from the solution by centrifugation at 5000 rpm for 20 minutes and dried in an oven at 40 °C for 24 hours and then the dried samples were stored in air-tight containers at room temperature for usage in the following steps in the research project [3].

### **3.8 Antimicrobial activity testing**

The antimicrobial activity evaluation was carried out using the Kirby-Bauer disc diffusion method.

The synthesized silver nanoparticles were compared against each other alongside the 8 mM silver nitrate solution and the Trimethoprim antibiotic standard. The bacterium which was used was *Escherichia coli* which is a gram-negative bacteria and *Staphylococcus aureus* which is a gram-positive bacteria. All the apparatus used during the course of the antimicrobial testing were firstly sterilized in an autoclave and ethanol was used for the step by step sterilization.

The *E. coli* and *S. aureus* subcultures were prepared on separate Mueller-Hinton agar plates and incubated for 24 hours [101]. After the culturing period, sterile 6 mm filter paper disks were used for the samples to be tested. Each of the solid samples was dissolved in the ratio, 2 mg per mL with distilled water and the paper disks were dipped into the samples and placed onto the disks using forceps [1]. The agar plates were carefully labelled according to the bacterium cultured and dissected according to the sample being tested and then incubated for 24 hours.

After 24 hours of incubation, the plates were collected, observed and the inhibition zones for each of the samples were measured (in millimetres) using a ruler. The data obtained from the antimicrobial tests was graphically represented and interpreted.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

#### 4.1 Introduction

This chapter gives an outline of the results obtained during the course of the research project and their detailed discussion. The results will include the extraction procedure results, the phytochemical tests results, the optimisation studies and synthesis results and the evaluation of the antimicrobial activity results.

#### 4.2 FTIR analysis of *Allium Ascalonicum* powder

A sample of the dried and powdered *Allium Ascalonicum* plant was analysed using FTIR analysis to identify the functional groups present in the crude sample. The functional groups were used to identify some of the potential functional groups present in the plant thus hypothesizing the ability of the plants extracts to reduce and cap the silver nanoparticles to be synthesized.

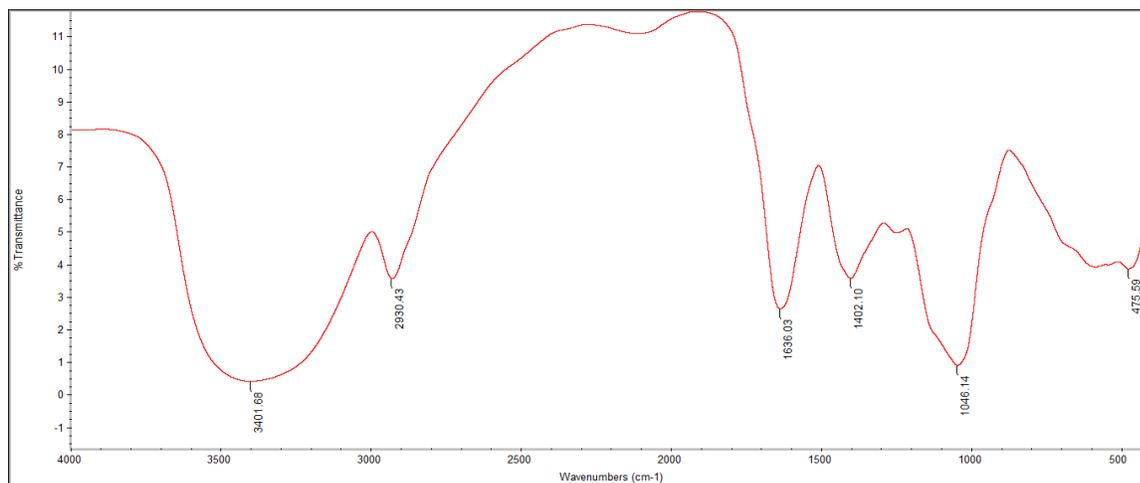


Figure 4.1: FTIR spectra of *Allium Ascalonicum* powder

FTIR analysis of powdered *Allium Ascalonicum* was carried out in order to identify the functional groups present in the plant sample and assist in the confirmation of which phytochemicals may be

present in the plant. The FTIR spectra of the *Allium Ascalonicum* plant is shown in Figure 4.1 and the functional groups determined to be present in the *Allium Ascalonicum* sample by the FTIR analysis are translated in Table 4.1. Phytochemicals mostly constitute of similar functional groups thus it is very important to carry out phytochemical tests to determine the specific phytochemicals present in the sample.

Table 4.1: FTIR interpretation of *Allium Ascalonicum* plant powder

Vibration $\text{cm}^{-1}$	Inference
3401.68	$\equiv\text{C}-\text{NH}_2$ primary $\text{NH}_2$ , strong amide $\equiv\text{C}-\text{NH}_2$ secondary N-H, weak amine R-O-H alcohol O-H O=C-O-H acid O-H
2930.43	C-H bond O-H acid O-H carboxylic acid stretch
1636.03	-C=C bond $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{R} \end{array}$ conjugated ketone $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{C}-\text{NR}_2 \end{array}$ saturated amide =C=N-
1402.10	$\equiv\text{C}-\text{O}-$ acyl and phenyl C-O $\begin{array}{c} \text{O} \\ \parallel \\ \text{R}-\text{N}^+ \\ \parallel \\ \text{O}^- \end{array}$ symmetric nitro
1046.14	-C-O alkoxy

The FTIR results show the presence of various polar functional groups such as the alkoxy group, hydroxyl group, the acyl group and the phenyl group from the plant. This coincides with the general structures of the phytochemicals discussed in the literature review. The presence of some functional groups that contain nitrogen such as the -NH<sub>2</sub> and -NH groups alongside C=C from aromatic rings and heterocyclic constituents (e.g. the alkaloids) impart a certain degree of non-polar behaviour amongst the compounds (phytochemicals).

The variation of polarity of the phytochemicals due to the presence of varying functional groups indicate that the phytochemicals mostly have large relative molecular structures and may be regarded as complex organic compounds (i.e. several cyclic and aromatic rings in the same molecule). Different solvents with varying polarity index values were then used during the extraction period and their corresponding yields are shown in Table 4.2.

Table 4.2: Polarity indices of the different solvents and extraction yields

	Ethyl Acetate	Cyclo-he xane	Distilled water	Hexane	Ethanol	Methanol	Methanol water <sup>50/50</sup>
Polarity index	4.3	0.04	10.2	0.1	4.4	5.1	7.65
% yield	35.09	23.00	58.80	27.03	45.38	49.98	63.29

### 4.3 *Allium Ascalonicum* phytochemicals extraction results

The phytochemicals from the *Allium Ascalonicum* plant were extracted using the maceration method with different solvents and Table 4.3 shows the calculated yield for each of the solvents used [87].

Table 4.3: Phytochemicals extraction using different solvents.

	Ethanol	Cyclo-h exane	Distilled water	Hexane	Ethyl acetate	Methanol	Methanol- water <sup>50/50</sup>
Initial	5.0248	5.0184	5.0152	5.0274	5.0232	5.0166	5.0016
mass/g							
Final	3.2614	3.8641	2.0665	3.6687	2.7439	2.5094	1.8363
mass/g							
Mass	1.7634	1.1543	2.9487	1.3587	2.2793	2.5072	3.1653
extracted/g							
% yield	35.09	23.00	58.80	27.03	45.38	49.98	63.29

The percentage yields for the different solvents extraction indicated that the more polar solvents had better extraction efficiency for the phytochemicals extraction. Differences in the structure of phytochemical compounds also determine their solubility in solvents of different polarity. Distilled water has a polarity index of 10.2 and an extraction yield of 49.98 % whilst methanol has a polarity index of 5.1 but an extraction yield of 58.90 %. Putting into consideration that the non-polar solvents, hexane and cyclohexane managed to extract some of the phytochemicals from the plant, it is deduced that the relatively lower polarity of methanol (relative to water) allowed it to interact more with the less polar functional groups on some of the phytochemicals thus maximising its extraction yield. Bioactive components such as carbohydrates and proteins have a very high solubility coefficient in methanol thus methanol resulted in a higher extraction yield as compared to distilled water despite it having a lower relative polarity index [87]. On the contrary, ethanol has a polarity index of 4.3 but resulted in a lesser relative yield than methanol which meant that the plants phytochemicals are polar in general. It can be deduced that most phytochemicals

have polar functional groups such as the -OH, -OCO and the -COOH by their interaction with the more polar solvents used during the extraction. The extraction efficiency was maximized by using a maceration time of 48 hours and the use of a shaker. This enable maximum interaction between the solvent and the powdered plant [85]. The plant had been ground and sieved to increase surface area and also obtain particles of a relatively homogeneous size thus enabling certain comparisons to be done [97].

#### **4.4 Phytochemical analysis**

Phytochemical tests were carried out on the different solvent extracts to qualitatively identify the specific phytochemicals present in the different plant extracts. The methods described in the methodology and literature were used during the phytochemical analysis. The information deduced from these tests would aid in the characterisation of the plant and identification of which of the phytochemicals were extracted by each of the solvents used. The purpose of standardized extraction procedures for medicinal plant parts is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum [91]. The phytochemical tests were carried out to also provide information on the selectivity of the various solvent in extracting specific phytochemicals. The results of the phytochemical tests are shown in Table 4.4.

Table 4.4: Phytochemical tests results

	Methanol -water <sup>50</sup> / <sub>50</sub>	Distilled water	Methanol	Ethanol	Ethyl acetate	Cyclo- hexane	Hexane
Steroids	+	+	+	+	+	-	+
Alkaloids	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+
Quinones	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	-	-	-
Phenols	+	+	+	+	+	-	+
Anthocyanins	+	+	+	-	-	-	-
Saponins	+	+	+	+	+	+	+
Terpenoids	+	+	+	+	-	-	-
Tannins	+	+	+	-	-	-	-
Coumarines	-	-	-	-	+	-	-
Emodins	-	-	-	-	-	-	-

In general, the results deduced that the more polar solvents (methanol/water <sup>50</sup>/<sub>50</sub>, distilled water, methanol, ethyl acetate and ethanol) were able to extract more phytochemicals than the non-polar solvents (hexane and cyclohexane) and this confirmed that solvents have different extraction capacities and different spectrum of solubility for the phytochemical constituents of plants, which are known to be biologically active [102].

The phytochemical tests were carried out for each of the 7 solvents used for the extraction and the significance (activity) of each of the phytochemicals is shown in Table 4.5 [103]. The functional

group constituents of the phytochemicals which are said to be the main characteristic of each of the phytochemical and also a very important molecular structure constituent of the phytochemicals as shown in table, were determined to be present by the FTIR analysis of the crude plant powder thus indicating a great correlation between the results obtained.

Table 4.5: Phytochemicals activity

Phytochemicals	Structural features	Examples	Activities
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Glycosides	Sugar + non-carbohydrate moiety	Amygdalin	Antidiarrhoeal
Flavonoids	Phenolic structure, one carbonyl group Hydroxylated phenols, C6 -C3 unit linked to an aromatic ring Flavones + 3 hydroxyl group	Chrysin, Quercetin, Rutin	Antimicrobial Antidiarrhoeal
Phenols and Polyphenols	C3 side chain, - OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal

Saponins	Amphipathic glycosides	Vina-ginsenosides-R5 and -R6	Antidiarrhoeal
Terpenoids and essential oils	Acetate units + fatty acids, extensive branching and cyclized	Capsaicin Berberine	Antimicrobial Antimicrobial Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt. 500-3000)	Ellagitannin	Antimicrobial, Anthelmintic, Antidiarrhoeal
Coumarines	Phenols made of fused benzene and $\alpha$ -pyrone rings	Warfarin	Antimicrobial

The general structures of the phytochemicals extracted from the *Allium Ascalonicum* plant are generally polar due to the functional groups present on their structures as indicated in Table 4.5 and this coincides with the results obtained from the extraction using different solvents. During extraction, solvents diffuse into the solid plant material and solubilize compounds with similar polarity [104]. These phytochemicals also possess natural antimicrobial, antioxidant, antimicrobial antidiarrhoeal and anthelmintic activity which is one of the main reason why green synthesis has been pursued to such an extent in recent years. The functional groups responsible the reduction and capping/stabilizing of silver nanoparticles during the plant-mediated synthesis procedure are the polar constituents such as the –OH and –COOH [105]. This then leads to a deduction that *Allium Ascalonicum* plant extract can be used in the synthesis of silver

nanoparticles.

From the above results it can be clearly seen that the methanol, distilled water and methanol-water <sup>50</sup>/<sub>50</sub> v/v solvents resulted in the highest yield of extraction and the most number of phytochemical species. The tests resulted in similar phytochemical tests positives with ethanol, excluding anthocyanins and tannins. Of the 5 solvents regarded as the polar solvents in this research project, ethanol had the lowest polarity index value of 4.3, this may have been a contributing factor when it comes to the extraction capability of the 2 phytochemicals.

An unexpected result was noted in the test for coumarines which only resulted in a positive test for the ethyl acetate extract. Coumarines are phenolic compounds made of fused benzene and  $\alpha$ -pyrone rings and it was highly expected that they would give a positive test result in the other polar solvents [102]. The absence of coumarines in the ethanol, methanol, distilled water and methanol-water <sup>50</sup>/<sub>50</sub> v/v solvents may have been due to the fact that some phytochemicals only exhibit a certain solubility in certain solvents, due to the properties of the solvent used during extraction, despite the relative polarity of the phytochemical and solvent (ethyl acetate is an ester). Since solvents diffuse into the solid plant material and solubilize compounds with similar polarity during extraction [91], it is highly probable that the polarity of the ethyl acetate is relatively similar to that of the coumarines thus they were soluble in it. Ethanol has a relatively similar polarity index as compared to ethyl acetate but the properties exhibited by the ethyl acetate as an ester may have induced a greater solubility potential to extract coumarines as compared to the alcohol, ethanol.

The general presence of steroids, alkaloids, glycosides flavonoids, phenols, anthocyanins, saponins, terpenoids, tannins and coumarines in the overall *Allium Ascalonicum* plant extract indicates that this plant has excellent potential for the reduction and capping of silver ion to silver nanoparticles.

#### 4.4.1 Column chromatography

The methanol/water <sup>50/50</sup> extract (the extract with the highest extraction yield) was distilled at a temperature of 70 °C to separate methanol (boiling point 64.7 °C) from the solution. This was carried out in order to allow the extract to undergo separation column chromatography using a polar stationary phase, silica gel mesh. The mobile phase used was a 1:4 ethyl acetate-hexane mixture. The distilled methanol was concentrated before the column chromatography procedure to enable the most efficient separation of phytochemicals to occur and this also allowed the colour of the extract to intensify and this was favourable as it imparted better clarity of fractions in the column [106]. Despite the effort, the column, however, did not produce the expected clear fractions during the separation. The extracts were then collected in 6 different flasks at 7 minute intervals and the phytochemical tests were carried out to determine which phytochemicals would be retained the least and the most in the column.

The fractions collected from the column were taken for phytochemical tests and the results are shown in Table 4.6.

Table 4.6: Methanol water 50/50 v/v column fractions phytochemical tests

	1	2	3	4	5	6
Steroids	-	-	+	+	+	-
Alkaloids	-	-	+	+	+	-
Glycosides	-	+	+	+	+	-
Quinones	-	-	-	-	-	-
Flavonoids	-	-	-	+	+	+
Phenols	-	+	+	+	-	-

Anthocyanins	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Terpenoids	-	+	+	+	-	-
Tannins	-	-	+	+	-	-
Coumarines	-	-	-	-	-	-
Emodins	-	-	-	-	-	-

The stationary phase of the column was also the polar phase thus it was highly expected that the first fractions collected would not give any positive result for the phytochemical tests. The first fraction, however, resulted in a positive test for saponins and the second fraction gave positive test results for glycosides, terpenoids, saponins and phenols. This showed that the molecular structures of the phytochemicals are highly complex and they are able to interact with different solvents of different polarities.

During the distillation process, the methanol was vapourised and condensed thus separating it from the water because water dissolves the stationery phase that was to be used (silica gel mesh). It is highly possible that some of the phytochemicals remained in the distillation flask with the water because the temperature used was not able to vapourise them (e.g. gallic acid, a tannin that boils at 260 °C) [26].

#### 4.5 Optimisation studies

Optimisation studies for the synthesis of silver nanoparticles using *Allium Ascalonicum* plant extract were carried out using the methanol water <sup>50</sup>/<sub>50</sub> extract which had previously resulted in the highest extraction yield. The parameter which were optimised were temperature, silver nitrate

concentration, volume of plant extract and reaction time. It is well known that silver nanoparticles exhibit yellowish- brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Reduction of the silver ion to silver nanoparticles during exposure to the plant leaf extracts could be followed by colour change and thus UV-VIS spectroscopy [107]. Since the peak wavelength did not shift during the reaction, it was possible to quantitatively monitor the concentrations of silver nanoparticles and thus conversion by measuring the absorbance at 400 nm [107].

Surface plasmon resonance is a collective excitation of the electrons in the conduction band around the nanoparticles surface. Electrons conform to a particular vibration mode due to shape and size of particles. Therefore, metallic nanoparticles display characteristic optical absorption spectra in the UV–visible region [108]. Silver nanoparticles also exhibit a specific surface plasmon resonance that is determined by the type of capping and reducing agent used during their synthesis. UV-VIS analysis was carried out in the 250nm – 600 nm wavelength range [15].

#### **4.5.1 Effect of plant extract volume**

The reduction of pure silver ions was observed by measuring the UV–Vis absorbance of the different plant extract volume reaction samples and the resultant spectra was plotted as shown in Figure 4.2. The effect of the plant extract volume was also determined using UV-VIS analysis at the SPR wavelength determined from the optimization of plant extract volume (Figure 4.3).

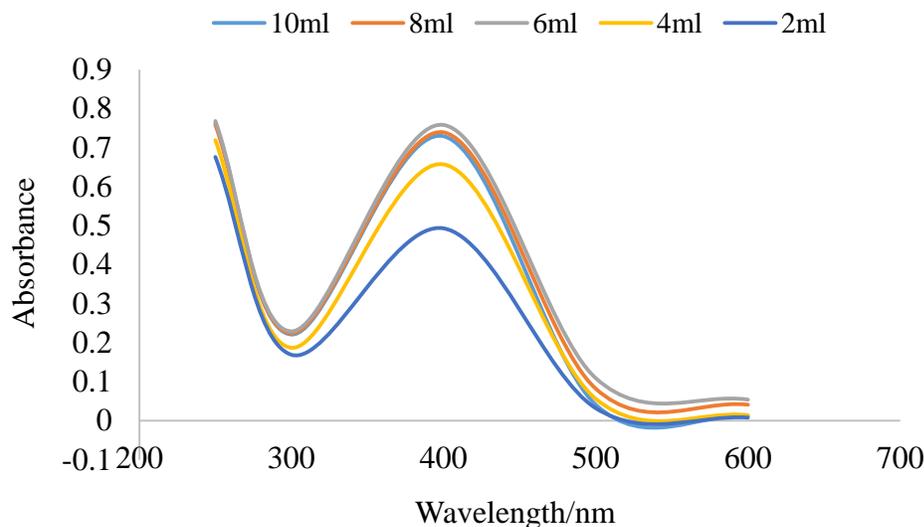


Figure 4.2: Optimization of plant extract volume

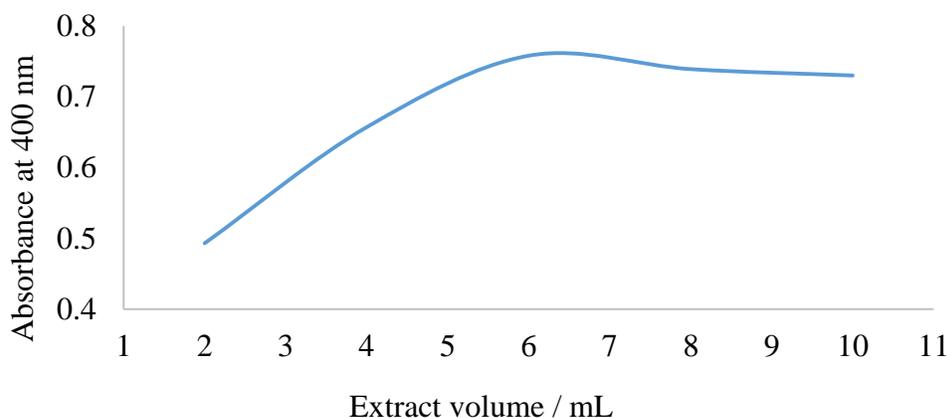


Figure 4.3: Effect of plant extract volume

In the UV/VIS absorption spectra of silver nanoparticles, synthesized using various reducing agents, narrow surface plasmon absorption peaks at the wavelengths 390-420 nm confirm the nanocrystalline character of the particles and the low degree of their polydispersity [20]. The results clearly showed that the synthesis of silver nanoparticles occurred with an optimum plant extract volume of 6 mL (Figure 4.2). This was also illustrated by the resultant colour of the varying reaction solutions. The 6 mL sample exhibited relatively the same colour intensity as the 8 mL sample and the 10 mL sample. This suggested that the reaction had completed. The SPR band was

centred on the 400nm wavelength. This band relatively coincided with that exhibited when a similar plant, onions were used in other studies for the synthesis of silver nanoparticles (SPR band at 397nm) [37].

The effect of the volume of the plant extract at 400nm (Figure 4.3) clearly shows that the reaction gives a maximum absorbance at the 6 mL point then remained relatively constant. This may have been caused by the reaction reaching equilibrium thus the reaction does not proceed any more. This was further confirmed by UV–Vis spectroscopic analysis [8]. The plant extract possessed various electronegative functional groups and other groups that are associated with resulting in the acidity of solutions such as the OH and COOH groups. This was confirmed by the reaction samples all having pH values less than 6. The NO<sub>3</sub> ion on the silver nitrate solution induces acidity (low pH of less than 7) alongside a relatively acidic plant extract solution which suggests that the 8 mL and 10 mL plant extract samples were highly acidic thus the reaction could not proceed to produce a concentration of silver nanoparticles higher than that given by the 6 mL sample.

The optimum volume was determined to be 6 mL of plant extract whilst the higher volumes (8 mL and 10 mL) showed relatively less absorbance at the SPR wavelength. When the concentration of the biological material mediating nanoparticle synthesis is increased, higher contents of the biomolecules involved in the metal reductive process are available resulting in a more intense colour, according to what has been reported with the bark extract of *Cinnamon zeylanicum* [36]. This, however, was not the case with the *Allium Ascalonicum* extract. The increased number of biomolecule resulted in agglomeration which reduced the absorption in the UV-Vis spectroscopy [93] thus a lesser absorbance was noted after the 6 mL of the plant extract.

A study carried out by Yong obtained sub-micro scale particles between 100 and 800 nm with high concentrations of leaf broth (more than 10 %), suggested that too many reducing agents cause

aggregation of the silver particles synthesized due to the interactions between capping molecules bound to the surface of particles and secondary reduction process on the surface of the pre-formed nuclei [35].

It is considered that particle size and shape are dependent on various conditions such as plant type, nanoparticle type, reaction temperature and composition [35]. A variation in the biological material and metal salt concentration is known to influence nanoparticle synthesis. When the concentration of the biological material mediating nanoparticle synthesis is increased, higher contents of the biomolecules involved in the metal reductive process are available resulting in a more intense colour. Such an effect has been reported with the bark extract of *Cinnamom zeylanicum* [36].

The spectra of the different volumes of the plant extract show an increase in the relative sharpness of the absorbance peak, from the lowest absorbance to the highest absorbance. The sharpness of a peak indicates the relative similarity in the size of the nanoparticles. The excess amount of plant extract (8 mL and 10 mL) lead to an agglomeration effect that lead the nanoparticles to vary slightly in their relative size. The broadening of peaks at a lower concentration of  $\text{AgNO}_3$  in the solution could be mainly attributed to the superposition of multi-ionic bands of Ag particles like  $\text{Ag}^+$ ,  $\text{Ag}_2^+$  and  $\text{Ag}_3^{2+}$  exhibiting different colloidal states [109].

#### **4.5.2 Effect of silver nitrate concentration**

The effect of silver nitrate concentration in the synthesis of silver nanoparticles was determined using a range of concentrations. The optimisation spectra for this particular parameter and the effect of silver nitrate concentration on the synthesis of silver nitrate are shown in Figure 4.4 and 4.5 respectively.

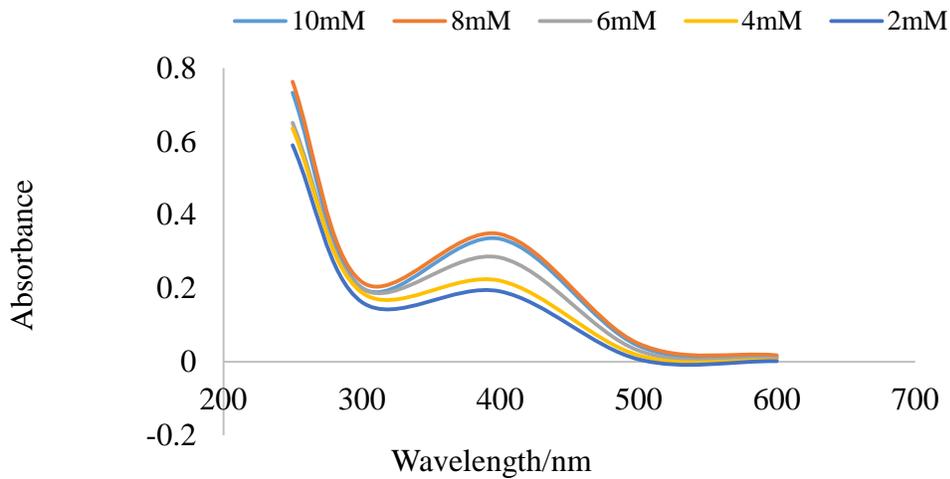


Figure 4.4: Optimization of silver nitrate concentration

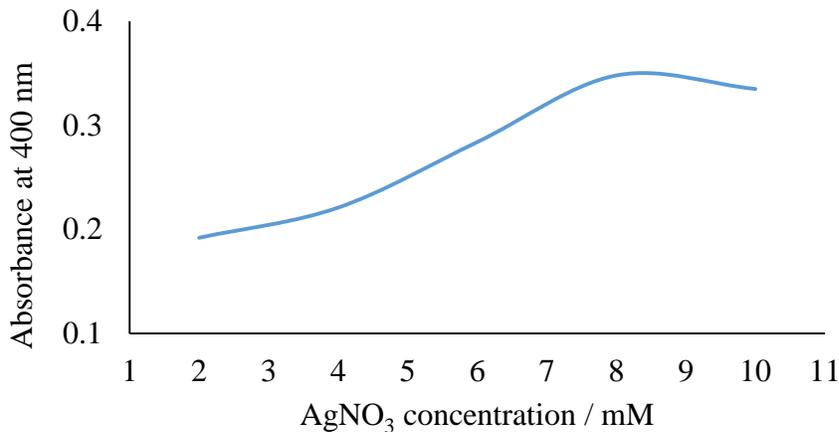


Figure 4.5: Effect of silver nitrate concentration

The highest absorbance was given off by the 8 mM  $\text{AgNO}_3$  sample with the SPR wavelength at 400nm. The absorbance peak usually arises as a result of the excitation of localized surface plasmon oscillations of the conduction electrons in case of noble metal nanoparticles such as gold and silver [41]. The increase in concentration illustrated the increase in absorbance in the UV-visible spectra which then showed a slight decrease in the 10 mM  $\text{AgNO}_3$  concentration sample. Considering Beer-Lamberts law, which deduces that absorbance is proportional to concentration, it was deduced that the highest concentration of silver nanoparticles was found in

the 8 mM sample thus it is the optimum AgNO<sub>3</sub> concentration. The observed colour intensity of the 8 mM appeared to be the darkest thus also coinciding with the UV-VIS spectra results. It is well known that silver nanoparticles exhibit yellowish- brown colour in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles [105]. Colourless-ness of the reaction mixture at the initial stage and the final deep reddish-brown colour after the completion of the reaction indicate the silver nanoparticles shape, size and the size distribution altering with the reaction time at different stages. The colour change might be due to the excitation of SPR in the production of silver nanoparticles [99].

The synthesis of silver nanoparticles is affected by factors such as the plant extract, silver nitrate concentration and temperature. The amount/concentration of reducing and capping phytochemicals present in an extract differ according to the plant, the environment the plant grows in and the conditions surrounding the plant during its growth. It has been reported that a 1 mM concentration of silver nitrate is best for the plant-mediated synthesis of silver nanoparticles as suggested by Balashanmugam [56]. Balashanmugam however, did carry out this study with other concentrations of silver nitrate (0.5 – 3 mM) and the results clearly showed that the best yield of silver nanoparticles does not necessarily come with the highest silver nitrate concentration.

This type of trend was observed in this research using *Allium Ascalonicum* and the study carried out by Obaid, who finally came up with the deduction that formation silver nanoparticles is not directly proportional to the Ag<sup>+</sup> concentration; small Ag<sup>+</sup> being enough to initiate the formation of the metal nucleation centre which acts as a catalyst for the reduction of another Ag<sup>+</sup> present in the reaction mixture [110].

#### **4.5.3 Effect of temperature**

The reactions for the optimisation of temperature were carried out at temperatures 20 °C, 30 °C, 40

°C, 50 °C and 60 °C [94]. A water bath was used to maintain the temperatures above room temperature whilst an ice bath was used to maintain the temperature below room temperature and the reactions were allowed to proceed for 4 hours [111]. The results are illustrated in Figures 4.6 and 4.7.

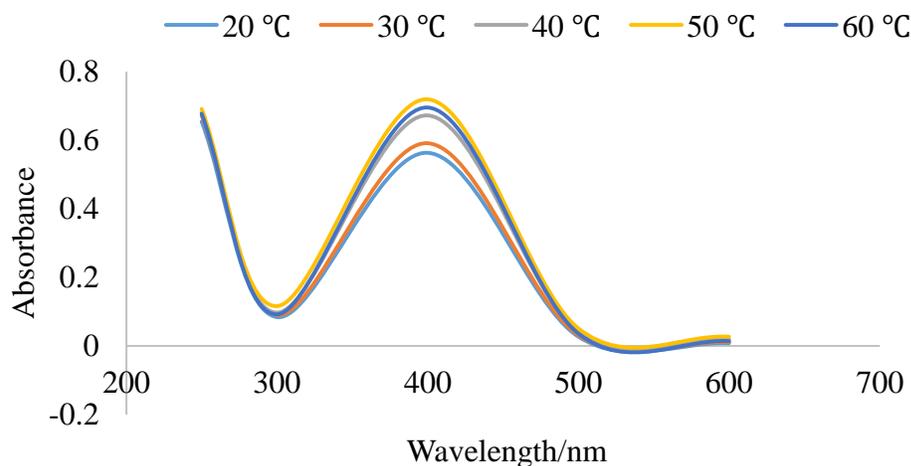


Figure 4.6: Optimisation of reaction temperature

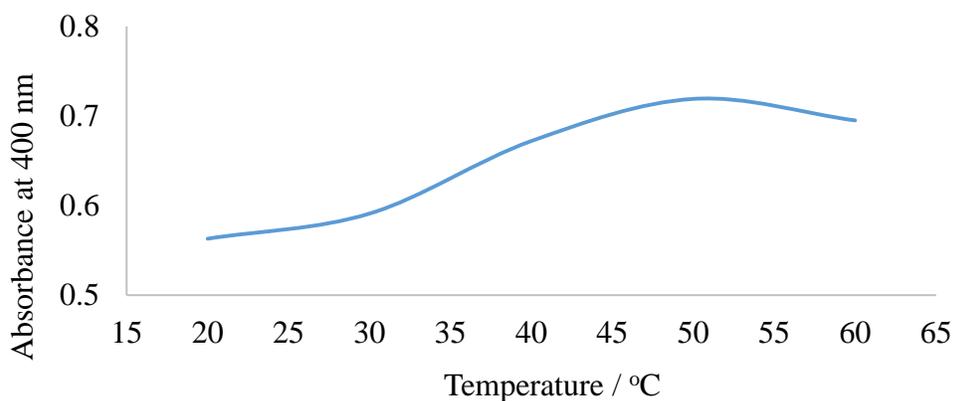


Figure 4.7: Effect of reaction temperature

The resultant curves from the UV-VIS analysis of the samples in the wavelength range of 250 nm-600 nm are shown in Figure 4.6. It has been noted that increasing temperature enabled the synthesis of silver nanoparticles to occur at a faster rate [6]. The highest absorbance was given by the 50 °C silver nanoparticles sample at the SPR wavelength of 400nm (Figure 4.7). This

suggested that an appropriate increase in temperature provided some activation energy to speed up the formation of the nanoparticles. The colour intensities of the sample were closely monitored and at the end of the 6 hours it was observed that the intensity of the 50 °C and 60 °C were the most intense and yet both looked the same. This suggested that the reaction had reached completion in both of them but the absorbance of the 50 °C was higher than given off by the 60 °C sample. It has been reported that the time required for complete reduction of the metal ions during biosynthesis of metal nanoparticles using bacteria and fungi range from 24 to 124 hours [100]. The rapid generation of nanoparticles was owing to the excellent reducing potential of the active components of the *Allium Ascalonicum* plant extract and their polymeric stabilization within a narrow size spectrum. It is possible that the reaction needed energy to speed up but the higher the temperature, the less effective the plant extract got because of some of the reducing and capping phytochemicals temperature degradation. An increase in temperature may have also resulted in some of the phytochemicals breaking down to smaller components which are not able to reduce and cap the synthesised silver nanoparticles.

Comparative study of various methods in the synthesis of AgNPs using *Amaranthus polygonoides* revealed that higher temperature method results in the rapid synthesis. Continuous stirring of silver nitrate solution with *Allium cepa* extract at 50-60 °C yielded average sized silver nanoparticles (33.6nm) and showed complete inhibition of *E. coli* and *Salmonella typhimurium* at 50 g/mL [12]. Silver nanoparticles synthesised using garlic extracts also showed that 60 °C the synthesis time was reduced, but an increase in the size and polydispersity the silver nanoparticles was observed [14].

As the reactions at 20 °C and 30 °C went through it was noted that the temperatures actually decreased to around 16 °C and 25 °C respectively. This may have been an indication that the

reaction between the *Allium Ascalonicum* plant extract and the silver nitrate solution was endothermic during the synthesis of the silver nanoparticles. This also explained why the temperatures above room temperature gave higher absorbance values at the SPR wavelength. The UV-VIS spectra given off by the five samples at the 400nm wavelength showed that the reaction for the synthesis of silver nanoparticles was maximum at 50 °C.

UV-VIS analysis confirmed that at the same extract concentration, the elevated temperature produced a slightly larger and more polydisperse population of silver nanoparticles. The increase in size and polydispersity is likely due to variation in the nucleation and growth rates of the nanoparticles during synthesis [14]. The temperatures used did not exceed 60 °C due to the plant extract used during the optimisation studies constituting of methanol which has a boiling point of 64.7 °C.

#### **4.5.4 Effect of reaction time**

The effect of the reaction time on the synthesis of silver nanoparticles was also studied. A timer was used to record all the times. Different samples were used in order to maintain the same conditions in each sample until the point at which it was analysed [8]. The results are shown in Figure 4.8 and 4.9 respectively.

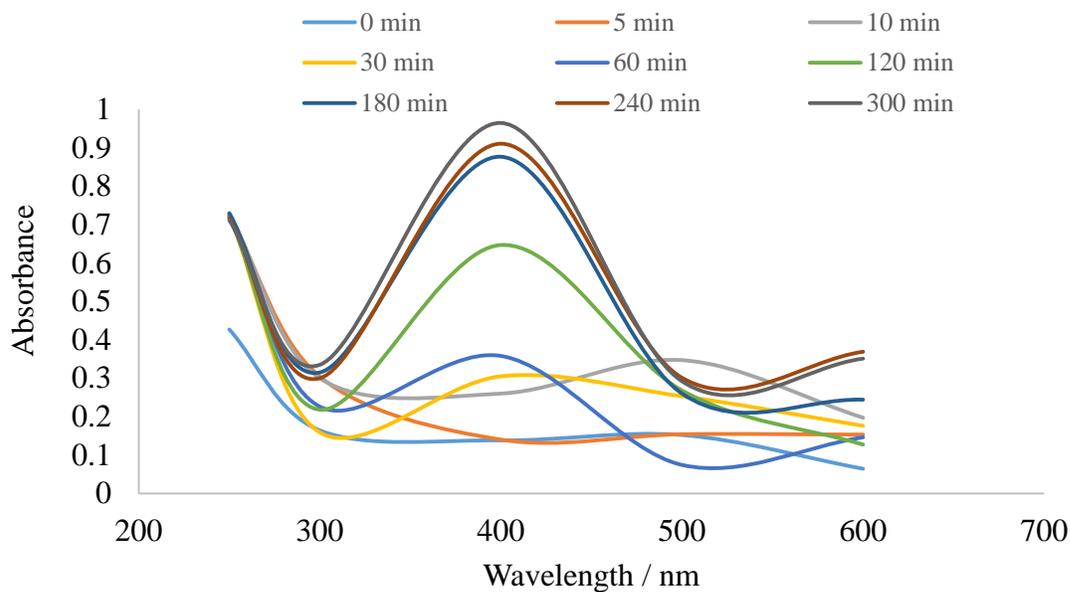


Figure 4.8: Optimisation of reaction time

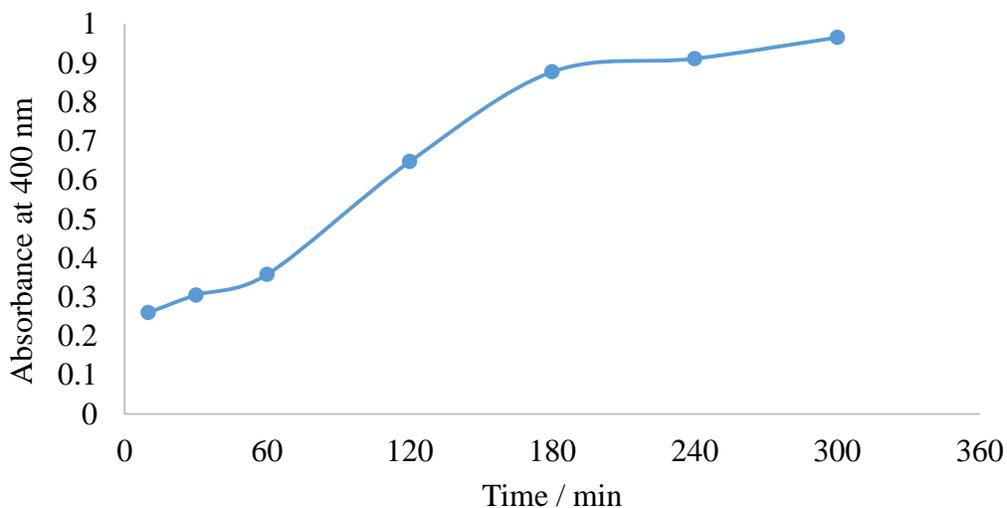


Figure 4.9: Effect of reaction time

The previously determined optimum conditions for the synthesis of silver nanoparticles were employed in the determination of the reaction time. The SPR peak wavelength determined from the previous optimisation synthesis procedures (400nm) was used to determine the effect of time. The synthesis of silver nanoparticles was first detected after 30 minutes and proceeded to rise at a relatively high and constant rate up until the 180 minute mark (Figure 4.9). The reaction proceeded

whilst showing a reasonable increase in the absorbance determined at 400nm but the rate of reaction has decreased. This may have been due to the  $\text{Ag}^+$  ions in solution reaching very low limits.

The UV-VIS spectra of the different samples indicated that the highest absorbance was given off by the highest time (300 minutes). This was an indication that, the synthesis reaction proceeds as time increases but this occurs at a very slow rate. It has been reported that the time required for complete reduction of the metal ions during biosynthesis of metal nanoparticles using bacteria and fungi range from 24 to 124 h [100]. The synthesis of silver nanoparticles mediated by the *Allium Ascalonicum* plant showed a significantly high reduction potential, as seen from the high absorbance at the highest reaction time. This is one of the reasons why the plant-mediated synthesis of silver nanoparticles has particularly been on the rise in recent years.

#### 4.5.5 Stability studies

The stability of the synthesised silver nanoparticles was determined over a period of 24 days whilst checking the absorbance at 400nm at different intervals. The stability of the nanoparticles gives a precise indication of the richness of phytochemicals with reducing and capping ability in the *Allium Ascalonicum* plant. The resultant stability graph is shown in Figure 4.10.

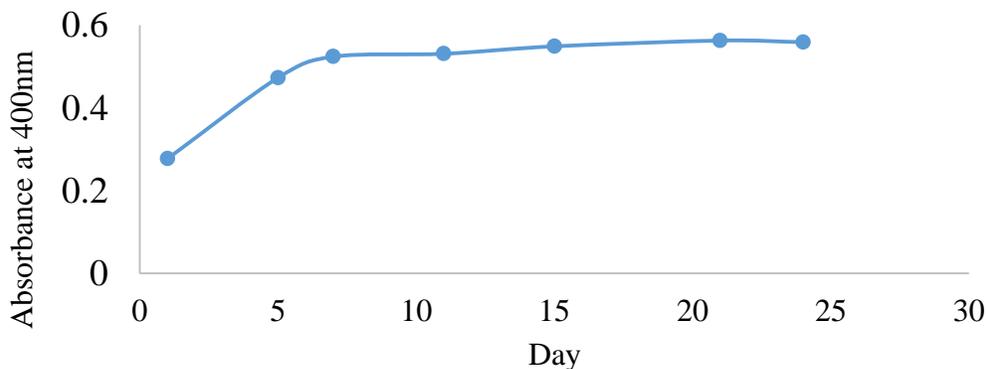


Figure 4.10: Stability studies

The silver nanoparticles showed a significant rise in the absorbance at 400nm for the first 7 days which indicated that the reaction was still in progress. This coincides with findings determined in the optimisation of reaction time, which showed that the reaction proceeds with time but at a slower rate than initially determined. The absorbance then remained significantly constant from day 7 to day 24. This indicated that the *Allium Ascalonicum* plants extracts were highly capable of capping the silver nanoparticles and maintaining the stability of the precipitate formed. This also coincides with the results obtained from other studies which determined that the presence of flavonoids, proteins and other reducing sugars in the plant extract are responsible for the capping of the silver nanoparticles thus inducing stability [29].

The almost constant absorbance given off by the silver nanoparticles at 400nm indicated that no agglomeration occurred at room temperature. This is mainly due to the excellent ability of the *Allium Ascalonicum* plants phytochemicals to cap the silver nanoparticles after reducing them. Agglomeration is defined as a collection of weakly bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components, the forces holding an agglomerate together are weak forces, for example van der Waals forces, or simple physical entanglement [112]. When agglomeration occurs, then peak absorbance at the SPR wavelength significantly decreases due to an increase in the particles size of the silver nanoparticles. The peak wavelength then shifts to the right indicating the presence of larger particles of silver nanoparticles.

Agglomeration is caused by factors such as an increase in the temperature of the silver nanoparticles whilst in solution and an increase in pH, leading to high alkalinity of the solution [113]. The reduction of silver ions in aqueous solution generally yields colloidal silver with particle diameters of several nanometres, initially the reduction of various complexes with  $\text{Ag}^+$

ions leads to the formation of silver atoms, which is followed by agglomeration into oligomeric clusters [18]. The ability of plant extracts to reduce silver ions and cap them thus inducing a high degree of stability is one of the major reasons why this biological synthesis technique has been deemed relatively favourable in recent years.

#### 4.6 Synthesis of silver nanoparticles from different solvent extracts

Silver nanoparticles were synthesised using all of the 7 solvents used in the extraction of the plants phytochemicals. The resultant silver nanoparticle solutions were scanned using UV-VIS spectroscopy to determine the formation of silver nanoparticles and compare the silver nanoparticle yield given off by the different solvents. The UV-VIS spectra given off by each of the solvents are shown in Figure 4.11 alongside the UV-VIS spectra of an 8 mM silver nitrate solution. The Observed colour changes after mixing and at 1 hour and 4 hours intervals are shown in Figure 4.12

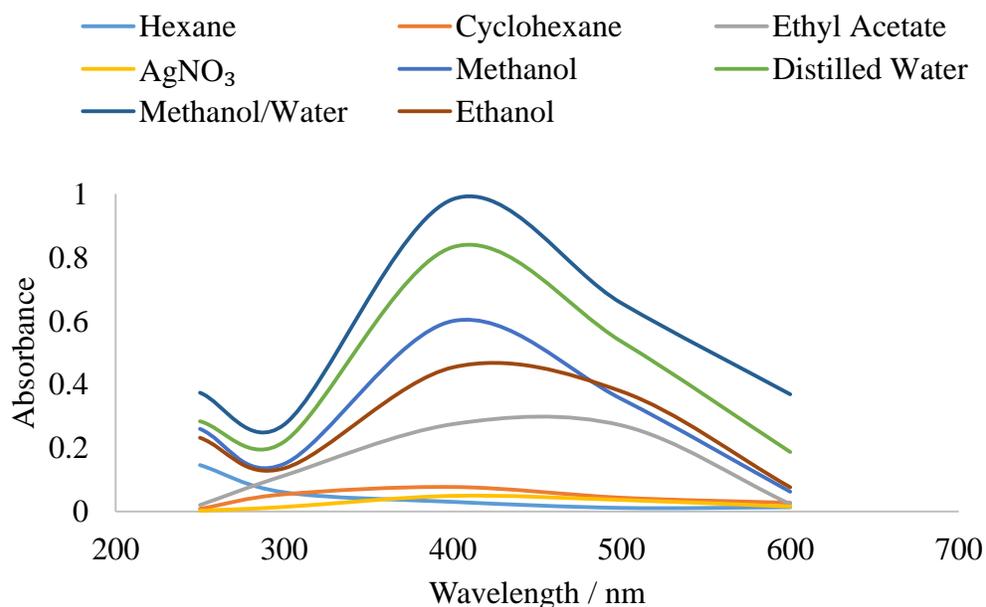


Figure 4.11: Synthesis of silver nanoparticles

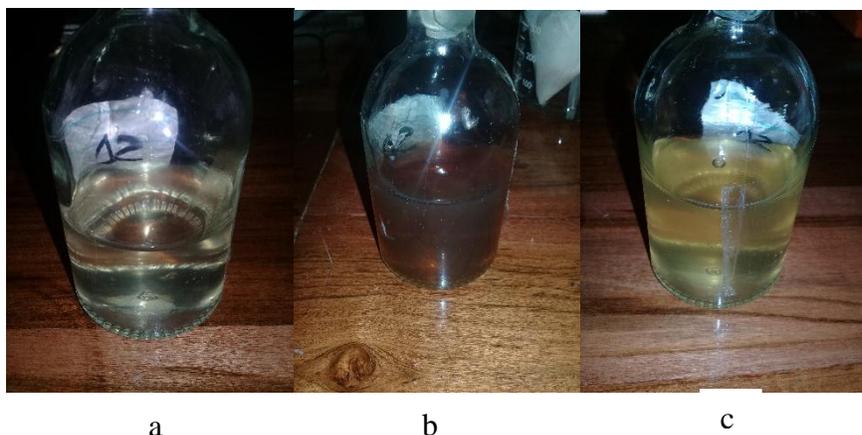


Figure 4.12: Observed colour changes a) After mixing b) After 1 hour c) After 4 hours

The optimum conditions determined using the highest yield extract, the methanol-water <sup>50</sup>/<sub>50</sub> solvent were used in the synthesis of all the nanoparticles and this could have led to slight deviations obtained in the overall synthesis using all the solvents due to the possibility that the different solvents possess different optimum conditions.

The silver nitrate solution showed no peaks when scanned between 250nm-600nm. The hexane and the cyclohexane extracts also indicated that no silver nanoparticles were formed. This may have been due to the absence of reducing functional groups in the phytochemicals extracted. The phytochemicals responsible for the reduction and capping of silver nanoparticles can be regarded as polar and it is understood that polar analytes are soluble in polar solvents thus enabling maximum extraction. The hexane and cyclohexane solvents are highly non-polar thus no phytochemicals with the ability to reduce and cap the silver to silver nanoparticles were extracted. Many studies have confirmed that also in other plant species where polar solvents produce a higher yield of phenolic concentration compared with the non-polar ones [114].

The ethyl acetate and ethanol solvents have a polarity index of 4.4 and 4.3 respectively. This degree of polarity suggested that a significant amount of the polar phytochemical with reducing and capping abilities were extracted. The ethanol and ethyl acetate UV-VIS spectra slightly

indicated the formation of silver nanoparticles but the spectra were relatively broad which indicated a high degree of agglomeration. Agglomeration affects the crystalline size and crystalline structure of plant extract synthesised silver nanoparticles [115]. This means that besides crystallite size, crystallinity also plays an important role in determining the effect of peak broadening [109]. Absolute polar solvents such as ethanol produce relatively lesser extraction yield [114]. It is possible that the phytochemicals extracted by the ethyl acetate and ethanol were insufficient to reduce and then cap all the silver nanoparticles thus leading to a high degree of agglomeration.

The methanol-water <sup>50</sup>/<sub>50</sub> mixture, methanol and water solvent extracts produced silver nanoparticles with the highest UV-VIS spectra absorbance and this indicated that they were able to successfully reduce and cap the silver ions. The methanol extract produced silver nanoparticles with the lowest yield, amongst the 3 solvents and this may have been due to its absolute alcoholic nature [114]. Highly-polar solvents (for example water) are not appropriate for extracting a high polar content. Moreover, the use of water as the only solvent yields an extract with a high content of impurities (for example organic acids, sugars, soluble proteins) along with polar compounds which could interfere in the identification and quantification of the extracts [114]. So the application of water combined with other organic solvents makes it a moderately polar medium ensuring the optimal conditions for extraction. Using water in combination with alcohols leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent finally improves the extraction yield [114].

The highest absorbance values at the SPR wavelength were given off by the methanol-water <sup>50</sup>/<sub>50</sub> solvent and the water solvent extracts. These confirmed the initially determined results that indicated that these two solvents had the highest extraction yields. The high extraction yield meant

that a high degree of phytochemicals with the ability to reduce and cap of silver nanoparticles was present in the extracts. These 2 extracts also tested positive for phytochemicals such as alkaloid, flavonoids, terpenoids and glycosides. which have been previously determined to exhibit excellent reducing and capping abilities in the plant-mediated synthesis of silver nanoparticles [99].

The spectra of the methanol/water <sup>50</sup>/<sub>50</sub> solvent and the water solvent silver nanoparticles were significantly sharp which indicated a high degree of capping of the silver nanoparticles thus inducing high stability. The presence of the phytochemicals in excess allowed the capping of the silver nanoparticles to be highly resistant to agglomeration and any other structural disturbances. Fourier transform infrared spectroscopy (FTIR) was used to determine the specific functional groups responsible for the reduction of silver nitrate to form silver nanoparticles and the capping agents present in the leaf extract [99]. The FTIR spectra of the extracts of the 4 highest yielding solvents used in the synthesis of silver nanoparticles are shown in Figure 4.13 - 4.16, alongside the FTIR spectra of their corresponding nanoparticles.

The presence of similar vibrations in the silver nanoparticles and solvent extracts FTIR spectra indicated that the phytochemicals responsible for the synthesis of the silver nanoparticles in the 3 solvents were significant enough to carry out both, the reduction and capping. The phytochemicals could have been in excess thus allowing all the silver ions to be reduced.

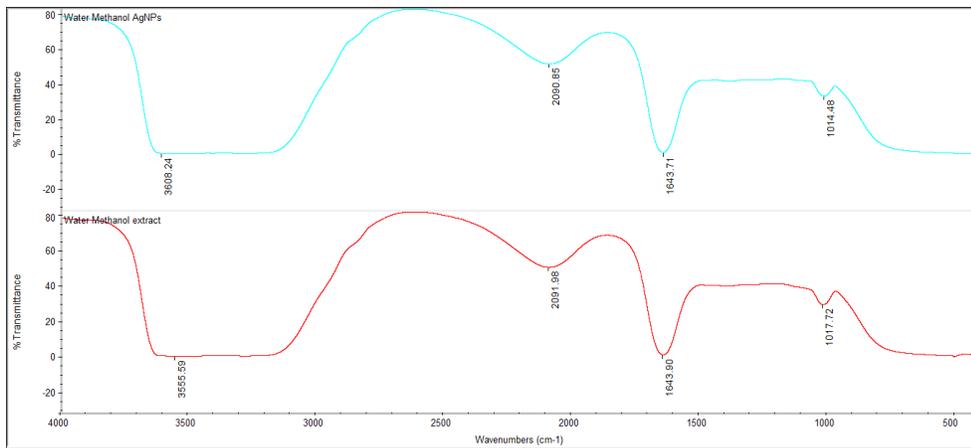


Figure 4.13: FTIR spectra of Methanol/Water extract and silver nanoparticles

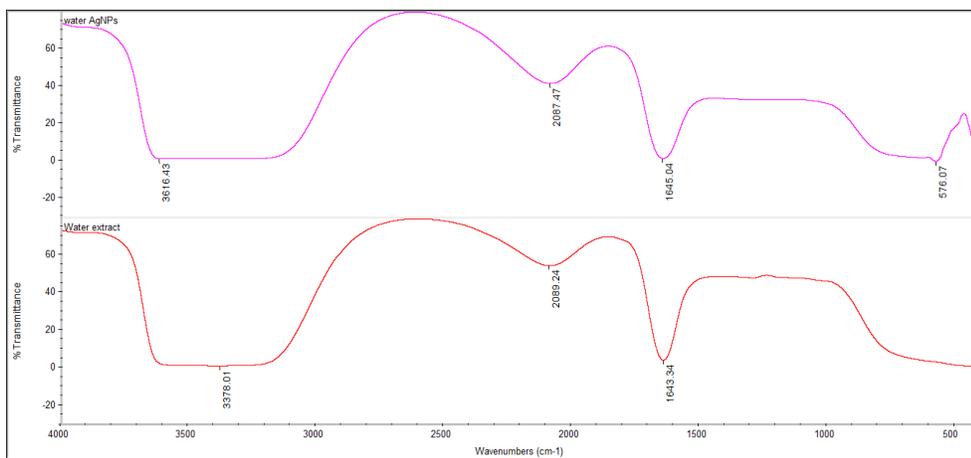


Figure 4.14: FTIR spectra of Water extract and silver nanoparticles

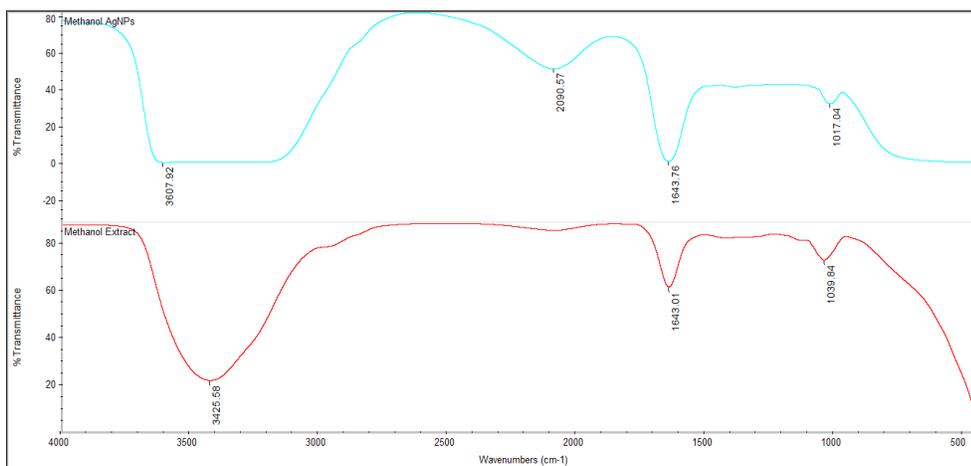


Figure 4.15: FTIR spectra of Methanol extract and silver nanoparticles

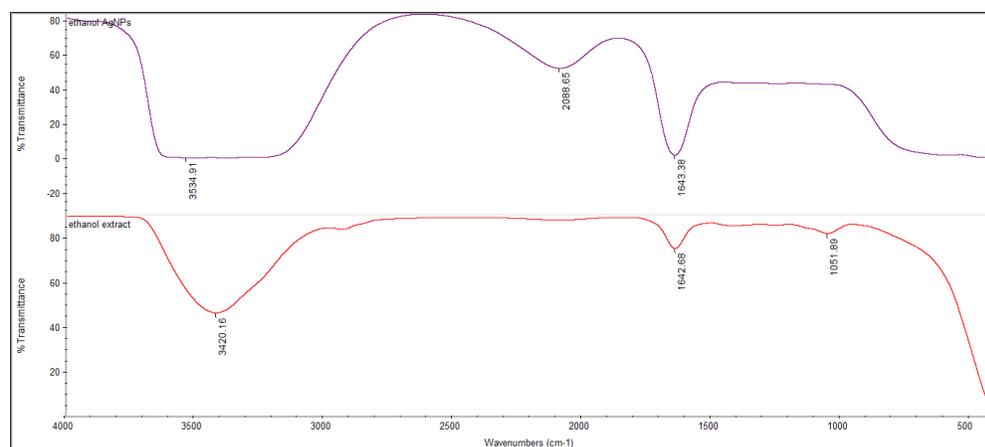


Figure 4.16: FTIR spectra of Ethanol extract and silver nanoparticles

Table 4.7: FTIR vibrational shifts interpretation

Vibration cm-1	Inference
3500	R-O-H alcohol O-H, pri. & sec. N-H, O=C-O-H acid O-H
2090	C≡C alkyne
1640	-C=C bond, =C=N-, conjugated ketone
1020	-C-O alkoxy

A slight shift in the FTIR vibrations (Table 4.7) indicated that the functional groups present in the extracts were still present but their bonding coordinates may have been altered slightly. The adsorption of certain functional groups, present in the phytochemicals molecular structure indicates the presence of phytochemicals on the surface of the silver nanoparticles and these are the functional groups which instigate the level of stability onto the silver nanoparticles [25]. The shift of the functional groups is a result of the capping of the silver nanoparticles whilst the disappearance of some of the functional groups gives information on which of the functional

groups are responsible for the reduction of the  $\text{Ag}^+$ .

The formation of an alkyne functional group at approximately  $2090\text{ cm}^{-1}$  indicates that under the appropriate conditions, some unexpected reducing groups such as the C=C have the ability to assist in the reduction of the silver nanoparticles as well. The appearance of C-O, O-H, N-H and the O=C-O-H functional groups suggests that phytochemicals such as alkaloids, flavonoids, glycosides and terpenoids are responsible for the reduction and capping of the silver nanoparticles.

#### 4.7 Antimicrobial Activity

Human beings are often infected by microorganisms such as bacteria, moulds, yeasts, and viruses present in their living environments [116]. Such problems and needs have led to a resurgence in the use of silver-based antiseptics that may be linked to a broad-spectrum activity and considerably lower propensity to induce microbial resistance compared with those of antibiotics [116]. The antimicrobial activity of the silver nanoparticles synthesised from the 4 extracts which showed the highest synthesis peak absorbance value was evaluated against a Trimethoprim antibiotic standard and a silver nitrate blank solution (Figure 4.18). The silver nanoparticles showed significant inhibition capability against the growth of *E. coli* and *S. aureus*. Table 4.8 shows the inhibition zones given off by each of the samples in millimetres.

Table 4.8: Inhibition zones

	$\text{AgNO}_3$	Trimethoprim	Methanol/Water AgNPs	Distilled water AgNPs	Methanol AgNPs	Ethanol AgNPs
<i>E. coli</i>	9 mm	27 mm	23 mm	18 mm	13 mm	15 mm
<i>S. aureus</i>	8 mm	25 mm	24 mm	21 mm	15 mm	18 mm



Figure 4.17: *Escherichia coli* plate and *Staphylococcus aureus* plate

The Mueller-Hinton agar plates (Figure 4.17) showed significant inhibition of bacterium growth due to the samples. An  $\text{AgNO}_3$  solution sample was also included in order to eliminate the possibility of observing inhibition zones due to unreacted silver nitrate on the silver nanoparticles surface. *S. aureus* is a gram-positive bacteria and *E. coli* is a gram negative bacteria and this results in slightly different susceptibility to antimicrobial agents. The Trimethoprim standard showed better inhibition for both bacteria than the silver nanoparticles which may be due to its mechanism of inhibition which starves the bacteria of folic acid. The Trimethoprim showed better inhibition of *E. coli* than *S. aureus* which was highly expected due to the cell structures of the two bacteria. The nanoparticle, in turn, showed better inhibition against *S. aureus* than *E. coli* and this indicates the high potential of silver nanoparticles in topical pharmaceuticals. The high bactericidal activity is due to the silver cations released from silver nanoparticles that act as reservoirs for the  $\text{Ag}^+$  bactericidal agent [100].

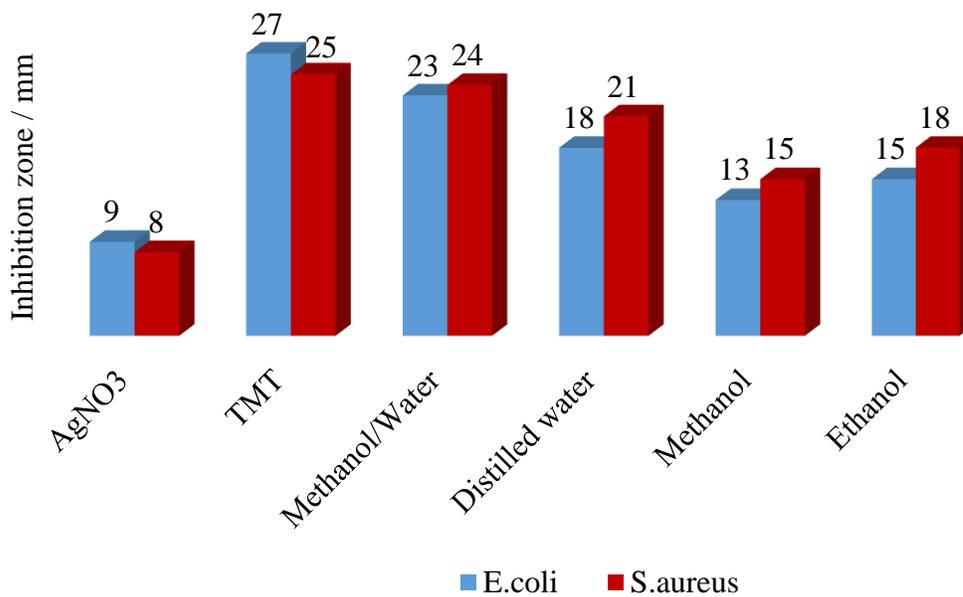


Figure 4.18: Graphical presentation of inhibition zones

A graphical presentation of the inhibition zones is shown in Figure 4.18. The high antimicrobial activity given off by the silver nanoparticles may also be attributed to the presence of the phytochemicals acting as surface capping agents. The phytochemicals may cap the nanoparticles in such a way that the pharmacophore structure on them which is responsible for their various therapeutic effects is not disrupted and therefore they remain therapeutically active in such an in-vitro study. The high surface area of the silver nanoparticles also allows maximum interaction between the bacteria and the nanoparticles thus the maximum possible inhibition is exhibited. The higher activity of silver nanoparticles may conceivably be due to the conformational changes persuaded in the membrane structure of bacterial cell wall by the action of silver nanoparticles resulting in increased membrane permeability, and as a result, leads to bacterial cell death [6]. Bacteria cell death arising out of exposure to silver nanoparticles might be due to the cytoplasmic membrane disorganization and the consequent leakage of various biomolecules such as amino acids, protein and carbohydrates [24].

Silver nanoparticles synthesised using *Crocus sativus L.* extracts showed no inhibition against *S. aureus* [11] and this indicated that the *Allium Ascalonicum* is very rich in phytochemicals and possess great potential as a plant-mediated synthesis technique.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

The research was successfully carried out as the main aim was achieved. *The Allium Ascalonicum* plants phytochemicals were successfully extracted using a methanol/water <sup>50/50</sup> extract with a high extraction yield of 63 %. The plants extract exhibited great capability of synthesizing silver nanoparticles by successfully reducing and capping the Ag<sup>+</sup> and the optimum conditions for this plant-mediated synthesis were found to be, 6 mL of plant extract, 8 mM silver nitrate solution at a 50 °C temperature and a minimum reaction time of 3 hours. The silver nanoparticles showed great inhibition against *Escherichia coli* and *Staphylococcus aureus* at 23 mm and 24 mm respectively. The silver nanoparticles synthesised using the plant extracts of the *Allium Ascalonicum* plant showed great potential as antibiotic alternatives against both gram-negative and gram-positive bacteria.

#### 5.2 Recommendations

The *Allium Ascalonicum* plants phytochemical constituents showed great ability in the synthesis of silver nanoparticles and great potential for up-scaling the production of silver nanoparticles using this plant. The nanoparticles exhibited significantly high inhibition of the bacteria *Escherichia coli* and *Staphylococcus aureus*. Further analysis of the silver nanoparticles exact morphology and particles size can be carried out. In-vivo and ex-vivo tests can be carried out to identify the potential of the nanoparticles as an alternative to antibiotics. Cytotoxicity tests may also be carried out to determine the pharmacodynamics and the bioaccumulation of the silver nanoparticles as a therapeutic agent.

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## APPENDICES

### Appendix A: Materials

#### List A1: Apparatus

Pestle and mortar, 150  $\mu\text{m}$  mesh sieve, weighing crucibles, measuring cylinder, volumetric flask, reagent bottles, filter paper, Buchner funnel, tests tube rack, test tubes, dropper, burette, agar plates, cotton swabs, tongs, thermometer, rubber stoppers

#### Table A1: Chemical reagents

Chemical name	Chemical Formular	Manufacturer	Mass/Concentration
Hexane	$\text{C}_6\text{H}_{14}$	A.C.E.	99 %
Cyclohexane	$\text{C}_6\text{H}_{12}$	A.C.E.	99 %
Ethyl acetate	$\text{C}_4\text{H}_8\text{O}_2$	Skylabs	99 %
Ethanol	$\text{C}_2\text{H}_6\text{O}$	Glassworld	75 %
Methanol	$\text{CH}_3\text{OH}$	Glassworld	32 %
Silica gel mesh	$\text{SiO}_2$	Aldrich Chemistry	50 g
Benzene	$\text{C}_6\text{H}_6$	A.C.E	99 %
Chloroform	$\text{CHCl}_3$	A.C.E	99 %
Potassium bromide	$\text{KBr}$	Cosmo Chemicals	1 g
Silver nitrate	$\text{AgNO}_3$	SaarChem	99 %
Lead acetate	$\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$	SaarChem	1 %
Sodium hydroxide	$\text{NaOH}$	Glassworld	10 %

Ferric chloride	FeCl <sub>3</sub>	SaarChem	5 %
Sulphuric acid	H <sub>2</sub> SO <sub>4</sub>	Glassworld	98 %
Potassium iodide	KI	Skylabs	7 g
Mercuric chloride	HgCl <sub>2</sub>	A.C.E.	1.36 g
Ammonium hydroxide	NH <sub>4</sub> OH	Glassworld	10 %
Acetic acid anhydride	C <sub>4</sub> H <sub>6</sub> O <sub>3</sub>	Glassworld	40 %
Trimethoprim	C <sub>14</sub> H <sub>18</sub> N <sub>4</sub> O <sub>3</sub>	Plus 5 Pharmacy	2 mg/mL

**Table A2: Instrumentation**

Name	Model	Manufacturer	Use
Water bath	ZWY 110X30	Zhicheng	Heating
Shaker	HY-4	Vision Electrical	Extraction
Analytical Balance	17250	AE Adam	Weighing reagents
Fridge	D50158	DEFY	Extracts storage
Ice maker	AF 80 AS	S. Frimont	Ice bath
FTIR	Nicolet 6700	Thermo scientific	Characterization
UV-VIS	UV 752	Perkin Elmer	Characterization
Sonicator	KQ-250B	China Corp	Ultra-agitation
Autoclave	RAU-123	Incotherm	Sterilization
Incubator	MISA	Labotec	Incubation
Centrifuge	200	Labofuge	Separation