GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *VANGUERIA INFAUSTA* AND *PARINARI CURATELLIFOLIA* LEAF EXTRACT AND EVALUATION ON THEIR ANTIFUNGAL ACTIVITIES

by

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DEDICATION

This is dedicated to my family especially my mother who supported me and never doubted my ability to make it in life and in memory of my father who had faith in me and gave me courage.

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My deepest gratitude goes to the Almighty Lord for giving me strength to pull through and be able to work hard to achieve this project. My sincere gratitude also extends to my supervisors Dr F.Chigondo and Miss T.Murinzi who guided and helped me throughout my research. Their hardwork, patience and unwavering enthusiasm for Chemistry kept me more engaged in my research so that my project becomes a success. My appreciation also goes to MSU Chemical Technology staff especially Mr and Mrs Mambanda, MSU Biosciences and Biotechnology staff, MSU Clinic staff, Laboratory Technicians and my classmates for their mentoring and encouragement which have been of great value in carrying out this research. I give my acknowledgements with gratitude to my family especially my mother and sister who by all means supported me even in the hardest times, I feel indebted to them for their Iove and their strength they offered throughout the entire degree. Finally I give would like to thank my friends for their support and assistance with this research, a special mention goes to Tapiwa Makodza and Nyasha Chichetu.

ABSTRACT

A green approach was used in the synthesis of silver nanoparticles which is an easy, cheap, eco-friendly and a fast method in achieving stable silver nanoparticles over a long period of time. Plant leaves extract from *Parinari Curatellifolia* and *Vangueria Infausta* were used in the synthesis after optimizing different parameters (temperature, silver nitrate concentration and amount of extract. The optimum conditions achieved for the synthesis of silver nanoparticles (AgNPs) using *Parinari Curatefollia* were at a temperature of 70 ^{o}C, 5 mM silver nitrate and 9 ml of the extract. For *Vangueria Infausta* the temperature was at 50 ^{o}C, 5 mM silver nitrate and 10 ml of the plant extract. The characterization of the synthesized AgNPs was done using Ultraviolet-Visible (UV-Vis) spectrophotometer and Fourier Transform Infrared (FTIR) spectroscopy. From the UV-Vis analysis the Surface Plasmon resonance (SPR) band of AgNPs using *Parinari Curatellifolia* leaf extract was found out to be at 430 nm and for *Vangueria Infausta* leaf extract it was at 415 nm. The groups which were responsible for the capping of silver nanoparticles as evidenced by the FTIR were the O-H, C-O and C=O bonds which were present at 3642.06 cm^{-1} and 1657.76 cm^{-1} respectively for the AgNPs obtained from *Parinari Curatellifolia* leaf extract and at 3638.45 cm^{-1} and 1655.73 cm^{-1} respectively for AgNPs obtained from *Vangueria Infausta* leaf extract. The synthesised silver nanoparticles from both plants were found out to be stable for over two months as evidenced by the UV-Vis Spectroscopy. The Agar disk diffusion method was used for antifungal studies and the synthesized AgNPs were effective against *Trichoderma*, *Penicillium*, *Fusarium* and *Saccharomyces Cerevisiae* fungi as indicated by the clear inhibition zones. Thus *Parinari Curatellifolia* and *Vangueria Infausta* extracts can be applied in the synthesis of AgNPs for application as fungicides as indicated by the clear inhibition zones.

Key words: Green synthesis, silver nanoparticles, *Parinari Curatellifolia, Vangueria Infausta*, antifungal activity.

DECLARATION

I, Evelyn Mariwowo , hereby declare that I am the sole author of this dissertation. I authorize Midlands State University to lend this dissertation to other institutions or individuals for the purpose of scholarly research.

Signature ……

Date …….

APPROVAL

This dissertation entitled "Green synthesis of silver nanoparticles using *Vangueria Infausta* and *Parinari Curatellifolia* leaf extract and evaluation on their antifungal activities" by Evelyn Mariwowo meets the regulations governing the award of the degree of Chemical Technology of Midlands State University, and is approved for its contribution to knowledge and literal presentation.

Signature of Supervisor……

Date……...

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LIST OF ABBREVIATIONS

AgNPs - Silver Nanoparticles

UV-VIS - Ultra Violet-Visible Spectroscopy

FTIR - Fourier Transform Infrared Spectroscopy

BSC - Biological Safety Cabinet

SPR - Surface Plasmon Resonance

ZOI - Zone Of Inhibition

XRD - X-Ray Diffraction

SEM -Scanning Electron Microscopy

PDA - Potato Dextrose Agar

DNA - Deoxyribonucleic acid

PSA - Particle Size Analyzer

TEM - Transmission Electron Microscopy

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CHAPTER ONE

INTRODUCTION

1.0 Introduction

This chapter gives an overview of the background to the research, the aims, objectives, problem statement and justification on why the research was carried out.

1.1 Background

Nanoparticles are particles with an average size of less than 100 nm and due to their potential application in various areas, the synthesis of noble metal nanoparticles has attracted much attention in recent years [1] .Noble metals like gold, silver and platinum have found many applications in information storage, electronics, biomedical, for example in targeted drug delivery and antimicrobial activity . Antimicrobial activity of silver nanoparticles (AgNPs) mostly depends on the relative surface area, since the smaller AgNPs can have greater toxic potentials [2]. Nano scale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties, which increases their contact with microbes and their ability to permeate cells [3].Silver is one of the most commercialized nano-material with five hundred tons of silver nanoparticles production per year. Silver and its compounds is well known to exhibit strong inhibitory and also bactericidal effects, in addition it has a broad spectrum of antimicrobial activities for bacteria, fungi, and viruses. It also has a higher toxicity to microorganisms while it exhibits lower toxicity to mammalian cells as compared to other metals. Lately, the recent advances in researches on metal nanoparticles appear to revive the use of AgNPs for antimicrobial applications and it has been shown that AgNPs prepared with a variety of synthetic methods have effective antimicrobial activity [4]. Thus there has been the application of

AgNPs to a wide range of healthcare products, for example in burn dressings, scaffold, water purification systems, and also medical devices. The toxic effects of silver on bacteria have been investigated for more than sixty years so has the acting mechanism of silver has been known in some extent, therefore, the preparation of uniform nano-sized silver particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products [4]. The silver nanoparticles have various and important applications. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items and it has been reported that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic micro-organism at low concentrations and without any side effects [5]. Various metallic nanoparticles of Silver, Gold, Zinc or Palladium, are being synthesized using biological systems. It has been suggested that the antimicrobial activity of Ag may be due to the binding of the Ag^{+} cation to electron donor groups in biological molecules containing sulfur, oxygen or nitrogen (e.g. enzymes) which, in turn, results in the loss of their function [6]. In small concentrations, silver is safe for human cells, but lethal for microorganisms [5]. Antimicrobial capability of AgNPs allows them to be suitably employed in numerous household products such as textiles, food storage containers, home appliances and in medical devices [5]. The most important application of silver and silver nanoparticles have proved to show other important applications among other metal nanoparticles such as antimicrobial, catalytic, and antifungal activity. This has led for their synthesis to gain much interest from researchers and chemists. Also other applications of these nanoparticles include their uses in detergents, toothpastes, shampoos, cosmetics and soaps. A wide range of methods have been used in the synthesis of these silver nanoparticles for example chemical methods, electrochemical methods, radiation chemical reduction and photochemical

methods [7]. These have proved to be very expensive, some uses high temperatures and pressures, others are destructive methods which uses toxic chemicals which are potentially hazardous to the environment and pose major biological risks [8].

In recent years, naturally occurring reagents such as plant extracts, sugars, enzymes [9], microorganisms (fungi, viruses, bacteria) [10] and biodegradable polymers (for example chitosan) [11] as reducing agents and capping agents are techniques that are used for obtaining nanoparticles which could be considered attractive for nanotechnology. Biological methods offer a very simple one step method which is rapid, relatively reproducible, widely acceptable, cheaper, often results in the production of more stable materials ecofriendly method than those mentioned earlier . However the synthesis of silver nanoparticles using plants has an added advantage of eliminating the elaborate process which involve microbial cultures maintenance [12]. The extract from plants may act as both reducing and stabilizing agents in the synthesis of silver nanoparticles [13]. The source of this extract is known to influence the characteristics of the nanoparticles [14], because different extracts contain different concentrations and combinations of organic reducing . Typically a plant extract mediated bio reduction involves mixing the aqueous extract with an aqueous solution of the relevant metal salt .The reaction occurs at room temperature and is generally complete within few minutes [14]. Various reports have been made in the green synthesis of silver nanoparticles using *Aloe vera* [5], *Acalypha Indica* [15], *Jatropha curcas* [16], *Zea Mays* [17] , *Calophyllum lnophyllum* leaves [18] and *Saraca lndiaca* bark [19], where the reducing and capping chemicals were found to be successful in the plant extracts. Different parts of the plants have been used in the green synthesis of these nanoparticles and these include the stem [20], bark [5], roots, flowers [21], and the leaves [22] and according to literature the synthesis of silver nanoparticles using any of these parts have proved to be successful. This research explores the use of *Parinari Curatellifolia*

and *Vangueria Infausta* extracts in the synthesis of AgNPs and the evaluation of their antifungal activity.

1.2 Aim

- Green synthesis of silver nanoparticles using the leaves extract of *Vangueria Infausta* and *Parinari Curatellifolia* and evaluation on their antifungal activity.

1.3 Objectives

- To extract phytochemicals from the leaves of *V.Infausta* and *Parinari Curatellifolia.*
- To carry out qualitative and the FTIR analysis on the leaf extract for both plants.
- To optimize the ideal conditions for the synthesis of silver nanoparticles using *V. Infausta* and *Parinari Curatellifolia* (temperature, amount of extract and silver nitrate concentration).
- To characterize the synthesized silver nanoparticles using FTIR and UV-Vis Spectroscopy.
- To study the effect of time on the stability of the synthesized nanoparticles
- To investigate on the antifungal activity of the leaf extract and the silver nanoparticles of both plants.

1.4 Problem Statement

Due to an increase in fungal disease in both humans caused by pathogens that are becoming more resistant to currently available drugs [23], it has become important that novel antifungal agents be identified and developed . The systemic and local infections that is caused by fungi, for example those concerning the skin and nails, are increasing [24]. Drugs which are being used for mycoses treatment such as Amphotericin B, Nystatin, Ketoconazole, Itraconazole , Fluconazole, among others , are having adverse side effects such as allergies, affecting liver or kidney function, causing liver damage or failure, weakening of the heart's ability to contract, cause itching, abdominal pain, headaches and severe skin reactions [4]. Due to the increase in the rates of fungal infections, the

changes in their epidemiology and the toxicities that some of the commercial antifungal drugs now have, has resulted in the need for an expanded arsenal of antifungal drugs. The treatment of these and many other fungal diseases require the use of antifungal medications that have serious side effects. The presence of fungi in food results in food spoilage and also they lead to rotting of stored crops, for example, the fungus *Claviceps purpurea* causes ergot, a disease of cereal crops which causes a reduction in the yield of these cereal crops, and has a serious toxic effect on humans and animals [25]. Also there has been a greater concern in some of the methods which are being used in the synthesis of silver nanoparticles and these include physical and chemical techniques which have proved to be expensive [26]. Chemical-based synthesis techniques are often discouraged as they involve the use of noxious reducing and/or stabilizing agents like sodium borohydride and toxic solvents [27]. The use microorganisms requires the elaborate process for cell culture maintaining, the process is not always successful, the production of nanoparticles occurs at a low rate and is also time consumption [10].

1.5 Justification

There has been a great concern to try and reduce the side effects caused by antifungal drugs and to maximize antifungal drug activity, thus the use of nanoparticles in drugs has been improved [28]. Different types of nanomaterials like copper, zinc, titanium , magnesium, gold , alginate have come up but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic microorganisms [16]. Synthesis of nanoparticles through biochemical routes, using plant extracts as reducing and capping agents, has received special attention among others, due to maintaining an aseptic environment during the process thus medicinal plants having well established therapeutic importance are being widely used for the size and shape controlled synthesis of silver nanoparticles [27]. There has been an increase in demand of

AgNPs due to their broad applications in many industries mainly in medicine [16], thus the implementation of green chemistry synthetic methods which are cheaper will benefit the world at large . The use of microorganisms such as fungi and bacteria has proven to be difficult since it involves the maintenance of cell culture during the synthesis thus there has been need to search for other alternatives [29] and plants have successfully been able to show a simple and viable way in the synthesis of these silver nanoparticles [26]. Historically, silver has been known to have a disinfecting effect and has been found in applications ranging from traditional medicines to culinary items, also there has been reports that silver nanoparticles are non-toxic to humans and most effective against bacteria, virus and other eukaryotic microorganism at low concentrations and without any side effects [5]. Colloidal silver, which contains silver of different concentrations and particle sizes, has historically been used for the treatment of wounds and infections [30] thus synthesis of these nanoparticles will help in dealing with fungal infections. Silver is the only noble metal at which its Surface Plasmon Resonance (SPR) frequency can be tuned to any desired wavelength in the visible region by changing its density distribution, size distribution and the dielectric properties of the surrounding medium [31] thus it is the suitable metal for the synthesis. Also the green methods in the synthesis of AgNPs tend to be safer to use compared to the risks that are associated with the use chemical or physical methods. The use of plants for the synthesis of nanoparticles offers a rapid, low cost, eco-friendly, and a single-step method [32]. The presence of phytochemicals in plants is responsible for their medicinal properties [33] and this in turn can be utilized for the synthesis of nanoparticles due to the ability of these phytochemicals to act as reducing and also stabilizing agents [34].

Due to the abundance of the plants used in this research (in Zimbabwe and all over the world) [35], it’s economic to use them in the synthesis of silver nanoparticles which would replace the antifungal

drugs which are. Also the plants have never been used before in silver nanoparticles synthesis and if they show promising results in antifungal activity *Vangueria Infausta* and *Parinari Curatellifolia* could be used at an industrial level as a source of silver nanoparticles which could be used for different medicinal purposes. *Vangueria Infausta* known in English as Velvet wild Medlar is a plant which is known to have antibacterial, antimalarial and antifungal activities [36] due to the presents of phytochemicals such as alkaloids, flavonoids and tannins [37]. The fruits of *V. Infausta* are eaten by both people and wild animals while different parts of this plant have been used traditionally for treatment of malaria, wounds, diarrhoea, abdominal pains, menstrual pain, pneumonia, swelling problems and genital swellings. Recent pharmacological reports have shown that extracts from leaves and roots of this plant exhibited significant antiplasmodial activity [35] and also the leaves are used as a ring worm remedy and as a relief for toothache. The pounded leaves are applied to tick- bite sores on livestock and dogs to speed up healing, also the poultice made of the leaves is used to treat swellings on the legs and inflammation of the navel in children [36].

Parinari Curatellifolia is known as Mobola Plum tree in English and it is considered a traditional food plant in Africa for both humans and animals, due to its fruits that are nutritious and tend to increase food supply in some African countries. It is known to have antimicrobial activities due to the presents of alkaloids, flavonoids, phenol, saponins, steroids, tannins and terpenes [36] which can be exploited in the synthesis of silver nanoparticles. The extracts from the leaves and the bark can be used as a remedy for the symptoms of pneumonia [38] or as a treatment for eye and ear ailments and also for tanning purposes for example in leather tanning. Traditional medicines mostly incooperate the bark of the Mobola Plum tree. The roots can be soaked in cold water for about an hour and used as eardrops for the treatment of earache.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter gives an introduction to nanotechnology which is the basis of this research, it goes on to give the different methods that are used in the synthesis of nanoparticles looking specifically at the silver nanoparticle and then some of the methods which are used for characterization such as UV-Vis spectroscopy, FTIR spectroscopy, SEM, XRD are briefly explained. The description of the plants that were used during this research and their medicinal uses are also explained. Lastly the chapter introduces some of the antifungal infections affecting the world and the serious side effects some of the antifungal drugs have on humans.

2.1 Introduction to nanotechnology

Nanotechnology is rapidly developing into an important field of modern research which deals with the synthesis, strategy and manipulation of particle's structure ranging from approximately 1 to 100 nm in size [39].The green process for synthesizing silver nanoparticles is widely evolving into an important branch of nanotechnology due to their wide applicability in catalysis, electrical conductivity ,antimicrobial activity, in optical polarizability and also in fields like cosmetics, electronics and health care due to their improved properties such as small size, distribution and morphology of the particles [29]. AgNPs have good chemical stability, good conductivity, antibacterial, anti-viral, antifungal and anti-inflammatory activities as some of their properties [40]. The synthesis of nanoparticles is achieved by using plants phytochemical constituents such as tannins, phenols, saponins, and flavonoids that act as reducing and capping agents which provide stability to the silver nanoparticles [41] . Literature reports that these phytochemicals breakdown silver nitrate which is a complex chemical into Ag^{+} and NO^{3-} ions , the Ag^{+} ions which are toxic

further enter a reduction process where they are reduced to non- toxic Ag^0 nanoparticles through the use of functional groups of reducing agents which are in the plant extract [42]. Maintenance of cell culture is not required in the green synthesis of silver nanoparticles unlike when using microbes in the synthesis [43].Nanoparticles play a crucial role in inhibiting bacterial and fungal growth in aqueous as well as solid media and they can easily penetrate inside the cell and may cause damage to DNA (Deoxyribonucleic acid) and protein releasing silver ions from AgNPs which makes it effective against various microorganisms [44].

2.2 Methods used in the synthesis of silver nanoparticles

Different methods have been applied in the synthesis of silver nanoparticles which can be grouped into chemical, biological and physical methods. Under physical and chemical methods reduction of silver ions in aqueous solutions has been carried out with or without stabilizing agents through thermal decomposition in inorganic solvents, chemical and photo reduction [45]. The biological synthesis of nanoparticles involves the use of algae, bacteria, fungi and plants [46]. Fig 2.1 shows the methods used in the synthesis of nanoparticles and some of the examples are shown under each method.

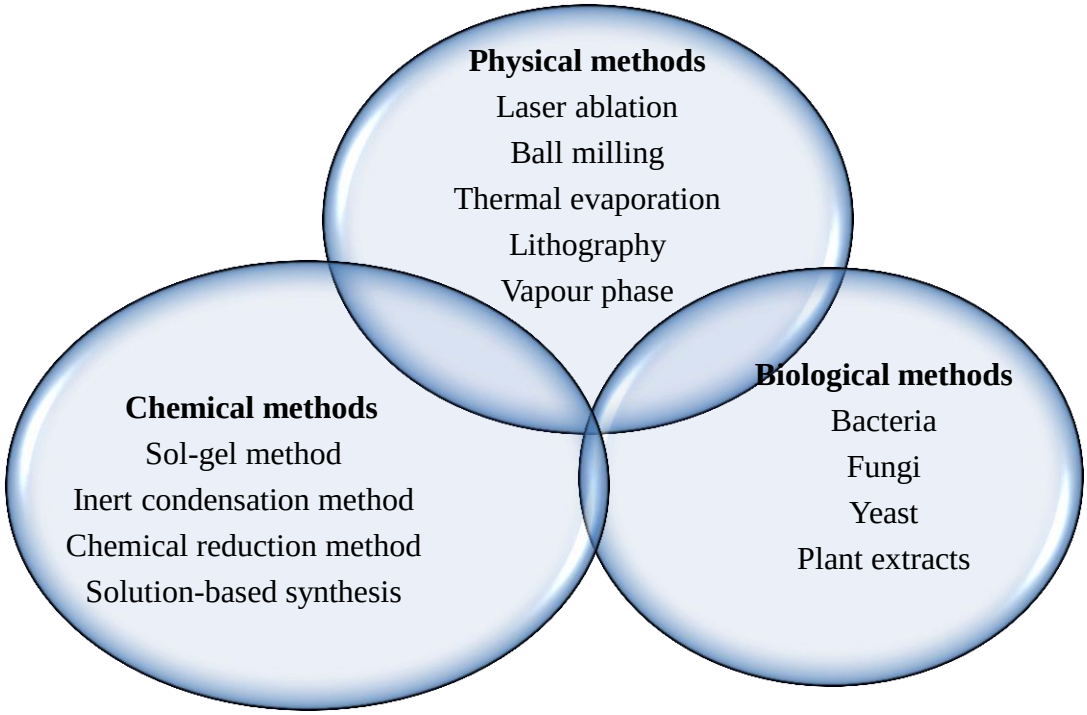


Fig 2.1: Methods used in nanoparticles synthesis

2.2.1 Physical methods

The most important physical methods used include evaporation-condensation and laser ablation. The advantages of physical methods in the synthesis of AgNPs are that there is the absence of solvent contamination in the prepared thin films and the uniformity of nanoparticles distribution [47]. Physical vapor deposition (PVD) is another method which involves the deposition of thin films of different metals on different surfaces and condensation from the vapour stage [48].

Various metal nanoparticles such as silver, gold, lead sulfide, cadmium sulfide, and fullerene have previously been synthesized using the evaporation-condensation method [49]. These methods have great advantages over chemical methods for example there is the absence of solvent contamination in the prepared thin films and there is uniformity in the nanoparticles distribution when physical methods are used in the synthesis. Alternatively silver nanoparticles could be synthesized by laser

ablation of metallic bulk materials in solution [50]. The ablation efficiency and the characteristics of produced nano-silver particles depend upon many factors such as the wavelength of the laser impinging the metallic target, the duration of the laser pulses, the laser fluence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants. One important advantage of laser ablation technique compared to other methods for production of metal colloids is the absence of chemical reagents in solutions [51]. Therefore, pure and uncontaminated metal colloids for further applications can be prepared by this technique.

2.2.2 Chemical methods

In the synthesis of nanoparticles using chemical approaches, chemical reduction by organic and inorganic reducing agents is most commonly used. There has been use of different reducing agents such as sodium citrate [52], ascorbate, hydrazine hydrate [53], sodium borohydride ($NaBH_4$) [34], elemental hydrogen [16], polyol process [54], Tollen's reagent [55] and many others are used for reduction of silver ions (Ag^+) in aqueous or non-aqueous solutions [49]. These reducing agents reduce silver ions (Ag^+) and lead to the formation of metallic silver (Ag^0), which is followed by agglomeration into oligomeric clusters which eventually lead to the formation of metallic colloidal silver particles . Silver nanoparticles synthesized via the chemical reduction of $AgNO_3$ by trisodium citrate and ascorbic acid as a surfactant proved to be uniform and well-dispersed [56]. The following equation shows the reduction that occurs during the silver nanoparticles synthesis using trisodium citrate:

$$4Ag^+ + C_6H_5O_7Na_3 + 2H_2O \longrightarrow 4Ag^0 + C_6H_5O_7H_3 + 3N\,a^+ + H^+ + O_2 \quad \text{.....(1)}$$

It is a requirement to use protective agents to stabilize dispersive nanoparticles during the course of metal nanoparticle preparation, and protect the nanoparticles that can be absorbed on or bind onto nanoparticle surfaces, avoiding their agglomeration [47] . Micro-emulsion techniques can be used to

obtain uniform and size controllable silver nanoparticles. A simple and effective method, UV-initiated photo reduction method, has been reported for synthesis of silver nanoparticles in the presence of citrate, polyvinylpyrrolidone, poly(acrylic acid), and collagen [47]. Electrochemical synthetic method can be used to synthesize silver nanoparticles [15] whereby the particle size can be controlled by adjusting the electrolysis parameters and changing the composition of the electrolytic solutions improve the homogeneity of silver nanoparticles. There has been reports on the synthesis of nano-sized silver particles with an average size of 8 nm with the use of photo - induced reduction using poly(styrene sulfonate)/poly(allylamine hydrochloride) polyelectrolyte capsules as micro reactors and this method could be used for converting silver nano-spheres into triangular silver nanocrystals (nano-prisms) with desired edge lengths in the range of 30-120 nm [49].Microwave assisted synthesis is a promising method for the synthesis of silver nanoparticles whereby nanoparticles could be synthesized by a microwave-assisted synthesis method employing carboxymethyl cellulose sodium as a reducing and stabilizing agent [47]. The concentration of sodium carboxymethyl cellulose and silver nitrate affect the size of . The resulting nanoparticles produced using this method were uniform and stable at room temperature for two months without any visible change [49].

2.2.3 Biological methods

There has been the development of efficient green chemistry methods in recent years which employ the use of natural reducing, capping, and stabilizing agents to prepare silver nanoparticles with desired morphology and size. Biological methods can be grouped into two namely one uses microorganisms such as bacteria, fungi or viruses and the other that uses parts of plants such as leaves, fruits or leaves. These methods can be used to synthesize silver nanoparticles without the use of any harsh, toxic and expensive chemical substances. The bio reduction of metal ions by

combinations of biomolecules found in the extracts of certain organisms (for example enzymes/proteins, amino acids, polysaccharides, and vitamins) is environmentally friendly, yet chemically complex. Many studies have reported successful synthesis of silver nanoparticle using organisms (microorganisms and biological systems) [32].There have been reports on the use of fungi such as *Fusarium oxysporum* [57], *Aspergillus oryzae* [43] on the synthesis of silver nanoparticles and there proved to be no evidence of flocculation of the particles even a month after the reaction . The long-term stability of the nanoparticle solution might be due to the stabilization of the silver particles by proteins. The morphology of nanoparticles is considered to be highly variable, with generally spherical and occasionally triangular shapes observed in the micrographs. The use of different plants also has been reported in the green synthesis of silver nanoparticles and these include *Aloe vera* [5], *Acalypha Indica* [15], *Jatropha curcas* [16], *Zea Mays* [17] and many others. The use of microorganisms tend to show more disadvantages than the use of plants for example there is need for culturing of the micro-organisms and this process is not always successful, the synthesis tends to be laborious and time consuming since there is need for maintenance of the micro-organism cell walls .Another shortcoming is that there is low nanoparticle production rate thus plants are proving to be more advantageous as they offer a very cheap, fast, simple and one step method in the synthesis of the nanoparticles. The advantages of green chemistry are shown in Fig 2.2.

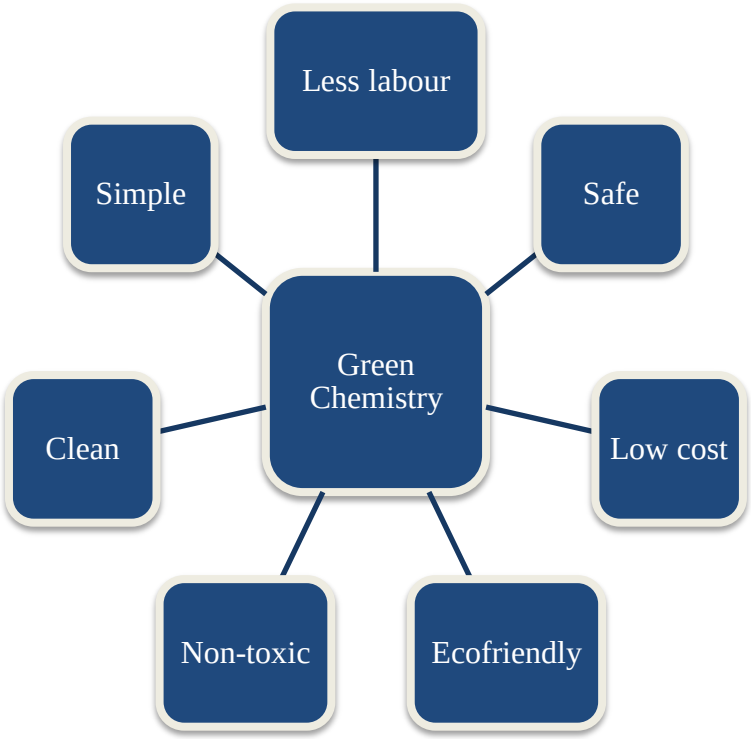


Fig 2.2: Advantages of green chemistry in nanoparticles synthesis

2.3 Instruments used for the characterization of nanoparticles

Different instruments are used for nanoparticles characterization and these include Scanning Electron Microscopy (SEM), UV-Vis spectroscopy, Fourier Transform Infrared (FTIR) Spectroscopy, X-ray Diffraction (XRD), Transmission Electron Microscopy (TEM), Particle Size Analyzer (PSA) and many others. Some of these methods are briefly described below.

2.3.1 Ultra violet- visible spectroscopy

This is a powerful technique used for the characterization of nanoparticles especially silver and gold nanoparticles due to their Surface Plasmon Resonance (SPR) as a result of their strong interaction with specific wavelengths of light [58]. The UV-Vis Spectroscopy quantifies the light which a sample absorbs. SPR is a phenomenon which occurs when an incident light beam strikes the surface of a substance at a particular angle or it can be defined as the effect of the oscillation of the

conducting electrons aligned in resonance to the wavelength of the irradiated light [59].Due to the unique optical properties of nanoparticles that are sensitive to the size, shape, concentration, agglomeration state, and refractive index near the nanoparticle surface, the use of a UV-Vis has become an important analytical technique for studying, identifying and characterizing metal nanoparticles. The sample is put between a source of light and a photodetector and the intensity of the light that is passed through a sample is measured as well as the light that passes through the sample [58]. This will then give the amount of the light absorbed. The relationship between absorbance and light intensity is shown below by the equation (i):

$$A = -\log\frac{I_0}{I} \quad \text{..... (i)}$$

Whereby A gives the absorbance, I_0 is the initial light intensity that is passed through the sample and I is light intensity that is emitted after portion of the initial light has been absorbed. Inorder to calculate the concentration of a given sample using the obtained absorbance, the Beer Lambert's law is used. This law indicates that absorbance is directly proportional to concentration as shown by equation (ii) below:

$$A = \mathcal{E}lc \quad \text{..... (ii)}$$

where A is the absorbance, $\mathcal{E}$ is the molar absorptivity coefficient, *c* is the sample concentration and l is the length of the cuvette [60]. A blank which is a cuvette that is filled with only the dispersing medium to guarantee that spectral features from the solvent are not included in the sample extinction spectrum is used at each spectrum for background correction [60].

2.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

In Infrared spectroscopy, Infrared radiation is passed through a sample where some of the radiation is absorbed by the sample and some of it is passed through (transmitted). The spectrum which results represents the molecular absorption and transmission, which creates a molecular fingerprint

of the sample. Due to the fact that different bonds / functional groups have different vibrational frequencies , the presence of the available bonds in a molecule can be detected by identifying this characteristic frequency as an absorption band in the infrared spectrum [61]. A plot of transmittance against the frequency is called infrared spectrum. In biomolecules, its usefulness lies in the ability to detect sample/compound`s chemical functional groups vibrational characteristics. The chemical bonds in a compound stretch when a particular compound interacts with infrared light resulting in the groups absorbing this light at specific wavenumbers [60]. In analyzing the phytochemicals in plants, the technique plays a role in the identification of the chemical groups present in the extract. For the nanoparticles synthesis using plant extracts, the biomolecules present in the extract which are responsible for the reduction of Ag^{+} to nanoparticles are determined and also the capping or stabilizing ability of these biomolecules on the nanoparticles is determined through the use of the FTIR spectroscopy [62].

2.3.3 X-ray Diffraction (XRD)

The usefulness of the technique lies in the fact that it determines the composition, orientation, texture and the crystalline structure of compounds [21]. X-rays pass through a compound and a diffraction pattern is produced which gives information on size and shape of a unit cell. When X-rays pass through the sample, they will be bent at different angles and the process is termed diffraction. The diffraction occurs when light is scattered by a periodic array with long-range order, producing constructive interference at specific angles [63]. This scattering of X-rays from the atoms results in the production of a diffraction pattern, which contains information about the atomic arrangement within the crystal [64]. The phase structure and also the particle purity of the nanoparticles can be determined using this technique and one advantage it offers is that it is a non-destructive technique. The pattern produced on XRD for pure substances is unique and it serves as a

fingerprint of that substance making the technique suitable for identification and characterization of crystalline substances. The peak area depends on the size of each crystal and the particular width of peaks of a given pattern can give information on the crystalline's average size. Sharper the peaks are obtained from larger crystallite.

2.3.4 Scanning Electron Microscope (SEM)

SEM is a very useful imaging technique that utilizes a beam of electrons to acquire high magnification images of specimens [65]. The SEM maps the reflected electrons and allows imaging of thick (~ mm) samples. The images which are formed by scanning a beam across the sample and forming the image point - by - point can provide the size, morphology, shape and size of the nanoparticles. The surface picture of high resolution offered by SEM makes the nanoparticles distribution easier to identify [66].

2.4 Introduction to medicinal plants

Plants like *Aloe vera* [5], *Acalypha Indica* [15], *Jatropha curcas* [16], *Zea Mays* [17] , *Calophyllum lnophyllum* leaves [18] and *Saraca lndiaca* bark [19] have been used in the synthesis of silver nanoparticles. Different plants have proved to have antimicrobial activity towards different bacteria and fungi like *E.Coli* [35], *Klebsiella pneumonia* [66] *Salmonella typhi* [67] , *C. mycoderma* [35] , *S.Cerevisiae* [14], *Trichoderma* [16] and many others. This research focuses on using *Vangueria Infausta* and *Parinari Curatellifolia* on the synthesis of silver nanoparticles and below a brief introduction to the plants is given together with their medicinal uses.

2.4.1 *Vangueria Infausta* and its traditional uses

Botanical name : *Vangueria Infausta*

English name: Velvet medlar

Shona name: Mudzvirungombe or Mutsviru

Ndebele name: Umviyo

Kingdom: Plantae

Family : Rubiaceae

Genus: *Vangueria*

Vangueria Infausta which is known in English as Velvet wild medlar is a plant which is found in most areas of Zimbabwe and in African countries like Zambia and Mozambique [35]. It is a deciduous tree which grows up to 7 m in height, having a short trunk and also hanging branchlets which are covered with short, woolly hairs. The bark is pale grey-brown, which peels in untidy flakes and the leaves are dull green in colour. The flowers are hairy greenish, the fruits are green when unripe, turning brownish after ripening and with a soft fleshy pulp [36]. Fig 2.3 shows the leaves and the fruits of *V.Infausta* [68].

It has various uses traditionally and these include treatment of abdominal pains, diarrhoea, malaria, pneumonia, stops menstrual pain or the root extract and the leaves are used as a ring worm remedy and as a relief for toothache [68]. The fruit of the tree is edible and has a pleasant sweet-sour, mealy taste. Dry fruits can be soaked in water, boiled, mashed and then used as a kind of porridge.

a)  b) 

Fig 2.3: *Vangueria Infausta* leaves (a) and fruits (b)

2.4.2 *Parinari Curatellifolia* and its traditional uses

Botanical name : *Parinari Curatellifolia*

English name: Mobola Plum

Shona name: Muchakata

Ndebele name: Umkhuna

Kingdom: Plantae

Family : Chrysobalanaceae

Genus: *Parinari*

Parinari Curatellifolia is an evergreen medium to large tropical tree found in various deciduous woodlands of Africa which are poorly drained and have a moderate altitude. It is mostly known as the Mobola Plum tree due to its tasty fruits which can be crushed and the pulp can be used as an ingredient in drinks and alcoholic drinks as it ferments well . The species is widespread in tropical Africa from Senegal to Kenya and southwards to northern South Africa, with the highest concentration in Zimbabwe and the low veld region in South Africa [38]. The tree can grow up to a

height of about 20-22m depending on the amount of rainfall in that area .The leaves are basically oblong shaped and have a dark green-grey surface. The fruits from the tree are yellow-orange and when ripe they appear to have grey speckles. It has heavy branches which can droop or grow erect resulting in an impressive shape for the tree .The bark is corky and rough consisting of yellow wholly hairs which are usually present in younger branches and twigs. Below is a picture of *Parinari Curatellifolia* leaves and fruits (Fig 2.4) [38].

Mobola Plum tree is considered a traditional food plant in Africa for both humans and animals, due to its fruits that are nutritious and tend to increase food supply in some African countries [38]. The edible fruits can be used to make nutritious syrup or can be used to make porridge. The crushed pulp can be used by some indigenous churches in Zimbabwe for faith healing. Also it can be used as an ingredient in drinks and alcohol as it ferments well. The seeds may be eaten raw in the form of nuts and basically they bear oil. The extracts from the leaves and the bark can be used as a remedy for the symptoms of pneumonia or as a treatment for eye or ear ailments and also for tanning purposes for example in leather tanning [38]. Traditional medicines mostly incooperate the bark of the Mobola Plum tree [69]. The roots can be soaked in cold water for about an hour and used as eardrops for the treatment of earache.

Fig 2.4: *Parinari Curatellifolia* leaves and fruits

2.5 Fungal diseases/ infections

Fungal diseases are more difficult to treat because unlike bacteria, fungi are eukaryotes. For antibiotics they only target prokaryotic cells, on the other hand compounds that kill fungi also harm the eukaryotic animal host. Fungi are widely distributed over the world and are affected by various environmental factors such as temperature, moisture, wind and geographical location [24]. They often attack humans and animals directly by colonizing and destroying tissues. Often topical and oral treatments are long term and may only be partially successful in controlling the fungus, if they work at all. Many infections will be chronic and if you are fortunate enough to rid the infection from your body, there is always the possibility of recurrence of the disease. Many serious bacterial diseases have been successfully treated and usually without side effects from the drugs used. This usually is not the case with treatment of fungal diseases. The reason for this is that fungi, like people, are eukaryotes, making the two types of cells similar, at least more similar than to bacterial

cells [37]. There is enough similarity that when attempts are made to rid your body of a fungal infection, with chemicals, it is difficult to find a treatment that can remove the fungus without doing significant damage to your own cells. Fungal diseases known as systemic mycoses, for example valley fever, Histoplasmosis, or pulmonary disease, are known to spread to internal organs and they mostly enter the body through the respiratory system [70]. In most cases the fungi spread via the bloodstream to multiple organs which include the skin, often causing multiple organs to fail and eventually resulting in the death of the patient. Opportunistic mycoses are fungal infections that are mostly common in all environments and mainly take advantage of individuals who have a compromised immune system, such as AIDS patients [71]. Fungi are ubiquitous in nature and vital for recycling of nutrients contained in organic matter. The vast majority of the known fungal species are strict saprophytes, although there are a few capable of causing disease in plants or humans. However, there are several fungal genera containing species that cause disease (for example infections, allergies, toxicity) in plants, animals and man. These fungi can be categorized into two groups in regards to infection: saprophytic fungi which can be opportunistic pathogens that enter *via* wounds or due to a weakened state of the host and true pathogens that may depend on living plant or human tissues for nutrients but can also survive outside of the hosts. Fungal diseases are often caused by fungi that are common in the environment and approximately there are about 1.5 million different species of fungi on earth. About 300 of these species are known to make people sick. These fungi grow best in warm, moist environment such as shoes, socks, swimming pools, locker rooms, and the floors of public showers. They can also be found in soil and on plants and trees as well as on many indoor surfaces and on human skin. They tend to cause skin damage and if not treated early the results may be deadly in humans and serious damage in agriculture. Ringworm is a commonly known fungal infection which causes itchy, red and circular rash on the

skin. It is also known as tinea or dermatophytosis. Depending on the affected body part, ringworm can be referred to by some other names for example athlete's foot or tinea pedia which is a fungal infection of the feet. If it's in the groin area it is named jock itch or tinea cruris. Fungi are also known to cause *mycetismus*, which is a disease that is caused by the ingestion of toxic mushrooms leading to poisoning. Food that is contaminated by fungal toxins (mycotoxins) cause *mycotoxicosis* which is the poisoning of humans (and other animals). Many of the fungal infections are superficial; that is, they tend to occur on the animal's skin. The decline of the world's frog population in recent years may be caused by the fungus *Batrachochytrium dendrobatidis*, which infects the skin of frogs and presumably interferes with gaseous exchange. Similarly, more than a million bats in the United States have been killed by white-nose syndrome, which appears as a white ring around the mouth of the bat. It is caused by the cold-loving fungus *Geomyces destructans*, which disseminates its deadly spores in caves where bats hibernate.

2.5.1 Athlete's foot

Athlete's foot (*tinea pedis*) is a skin infection caused by a type of fungus called a *dermatophyte* [72] and this fungal skin infection is confined to the foot where it can occur on the sole, back of the foot or toe webs to both athletes and non-athletes (Fig 2.5). This infection can be contagious and the species which cause this disease are mostly found in the soil or on decaying vegetation and they typically enter the foot through wounds or from walking bare-footed. It is usually caused by irritants, contact allergens, sweat , poorly fitting shoes, rash, sharing shoes or socks with someone who is infected can lead to contraction of the infection and so on [72]. The fungus that causes the infection is found in swimming pools and it also grows on the floors of public showers and of locker rooms. It is most common to people who wear closed shoes and also it's common to older males. Its signs and symptoms include white, moist, cracks, itchiness and scaly sores between the

toes. Other symptoms may involve painful blistering lesions if the athlete's foot becomes super infected with bacteria which lead to the swelling of the foot. Having other diseases or any other type of illness such as diabetes might make it very difficult to fight the infection thereby causing more serious skin problems that might take time to heal permanently.

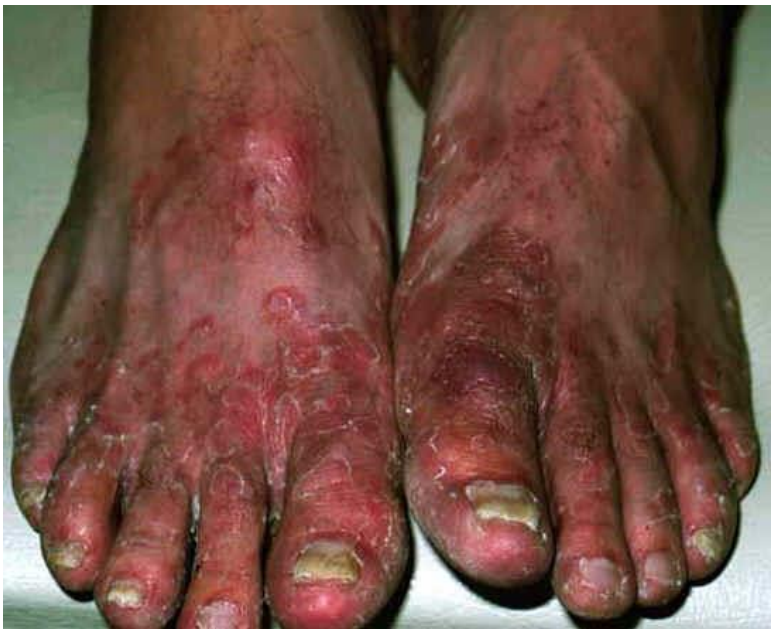


Fig 2.5: A person with athlete's foot

2.5.2 Candidiasis

Candidiasis also known as thrush is a form of fungal infection (dimorphic fungus it grows as both mycelium and yeasts) which is caused by yeasts that belong to the genus *Candida* .Basically many of these species of *Candida* yeasts cause infection in humans, but the most common of them is *Candida albicans* .This type of fungus normally occurs in genital organs for example the vagina, mouth (Fig 2.6) and digestive tract of even the healthy people. For some unknown reasons and also under some circumstances this can eventually result in severe and fatal infections, which may lead to lesions and eruptions of the mouth ,lungs, skin , bronchial tubes and nails [24]. Candidiasis genital is an infection that is much more common in females than in males. When it occurs in men

candidiasis affects the head of the penis and the foreskin [73]. The *Candida* fungi are naturally found inside the body and on the skin and almost all humans are colonized by the fungus, but at minimum levels this does not usually cause problems. Naturally the immune system of humans and the natural ecology of bacteria normally keep the fungal population in check, however disturbing this balance causes the fungus to thrive .This is commonly seen with newborn babies that can be affected by thrush while they develop a balanced microbial flora [24]. Problems with Candida albicans only arise under certain conditions that allow for its thriving and growth to numbers that result in candidiasis and with the fungal cells producing hyphae, structures that penetrate the tissue [74] .Candidiasis is most likely to occur from any one of the following cases:

- Due to a broad-spectrum in antibiotic use, their continuous usage result in a change in the normal microbial flora balance which will upset its balance thereby causing the Candida to overgrow [73].
- Due to the weakening of the immune system (immunosuppression) for example in people with HIV infection, or the taking of medication such as chemotherapy or corticosteroids results in a reduced defence mechanism from the body against the fungus thereby allowing the Candida to thrive .
- For uncircumcised men with poor hygiene yeast growth is promoted in their moist, dark, warm space underneath the foreskin. The irritation of the penis can lead to fungal infection maybe as a result of chronic local irritants such as bath foam, soaps, shower gels and lubricants. Also fungi can thrive very well on the penis when men don't dry carefully after washing [23].
- If diabetes is poorly controlled, higher levels of blood sugar will allow a more conducive environment for the yeasts to thrive [24].

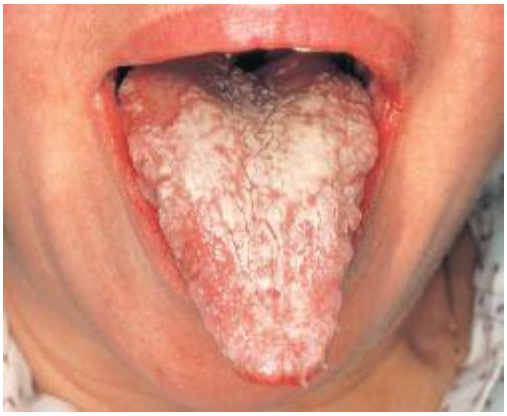


Fig 2.6 : Candidiasis of the mouth

2.5.3 Histoplasmosis

Histoplasmosis is a systemic disease caused by the fungus *Histoplasma capsulatum* and it occurs due to two varieties of the pathogen, *Histoplasma capsulatum var. capsulatum* and *Histoplasma capsulatum var. duboisii* [75]. The infections normally occur from inhalation of spores of fungus, also by the direct inoculation into skin or mucous membrane and once entry is achieved, it is thought to enter bloodstream in some cases where many body parts involved. The microscopic fungal spores grow in soils and materials contaminated with bird or bat droppings and if these are disturbed the spores can become airborne and be breathed into the lungs [76].The disease cannot be transmitted from person to person or from animals to people. The symptoms are similar to pneumonia and may include fever, chest pain, and a dry cough and the infection can sometimes become serious if it is not treated, especially if the infection spreads from the lungs to other parts of the body [77].

2.5.4 Aspergillosis

The *Aspergillosis* is a large spectrum of fungal diseases, which primarily affect the lungs and are caused by members of the genus *Aspergillus* [78]. The genus are saprophytes which are found worldwide in soil, forage products, food products, dust, organic debris and also in decomposing

matter. They are considered weak plant pathogens and there are two species namely *Aspergillus flavus* and *Aspergillus parasiticus*, which produce potent toxins (aflatoxins) on certain crops [25].The fungus grows best in moist environments, although spore aerosolization and dispersion occur most effectively in dry climates [79].

2.6 Antifungal drugs used on humans and their side effects

For oral antifungals for example capsules, the side effects include headache, abdominal pain, diarrhoea, rash and indigestion. Severe reactions can occur as a result of using these antifungals such as swelling of the face, tongue or neck, blistering or peeling of the skin and also some patients may suffer from breathing difficulties.

2.6.1 Effects of antifungal drugs

The side effects of antifungal medicines depend on the type of medicine one is using. Some creams for example causes redness, itching and mild burning sensation to the skin [80]. Oral antifungals may cause more serious side effects such as liver damage. The signs and symptoms for liver damage due to these antifungals may include jaundice(this is the yellowing of the skin or eyes), unusual tiredness or weakness, urine which is dark and feaces which are pale than usual, vomiting, a serious loss in appetite and continuously feeling sick. For intravenous antifungal (medicine that is applied as a continuous drip into the vein of the arm) such as Amphotericin B, the side effects include , epigastric pain(a pain in the upper part of the tummy), a fever (high temperature), muscle and joint pain, anemia (this is a reduced number of red blood cells) , chills and many other side effects. Also in rare circumstances it can affect the heart by causing an irregular heartbeat or causes blood pressure changes and also might affect the liver functions. Also some antifungals might interact with other medicines; this is known as drug or pharmacokinetic interaction that is taking two or more medicines at the same time thereby altering the functions of the other. Some of the

medicines that antifungals may interact with include benzodiazepines which are a group of medicines that are used to help sleep or reduce anxiety, hydrochlorothiazide which is used for treatment of high blood pressure, rifampicin which is an antibiotic used in the treatment of bacterial infections, theophylline which is used asthma treatment and cimetidine which is used in indigestion treatment.

2.7 Phytochemicals

Phytochemicals can be defined as natural chemicals that plants produce for defense and control purposes against pathogens and diseases but the phytochemicals are non-nutritive to the plant. These phytochemicals tend to be important to human health due to their ability to display different biological properties such as anti-inflammatory, anti- microbial, anti-oxidant, anti-cancerous activities and many others. The source of these biochemicals are the vegetables and the fruits that are consumed everyday by humans. The effect of the extracted plant phytochemicals mainly depends on :

- The origin and the nature of the plant
- Its degree of processing
- The moisture content in the sample

2.7.1 Flavonoids

Flavonoids are widely found in nature and they are polyphenolic molecules which are soluble in water soluble. They constitute of 15 carbon atoms (C_{15}) and they are aromatic containing at least two benzene rings which are joined together with a short three carbon chain. One of the carbons of the short chain is always connected to a carbon of one of the benzene rings, either directly or through an oxygen bridge, thereby forming a third middle ring, which can be five or six-membered. The flavonoids are sub divided into 6 major subgroups namely, flavonols, flavones, flavanones,

isoflavones, catechins, anthocyanidins and chalcones [81]. Flavanoids are responsible for the coloring of fruits, vegetables and herbs. Reports have been made on the activities of these compounds which include their antioxidant, antitumor, antinflammatory, radical scavengers, antimicrobial and anti-diarrhoea properties. Flavones and isoflavones are said to have anti-malaria properties. An example of a flavonoid is Quercetin shown in Fig 2.7:

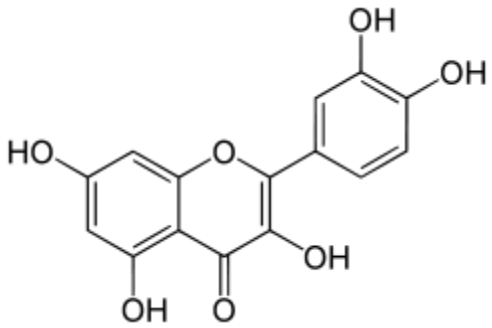


Fig 2.7: Structure of Quercetin a flavonoid

2.7.2 Terpenoids/terpenes

The two compounds are among the largest group of phytochemicals, though the words are used interchangeably, they have different meanings. Their main difference lies in the fact that terpenes are hydrocarbons (meaning the only elements present are carbon and hydrogen); on the other hand terpenoids are considered to be denatured by oxidation during drying and curing the flowers or they are chemically modified, they contain extra functional groups that could be comprised of a range of chemical elements having the general formula $(C_5H_8)_n$ [82]. They are aromatic compounds that are found in thousands of plant species, and are responsible for the various flavors and fragrances of cannabis. Terpenes are known to be the major constituents of plant resin and also essential oils extracted from such plants, an example is given in Fig 2.8 below. Their potential therapeutic properties only begun to expand recently, though their presence in cannabis has been known for decades.

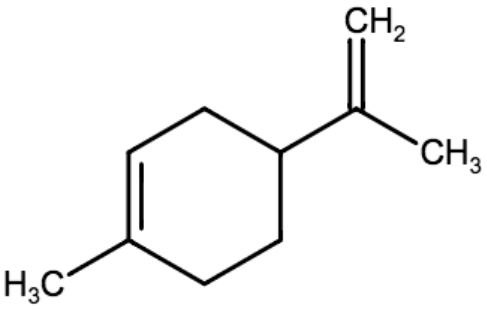


Fig 2.8 : Structure of Limonene a monocyclic terpenoid

2.7.3 Steroids

These are also termed cardiac glycosides and they represent one group of phytochemicals which have medicinal properties. They can be applied as cardiac drugs and they can be administered by injections. The excessive dosages of these steroids is harmful to humans and it can lead to death. Taking small amounts of the drug is enough to treat heart disease. The structure of a steroid is shown in Fig 2.9:

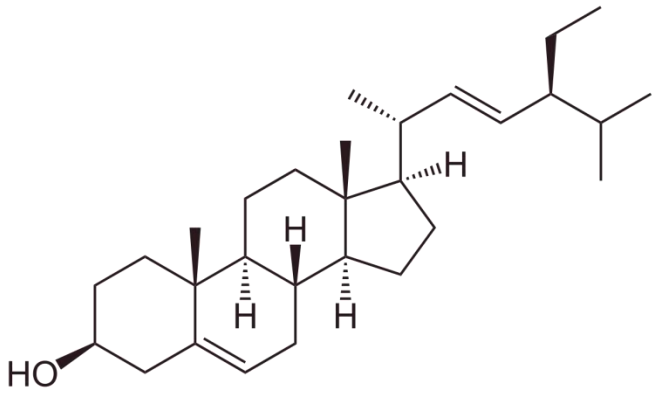


Fig 2.9 : Structure of a stigmasterol

2.7.4 Coumarins

These are phytochemicals that are present in dicotyledonous plant families that have a vanilla like flavor and is an oxygen heterocyclic compound [83]. They have blood-thinning, antimicrobial,

sedative, anticoagulant, analgesic, estrogenic, antimalarial activities, anti-fungicidal and anti-tumor properties and it increases the blood flow in the veins and also decreases capillary permeability. These compounds can be toxic when they are used at high doses for a long period. The structure of coumarines is shown below in Fig 2.10. Many activities associated with coumarines have been reported and these include antimicrobial, sedative, hypnotic, anticoagulant, analgesic, estrogenic and antimalarial activities. The antifungal properties of furocoumarins and other types of coumarins have been reported.

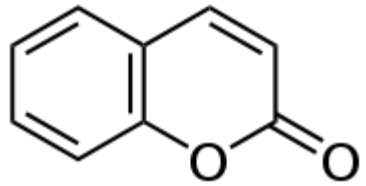


Fig 2.10: Structure of a simple coumarin

2.7.5 Phenolics

Phenolic compounds have at least one aromatic ring which is bonded to few hydroxyl groups (Fig 2.11). Mostly they are found in pigments and are responsible for the color of fruits and also for protecting the plants from pathogens. They are known to have antioxidant properties. Extracts rich in phenolics can reverse brain related damages [83].

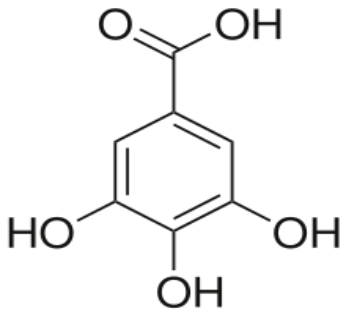


Fig 2.11: Structure of gallic acid a phenolic compound

2.7.6 Alkaloids

These are a group of naturally occurring compounds of organic nitrogen-containing bases. The alkalinity nature of these alkaloids mainly depends on the position of the functional group. Examples of these alkaloids are nicotine (a stimulant), quinine, codeine (an analgesic), ephedrine, morphine (a narcotic) used for pain relief and strychnine. They are the secondary metabolites of plants and secondary metabolites refer to the organic compounds or chemicals that are produced by the plant but are not directly involved in the development, normal growth or reproduction of the plant [84]. The seeds and the roots of a plant are the main constituents of these alkaloids. They are used as pesticides or insecticides, as addicting drugs, as medicine and also in research and scientific studies. Nicotine which is a drug is an example of an alkaloid and its structure is shown in Fig 2.12 below. They offer a protection to the plants against pathogens and some herbivores. They are mostly found in solid form for example atrophine, which is soluble in alcohol though some of them are liquids in nature containing carbon, hydrogen and nitrogen. Different screening methods have been used in the detection of alkaloids in plants and these include the use of Meyer's reagent (which is a potassiomercuric iodide solution) ,Wagner's reagent

(iodine in potassium iodide) ,Tannic acid ,Hager's reagent (a saturated solution of picric acid) and Dragendorff's reagent [85]. The presence of alkaloids have been confirmed by colour changes which occur with the use of any of the screening methods.

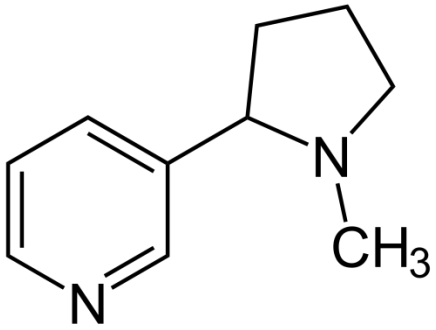


Fig 2.12: Structure of a pyridine alkaloid (Nicotine)

2.7.7 Quinones

These compounds occur as biochromes that is they are biological pigments. Examples include benzoquinones, anthraquinones, naphthoquinones and polycyclic quinones. An example of a quinone (halenaquinone) is given below in Fig 2.13. These quinones are usually found in bacteria, also in fungi and appear in higher plant forms, but they are few in animals.

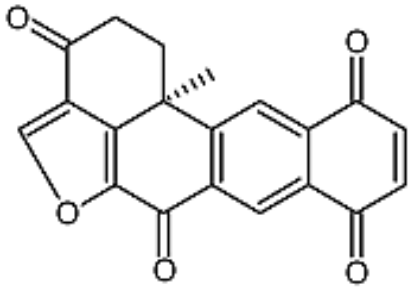


Fig 2.13: Structure of halenaquinone

2.7.8 Anthocyanins

Anthocyanins are regarded as the largest and the most important group of water-soluble pigments in nature and they are responsible for the blue, purple, red and orange colors of many fruits and vegetables [86]. They are polyphenols in nature with known antioxidant activity which might be responsible for some of the biological activities which include the prevention or lowering the risk of cardiovascular disease, diabetes, arthritis and cancer. Fig 2.14 shows an example of an anthocyanin. The anthocyanins belong to the family of compounds known as flavonoids, but they are distinguished from other flavonoids due to their capacity to form flavylium cations [86].

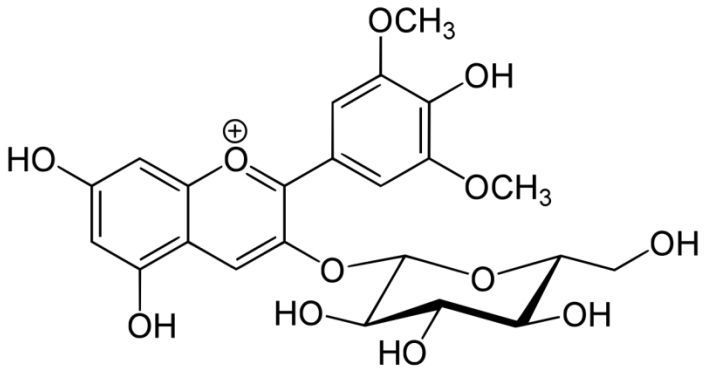


Fig 2.14: Chemical structure of the Malvidin-3-O-glucoside (Oenin) an anthocyanin

2.8 Summary

The availability of phytochemicals such as alkaloids, tannins, saponins and many others in plants could be exploited in the synthesis of silver nanoparticles which could be used in the preparation of antifungal drugs and thus eliminating the side effects of the traditional chemical methods. The plants used in this research have antimicrobial properties and when they are used for silver nanoparticles synthesis for antifungal drugs, the resulting antifungals have a promising high potency. The use of green chemistry would reduce the use of toxic and expensive physical and chemical methods thereby reducing the levels of pollution in the environment.

CHAPTER THREE

METHODOLOGY

3.0 Introduction

This chapter gives the methods used for the plant leaves collection and preparation, synthesis of the silver nanoparticles, characterization methods used and antifungal tests done on the silver nanoparticles. Fig 3.1 shows a flow diagram of the steps taken in the research:

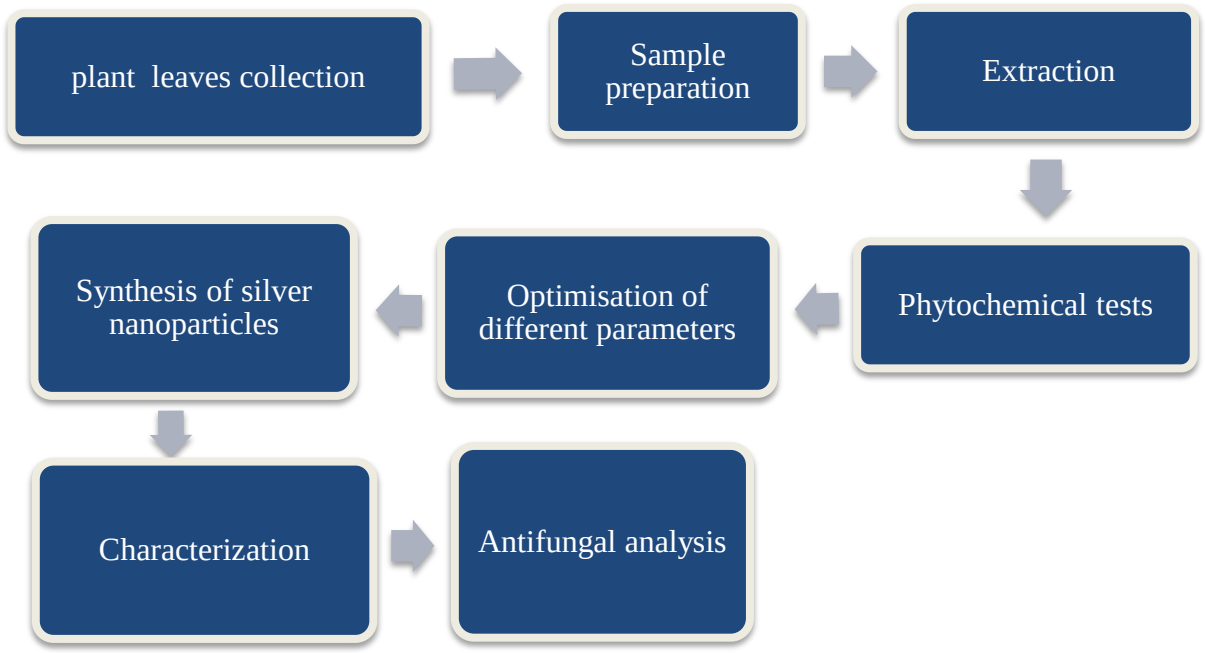


Fig 3.1: Flow diagram of the steps taken in the synthesis of silver nanoparticles for both plants

3.1 Reagents

All chemicals used for the experiments carried out in this research were of analytical reagent grade and were used without further purification. Distilled water was used throughout. The following reagents were used: silver nitrate ($AgNO_3$), Acetic anhydride ($(CH_3CO)_2O$, 40%), ferric chloride ($FeCl_3$, 2 %), sodium hydroxide (NaOH, 2% and 10 %), sulphuric acid (H_2SO_4, 98 %), mercuric

chloride ($HgCl_2$, 99.9 %), potassium iodide (KI, 99.9 %), chloroform ($CHCl_3$, 55 %) and potassium bromide (FT-IR grade, KBr, 99.9 %).

3.2 Sample collection and preparation

The leaves of both *Vangueria Infausta* and *Parinari Curatellifolia* were collected from Lower Gweru in Zimbabwe. They were washed thoroughly with tap water then with deionized water to remove any dirt and any form of debris. The leaves were sun dried for one week to completely remove any moisture and make sure they were completed dry. They were then ground into a fine powder (Fig 3.2) using a 180 μm mesh sieve inorder to increase the surface area of the sample for extraction, to obtain a homogenous sample and to increase the contact of the sample with the solvent system [7]. The powder from each tree was used for further studies. All the glassware which was used in was washed with soap and tap water then rinsed with distilled water and dried in an oven before uses.

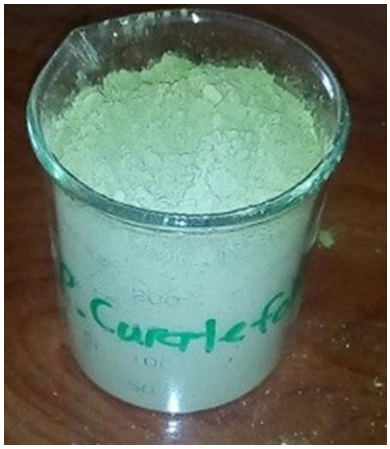
a)  b) 

Fig 3.2: Powder of *V.Infausta* (a) and *P.Curatellifolia* (b)

3.2.1 Preparation of the leaf extract

The extraction of the phytochemicals from the leaf powder, was done using the maceration .Using a measuring cylinder, 100ml of distilled water was added in 20g of *V.Infausta* powder in a 1000ml volumetric flask and again the same amount of water was added in 20g of *P.Curatellifolia* powder in another volumetric flask [57]. The mixtures were placed on a rotary shaker for 24hrs and then filtered using a filter paper. This was done twice inorder to make sure that all the phytochemicals were extracted. The obtained extract was then used for further studies.

3.3 FTIR Analysis of extract

The method which was used is called liquid membrane method and it was carried out by dripping several drops of the extract onto a KBr aperture plate and sandwiching it under another aperture plate, such that no gas bubbles were trapped. The plate was place in the FTIR spectroscopy and scanned in the range of 400 cm^{-1} - 4000 cm^{-1} .

3.4 Phytochemical analysis

The presence of different phytochemicals was tested with the use of different methods which were in accordance to those found in literature. The presence of phytochemicals such as steroids, tannins, alkaloids, coumarines, anthocyanins, saponins, flavonoids, phenols, terpenoids, glycosides, emodins and quinones was tested as follows:

3.4.1 Test for steroids

By using a syringe, 1ml of plant extract was measured and dissolved in 10 ml chloroform and 10 ml concentrated sulphuric acid (98%) in a test tube [87]. The acid was added dropwise. The resulting mixture was shaken vigorously. The existence of two layers indicated the presence of steroids. The top layer displays a red color while the bottom acid layer displays a yellow colour with green fluorescence [84].

3.4.2Test for alkaloids

With the use of a syringe, 1ml of plant extract was measured and mixed with iodine in potassium iodide solution in a test tube and the formation of a cream/ reddish precipitate indicates the presence of alkaloids in the test sample [88].

3.4.3 Test for terpenoids

By using a syringe, 2 ml of plant extract was added to a solution of 2 ml acetic acid anhydride and drops of concentrated sulphuric acid (98%) [89]. The formation of a blue ring indicates the presence of terpenoids [87].

3.4.4 Test for glycosides

The presence or absence of glycosides was determined by using Salkowski's test. The extract (2 ml) was mixed with 2 ml chloroform and then 2 ml of concentrated sulphuric acid (98%) was added. The mixture was shaken gently [90]. The presence of glycosides is indicated by appearance of a reddish brown coloration [88].

3.4.5 Test for quinones

The presence of quinones was determined by use of the Mayer's reagent which is prepared by mixing 1.36 g $HgCl_2$ and 5.007 g KI in 1000 ml distilled H_2O. This is then added to the extract. A cream precipitate indicates the presence of quinones [83] .

3.4.6 Test for saponins

To 1 ml of the plant extract in a test tube, 20 ml distilled water was measured using a measuring cylinder and added to the extract [87]. The mixture was vigorously agitated for about 15 minutes. The formation of a dense layer of foam shows the presence of saponins [91] .

3.4.7 Test for flavonoids

For determining the presence of flavonoids, the Alkaline reagent test was carried out. The extract (2 ml) was mixed with 2 ml of a solution of sodium hydroxide (2 %). Presence of flavonoids is indicated by formation of a yellow colour which is intense but should dissolve on addition of drops of acid [84].

3.4.8 Test for phenols

To 2 ml of the crude extract, a few drops of ferric chloride (2 %) was added and the mixture was agitated lightly. The presence of phenols was denoted by the appearance of an intense blue black colour [88].

3.4.9 Test for tannins

To 2 ml of the plant extract a few drops of lead acetate (1 %) were added in a test tube. The presence of tannins shows a yellow precipitate [87].

3.4.10 Test for coumarins

To 1 ml of extract, 1 ml of 10 % sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color [83].

3.4.11 Test for anthocyanins

By using a syringe,2 ml of extract was measured and added to 2 ml of 2M HCl with ammonia in a test tube [83]. A pink red colour which turns blue violet is the indication of the presence of anthocyanins [85].

3.4.12 Test for emodins

To 2 ml of the plant extract, 2 ml benzene and 2 ml ammonium hydroxide was added and the mixture was shaken. The appearance of a red colour shows presence of emodins [90].

3.5 Optimization of different parameters

Factors which affect the synthesis of silver nanoparticles namely silver nitrate concentration, amount of extract and temperature were optimized. The aqueous solutions were prepared using distilled water [7]**.** Distilled water was also used for background correction in UV-Vis studies.

3.5.1 Effects of silver nitrate concentration

Different concentrations of 0.5 mM, 1 mM, 2 mM, 3 mM, 4 mM and 5 mM of silver nitrate [92] were used to synthesize the silver nanoparticles. To 10 ml of the plant extract, 90 ml of silver nitrate was added [57] in a conical flask using the five different concentrations at room temperature [53]. UV-visible spectroscopy was used to monitor the rate of metal reduction and progress of nanoparticles biosynthesis after 24 hours [93]. Aliquots (1 ml) were taken from the reaction mixture and were scanned at wavelengths between 200 nm-600 nm to determine the absorbances of the silver nanoparticles produced using the different silver nitrate concentrations [94].

3.5.2 Effects of the amount of extract

Different amounts of extract of 2 ml, 4 ml, 6 ml , 8 ml and 10 ml were used in the synthesis of the silver nanoparticles using 90 ml of 5 mM silver nitrate for both the plant extracts at room temperature. This was monitored again by the use of a UV-Vis using the same range of wavelengths (200 nm- 600 nm) after 24 hours of synthesis. The results were used in the plotting of the graphs of absorbance against wavelength and determining the amount with the highest yields of the nanoparticles.

3.5.3 Effects of temperature

A water bath was used to set a temperature range of 20 ^{o}C, 40 ^{o}C, 60 ^{o}C and 80 ^{o}C using 10 ml of the plant extract and 90 ml of 5 mM of silver nitrate. UV-Vis was used in the monitoring of the silver nanoparticles produced after 24 hours of synthesis.

3.6 Synthesizing silver nanoparticles and their characterization

Silver nanoparticles were synthesized from the leaf extract of the two trees using the optimum conditions obtained above. With the use of a measuring cylinder 9 ml of *P.Curatellifolia* leaf extract was mixed with 81 ml of 5 mM silver nitrate and heated on hot plate at 70 ^{o}C. With *V.Infausta* 10 ml of plant extract was mixed with 90 ml of 5 mM silver nitrate at a temperature of 50 ^{o}C.

3.7 Characterization

3.7.1 UV-Vis Spectroscopy

The synthesis was monitored visually and with the use of a UV-Vis spectrometry [9] at different time intervals from 10 minutes up to 6 hours. The bio-reduction of silver ions was monitored by periodic sampling by the UV- Vis spectroscopy. Deionized water was used as reference for background correction of experiments [1] . The wavelength of the reaction mixture in the UV-Vis spectrum was measured at a range of 200 nm to 600 nm in quartz cuvettes with 1 cm path length [93].

3.7.2 FTIR analysis of silver nanoparticles

The possible groups responsible for the interaction between the capping agents and silver nanoparticles were confirmed by FTIR [12]. Powdered AgNPs were pelleted with potassium bromide (KBr) in the ratio of 1 : 10 respectively [57] . A mortar and pestle was used for grinding. The samples were scanned using infrared in range of 4000- 400 cm $^{-1}$ using FTIR. The spectrum obtained was compared with a reference IR chart to identify functional groups present in sample.

3.8 Effect of reaction time

To study the effect of the reaction time, the UV-Vis spectroscopy was used to monitor how time affects the rate at which the silver nanoparticles are produced [32]. After addition of silver nitrate to the plant extracts, the bio-reduction of the Ag^{+} ions in solutions was monitored by periodic sampling of aliquots (1 ml) of the aqueous components from 10 minutes to 6 hours using UV-Vis spectroscopy for both *Vangueria Infausta* and *Parinari Curatellifolia* and their absorbances were noted at a wavelength range of 200 nm to 600 nm. The resulting spectra were used to analyze the effect of reaction time on the synthesis of silver nanoparticles.

3.9 Stability tests

The stability studies of the silver nanoparticles were monitored periodically by the use of a UV-Vis spectroscopy [12] from day 1 up to 60 days . Small aliquots (1 ml) from the synthesised nanoparticles were used at each test and the absorbances were noted at a wavelength range of 200 nm to 600 nm. The resulting spectra graphs were used to show how the SPR of the synthesised AgNPs change with time and this would determine the stability of these AgNPs [31].

3.10 Antifungal activity testing

The agar disk diffusion method was used for evaluating the antifungal properties of the synthesized AgNPs and the leaf extract. The agar was prepared by adding 39 g of Potato Dextrose Agar (PDA) in 1litre of distilled water and boiling the mixture. After sterilization in an autoclave for 40mins at 121 ^{o}C, the PDA was poured in petri dishes, this was carried out in a Biological Safety Cabinet (BSC) to avoid any contamination to the agar plates [23]. All the other apparatus used in the antifungal analysis were also autoclaved inorder to sterilize them including the used paper discs. The agar plates were solidified, after solidification; four fungal cultures namely *Trichoderma, Saccharomyces Cerevisiae, Penicillium* and *Fusarium* were inoculated and swabbed on the agar

plates. Sterile Whatman filter paper discs (6 mm in diameter) were used [23] which were impregnated with plant extract, 1 mg/ml silver nanoparticles solution [5] , silver nitrate and standard antifungal drugs (Itraconazole and Griseofulvin) were also placed onto the agar plates and the plates were closed. The plates were inverted and the incubated at 37 ^{o}C for 48 hours .Fungal inhibition on different plates was checked after incubation. The formation of a clear zone (restricted fungal growth) around the cavity was an indication of antifungal activity [95]. The antifungal activity of AgNPs against *Trichoderma, Fusarium, Penicillium and Saccharomyces cerevisiae* as models for fungi was investigated. The activities of AgNPs were compared with antifungal drugs namely Itraconazole and Griseofulvin. All analyses were carried out in triplicates as reported [96]. The zones of inhibition were measured in millimeters with the use of a ruler and the results obtained were recorded [53].

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

This chapter gives the results obtained during the research and their detailed discussion. These include phytochemical analysis results, synthesis of silver nanoparticles, FTIR results and UV-Vis studies and antifungal activity. The results are represented in the form of graphs, tables and pictures.

4.1 Phytochemical Results of *Vangueria Infausta* and *Parinari Curatelliffolia*

Qualitative analysis of the phytochemicals in the crude extracts of both plants was carried out to find out the constituents responsible for the bio-reduction of silver. The crude extracts for both plants and the colour changes which were used for inference are shown in Figs 4.1 to 4.3. The inferred results are shown in Table 4.1.

Table 4.1 : Phytochemical analysis results

Phyto-constituents	Confirmation (Inference)	
	V. Infausta extract	*P.Curatellifolia* extract
Steroids	+ve (present)	+ve (present)
Terpenoids	-ve (absent)	-ve (absent)
Glycosides	+ve (present)	+ve (present)
Saponins	+ve (present)	+ve (present)
Alkaloids	-ve (absent)	-ve (absent)
Flavonoids	+ve (present)	+ve (present)
Phenols	+ve (present)	+ve (present)
Cumarines	-ve (absent)	+ve (present)

Tannins	+ve (present)	+ve (present)
Anthocyanines	-ve (absent)	-ve (absent)
Quinones	-ve (absent)	-ve (absent)
Emodins	-ve (absent)	-ve (absent)

Key : + means present and – means absence

Fig 4. 1: *P.Curatellifolia* and *V.Infausta* leaf extract

Fig 4.2: Phytochemical tests on *P.Curatellifolia* leaf extract

Fig 4.3: Phytochemical tests on *V.Infausta* leaf extract

The phytochemical results showed the presence of steroids, glycosides, phenols, saponins ,flavonoids and tannins in *V.Infausta* extract and steroids, glycosides, phenols, saponins ,flavonoids ,tannins and cumarines in *P.Curatellifolia*. The presence of hydroxyl group containing compounds such as phenols, flavonoids in *P.Curatellifolia* and *V.Infausta* leaf extract and also the cumarines found in the *P.Curatellifolia* can be attributed to the successful reduction of Ag^+ ions. Literature indicates that the hydroxyl group is useful in the reduction of Ag^+ ions to nanoparticles in solution [97]. The extracts also confirmed the presence of flavonoids and saponins which are known to have antimicrobial activities. The reactions which are thought to occur after the addition of plant extract are as shown in equation (2) and (3) below according to [93]:

$$Ag(NO_3)_2 + \text{Plant aqueous extract} \longrightarrow Ag^+ + NO \quad \text{.....(2)}$$

$$\text{Biomolecules of phenols} + Ag^+ + e^- \xrightarrow{\text{Reduction}} Ag^0 \quad \text{.....(3)}$$

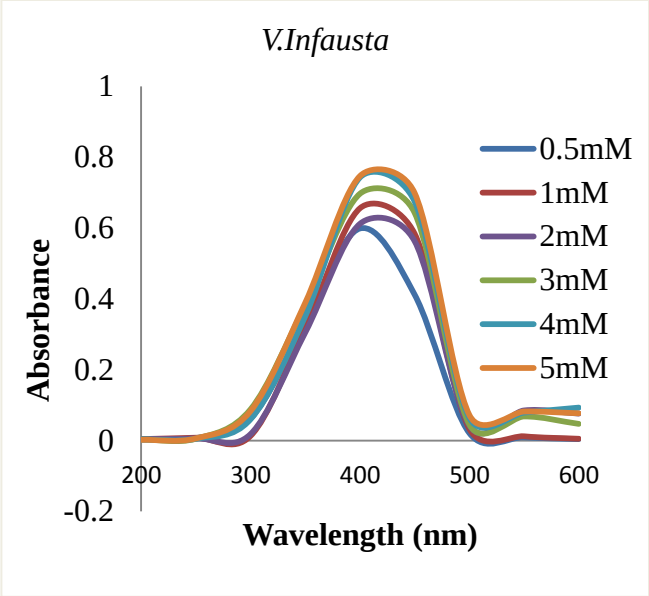
4.2 Optimization of different parameters

The effect on silver nitrate concentration, amount of the extract and reaction temperature on the synthesis of silver nanoparticles for both plants was optimized inorder to come up with the best

conditions for the reaction. UV-Vis spectrometry was used qualitatively to measure the amount of the synthesized nanoparticles and the results are discussed below.

4.2.1 Effects of silver nitrate concentration

Different concentrations of silver nitrate were used on both the plant extracts ranging from 0.5 mM to 5 mM using 10 ml of the plant extract and 90 ml of silver nitrate at room temperature. The reaction mixtures were left for 24 hours to allow for the maximum formation of silver nanoparticles [40]. It was deduced that more silver nanoparticles were achieved at a higher concentration of silver nitrate (Fig 4.4 and Fig 4.5), thus a concentration of 5 mM silver nitrate was used on both the extracts. This is in accordance with [92] where silver nanoparticles were synthesized using *Asiatic Pennywort* and *Bryophyllum* leaves extract and a comparable concentration of silver nitrate was found out to be more effective. According to literature synthesizing these silver nanoparticles at a higher concentration leads to the formation of more stable silver nanoparticles than those synthesised at a lower concentration [62].



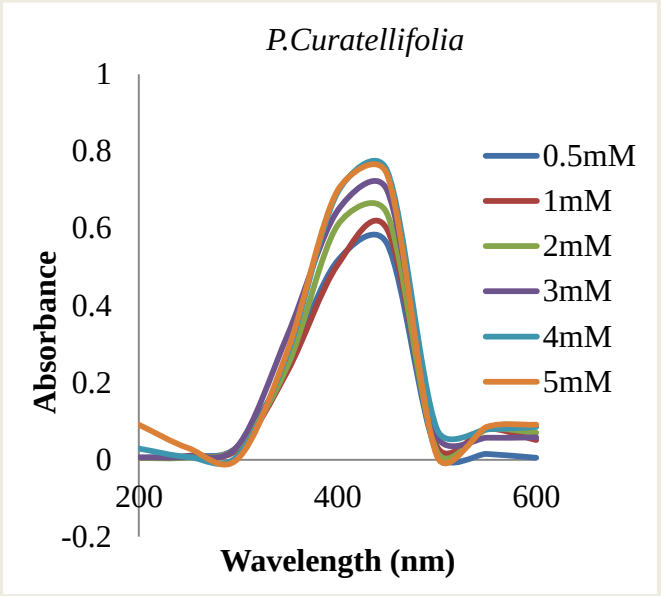
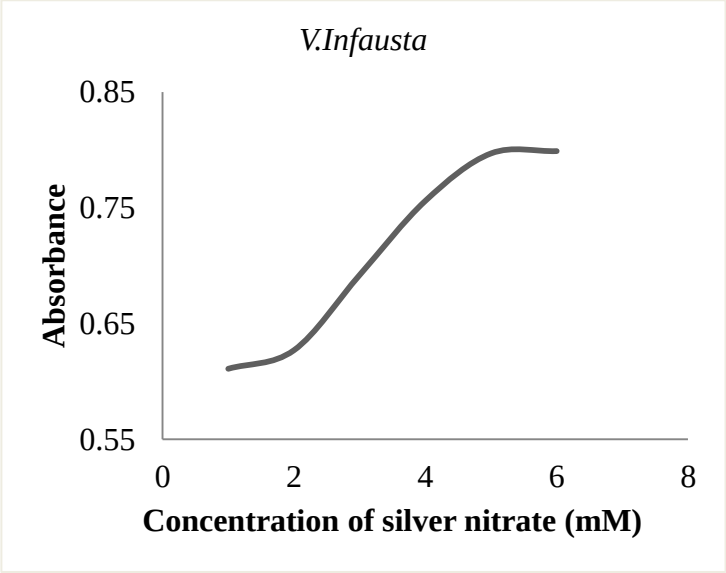


Fig 4.4: Effect of silver nitrate concentration at different wavelengths for *V.Infausta* and *P.Curatellifolia*



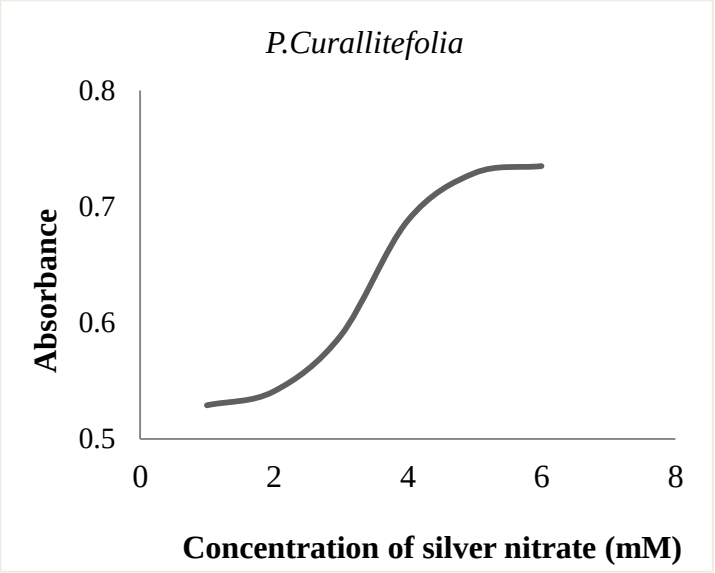


Fig 4.5: Relationship between silver nitrate concentration and absorbance at maximum wavelength for *V.Infausta* and *P.Curatellifolia*

4.2.2 Effects of amount of extract

The effect of the amount of extract of *P. Curatellifolia* and *V.Infausta* on the reaction was studied by the UV-Vis spectroscopy at varied volumes of 2 ml to 10 ml. A concentration of 5 mM of silver nitrate (90 ml) was used for both plant extracts, at room temperature and the reaction was left to occur for 24 hours. An increase in the amount of extract increases the intensity of absorbance which means there is an increase in the amount of silver nanoparticles obtained (Fig 4.6 and 4.7). The higher the amount of extract being added meant that there was a higher possibility of the green bio-reduction mechanism of the silver nitrate which was occurring which would lead to the formation of stable and well defined silver nanoparticles. There was no further increase in amount of the silver nanoparticles formed at higher extract amounts of 9 ml extract for *P.Curatellifolia* and 10 ml extract for *V.Infausta*. This can be attributed to the fact that Ag^+ ions in the solution had been used up by the phytochemicals in the leaf extract thus the reduction process had to an end, due to the fact that Ag^+ ions in the in the solution had been used up by the phytochemicals in the leaf extract thus the reduction process had come to an end, so the graphs flatten as shown in Fig 4.7. There is a considerable increase in absorbance as the amount of extract increases and the graphs then levels off when the silver nitrate gets used up during the reaction. the graphs are showing a considerable increase in absorbance as the amount of extract increases but then levels off when the silver nitrate gets used up during the reaction. The Ag^+ in solution now becomes the limiting factor as the addition of more phytochemicals cause no further effect to the production of silver nanoparticles. Higher amounts of extract also increase the concentration of reducing agents from the extract , thus the higher the possibility of the green reduction mechanism of the silver nitrate occurring, which would lead to the formation of well-defined and stable silver nanoparticles [98]. The variation in values of absorbance suggested the increase in the amount of nanoparticles produced and the

shifting of absorbance bands to longer wavelengths suggested the increase in the particle size of the silver nanoparticles [94].

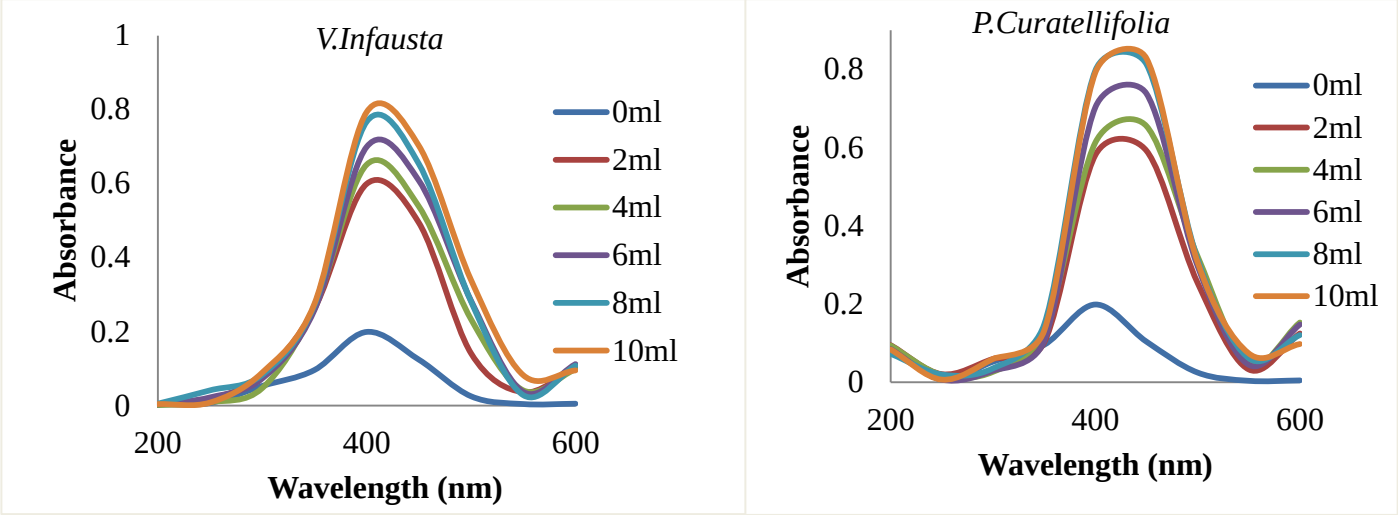


Fig 4.6: Effect of the amount of extract at different wavelengths for *V.Infausta* and *P.Curatellifolia*

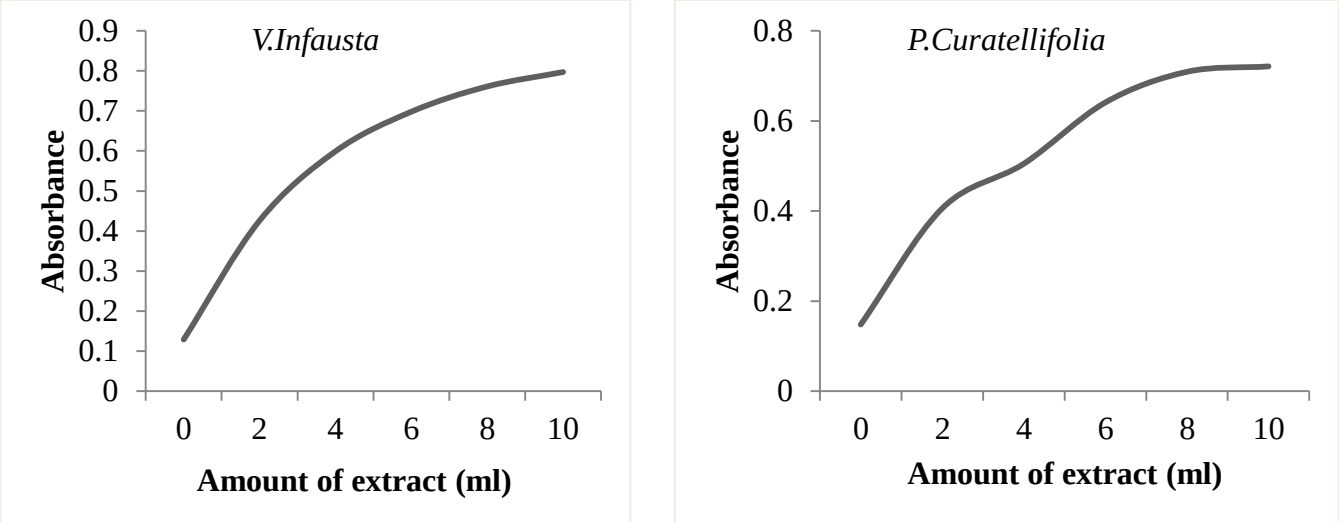


Fig 4.7 : Relationship between the amount of extract and absorbance at a maximum wavelength for *V.Infausta* and P.*Curatellifolia*

4.2.3 Effect of temperature

The effect of temperature was carried out in a water bath at varied temperatures of 20 ^{0}C to 80 ^{0}C using 90 ml of 5 mM silver nitrate and 10 ml of plant extract.. An increase in temperature increases the amount of silver nanoparticles produced as evidenced by an increase in absorbance (Fig 4.8 and 4.9). This might be due to an increase in kinetic energy of the reacting molecules which means more Ag^+ were in collision with the reducing molecules of the extract, thereby resulting in the reduction of Ag^+ occurring at a faster rate. As the temperature is increased, there is a lowering in the activation energy of reacting molecules, causing the reaction to proceed at faster rate. A further increase in temperature beyond 50 ^{0}C and 70 ^{0}C for *V.Infausta* and *P.Curatellifolia* respectively however does not show a considerable increase in the absorbance as evidenced by the graphs in Fig 4.8 below. This is because the reacting molecules become the limiting factor as the temperature is increased thus the graphs of *V.Infausta* and *P.Curatellifolia* flattens after a temperature of 50 ^{0}C and 70 ^{0}C respectively showing that there is no further AgNPs production as indicated by Fig 4.9 below. It shows that after those temperatures, the silver nanoparticles are no longer being produced therefore there is no further increase in absorbance hence the flattening of the graphs [99].

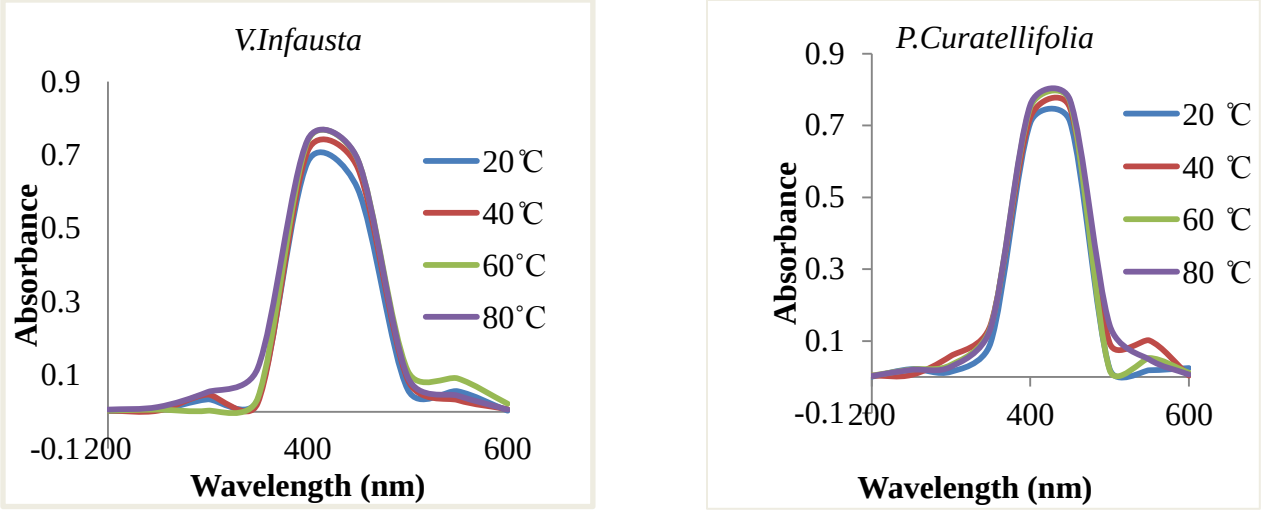


Fig 4.8 : Effect of temperature at different wavelengths for *V.Infausta* and *P.Curatellifolia*

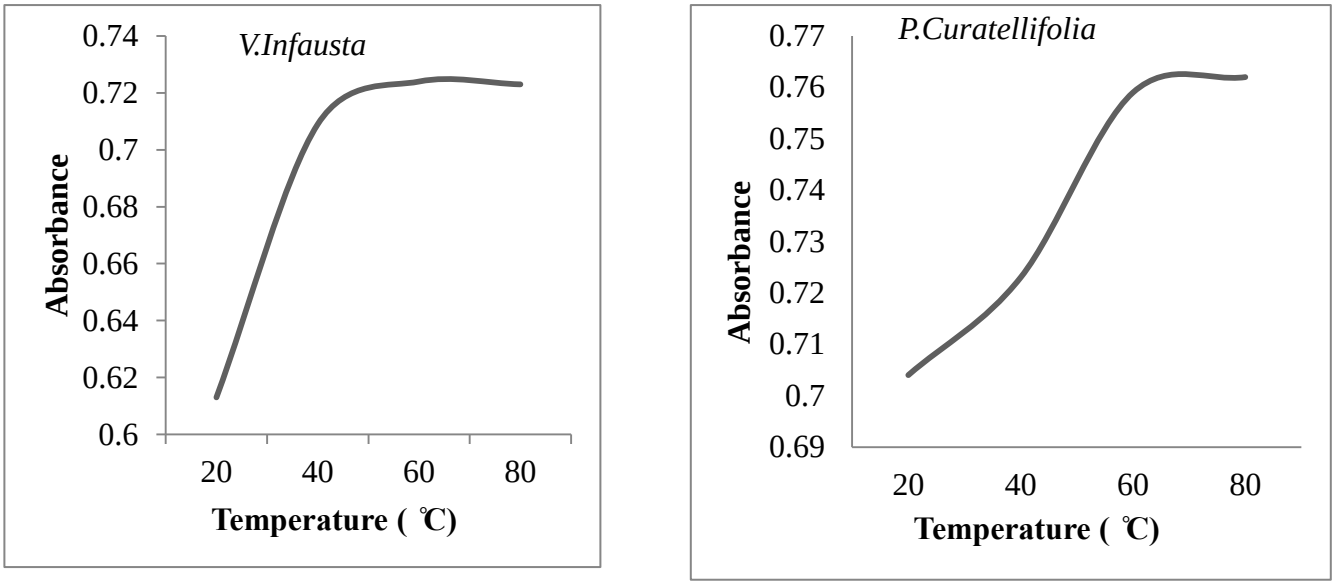
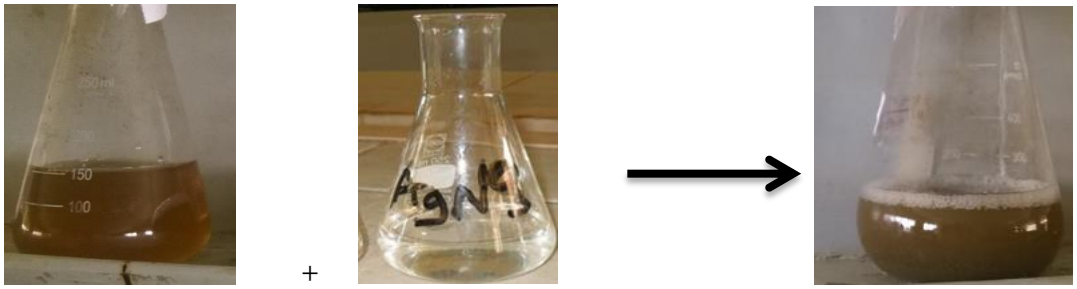


Fig 4.9: Relationship between temperature and absorbance at a maximum wavelength for *V.Infausta* and *P.Curatellifolia*

4.3 Synthesis of silver nanoparticles

The colour changes which occurred after the addition of silver nitrate to the plant extract are shown in Fig 4.10 (a) and (b) below. The extracts of the plant leaves acts as a reducing agent and also as capping agent, thus mediating synthesis as well as stabilization of the silver nanoparticles [41].

Leaf extract + silver nitrate ⟶ silver nanoparticles

a) 

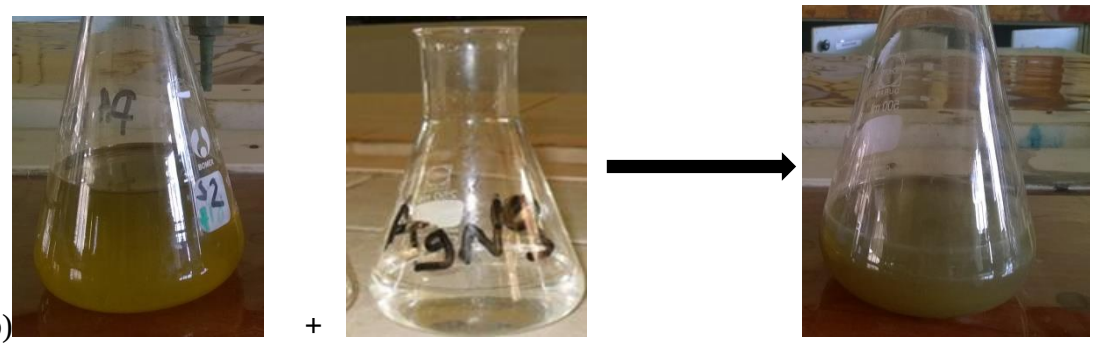
b) 

Fig 4.10: Synthesis of silver nanoparticles using plant leaf extract (a) *P.Curatellifolia* (b) *V.Infausta*

4.3.1 Visual examination of silver nanoparticles

The visual observation of nanoparticles showed that they exhibit a yellow- dark brown color in the aqueous solution due to the excitation of the Surface Plasmon resonance phenomenon as reported by literature [9] . The reduction of silver ions on exposure to the plant extract could be followed by a colour change as shown in Fig 10. The appearance of a brown colour confirms the existence of silver nanoparticles in the solution [18] .This formation indicates that silver ions in reaction medium have been converted to elemental silver having the size of nanometric range [57]. The appearance of different colours at different time intervals is an indication that the morphology (shape and the size distribution) of silver nanoparticles alters with the reaction time. With the

duration of time the colour intensity also increased as a result of formation of more silver nanoparticles in aqueous solution [100].

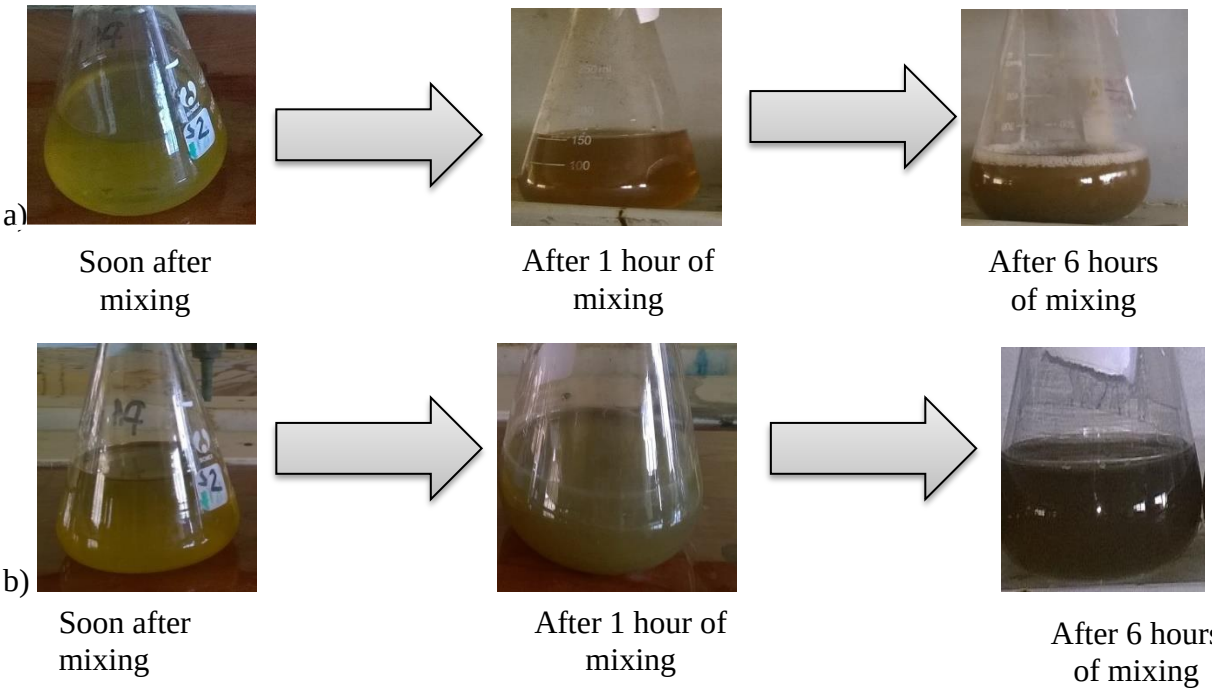


Fig 4.11 : Visual observation on the colour of synthesised silver nanoparticles with time

a) *P. Curatellifolia* b)*V.Infausta*

4.4 Characterization of the synthesised silver nanoparticles

The characterization of the synthesised silver nanoparticles was carried out with the use of a UV-Vis spectroscopy and an FTIR spectrophotometer. The results are given in form of spectra, graphs and tables.

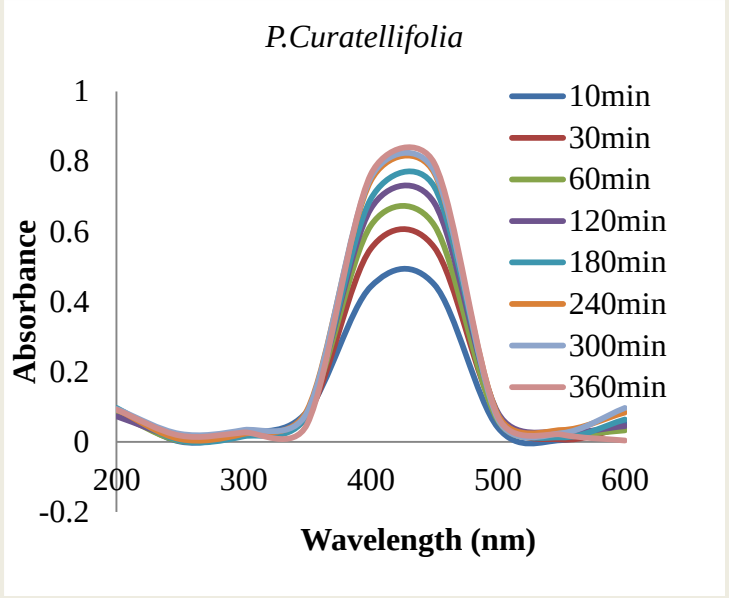
4.4.1 UV-Vis spectroscopy in monitoring the effect of reaction time

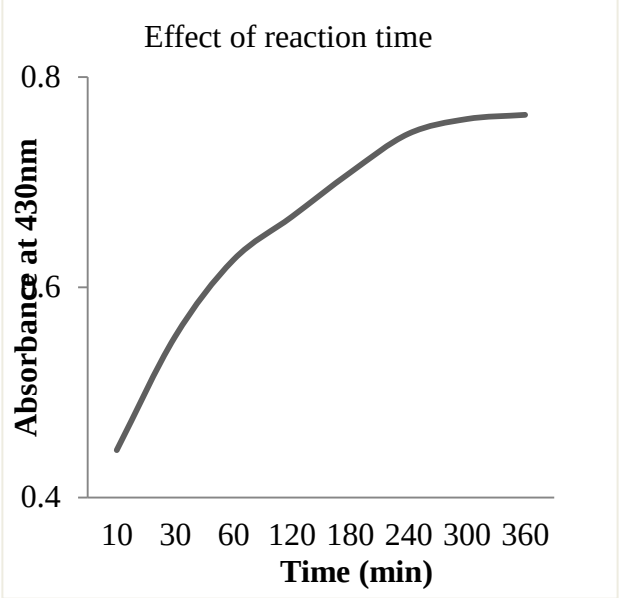
The formation and stability of silver nanoparticles in aqueous solution was confirmed by using UV-Vis spectroscopy analysis. It is generally recognized that UV-Vis spectroscopy could be used to

examine size and shape-controlled nanoparticles in aqueous suspensions [101] and also for the characterization of colloidal particles [44]. The intensity of the dark brown color observed on the synthesised nanoparticles can be said to be directly proportional to the incubation time of reaction mixture. During the first 60mimutes, the rate of silver ions reduction was taking place at a slow rate as indicated by the low absorbance values and also by the colour intensity of the reaction mixtures which were pale. The UV-Vis spectra of silver nanoparticle after 10min, 30min, 60min, 120min, 180min, 240 min, 300min and 360min of the reaction were recorded, indicating the formation of silver nanoparticles due to excitation of surface plasmon vibrations in silver nanoparticles. It was observed that the absorption peaks increase with the time which is in accordance with the color change observed in visual examination [9] . The Surface Plasmon Resonance (SPR) band observed at 415 nm for *V.Infausta* was close to that reported in [100] and 430 nm for *P.Curatellifolia* which was also comparable with those in literature [89]. As reported by literature the SPR bands are said to be influenced by the size, shape, morphology ,composition and the dielectrical environment of the prepared silver nanoparticles. The appearance of only a single SPR band in the absorption spectra of the nanoparticles indicates that they are spherical in shape [57]. It was observed that during the reaction period, there was an increase in absorbance without any change in the peak position as a function of time, which was an indication of an increase in production of colloidal AgNPs and the stability of the AgNPs [93] as shown in Fig 4.12 (a) and (b) below which represent the plot of absorbance at the maximum wavelength against reaction time [57]. An increase in the color intensity also increased the absorbance of the silver nanoparticles and this was revealed by the graphs below of *V.Infausta* and *P.Curatellifolia*. This increase in absorbance along with color intensity could be ascribed to an increase in the amount of silver nanoparticles with time [97]. The complete reduction of the silver ions to the respective nanoparticles took place after 6 hours for

V.Infausta and after 4 hours for *P.Curatellifolia.* 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 [102],thus the rapid generation of nanoparticles with the use of *V.Infausta* and *P.Curatellifolia* showed the excellent reducing potential of the active components of the extracts and their polymeric stabilization within a narrow size spectrum [44]. Literature review reports that the properties of the absorption peaks are related to surface plasmon resonance of metallic nanoparticles are largely governed and in fluenced by the particle size, shape, distribution and also depend on the dielectric constant of the surrounding media [103].

a)





b)

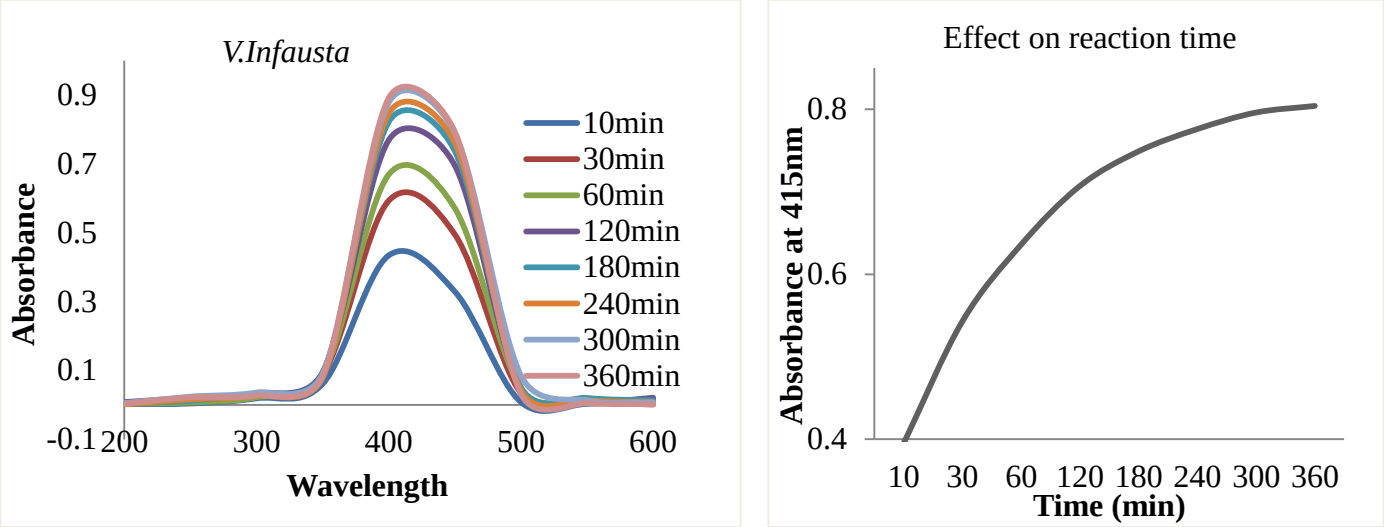


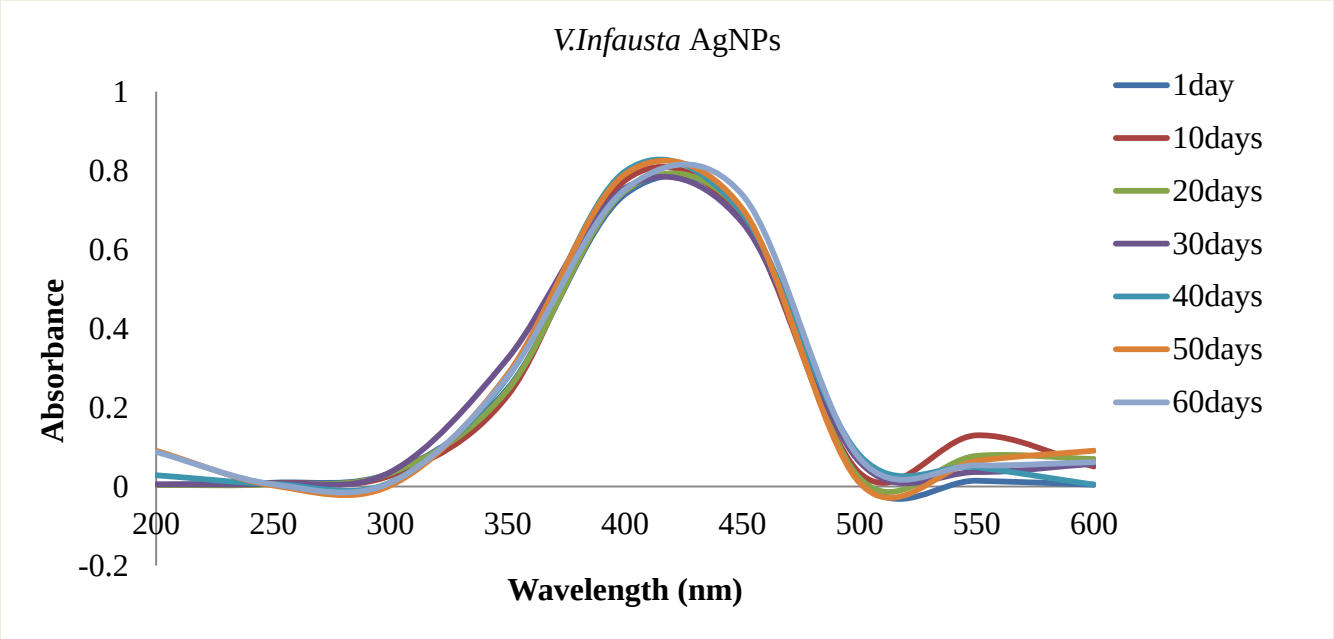
Fig 4.12: Effect of reaction time on the synthesised silver nanoparticles a*) V.Infausta* b) *P.Curatellifolia*

4.4.2 UV-Vis Spectroscopy in monitoring the stability studies of the silver nanoparticles

UV-Visible spectroscopy offers a simple and reliable method which can be used for monitoring of the stability of aqueous solutions of nanoparticle. Stability of AgNPs can be obtained by either electro- static/charge stabilization or polymeric stabilization [36]. The electrostatic stabilization of the silver nanoparticles is based on the formation of a charged layer through adsorption of ionic groups present in the medium to the surface of AgNPs, thus resulting in a repulsive force among each other thereby preventing aggregation [59]. The optical properties of silver nanoparticles change when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighboring particles [104]. This causes the surface plasmon

resonance to shift to lower energies, thereby causing the absorption and scattering peaks of the AgNPs to red-shift to longer wavelengths as shown by graphs (a) and (b) below after 50 days.

a)



b)

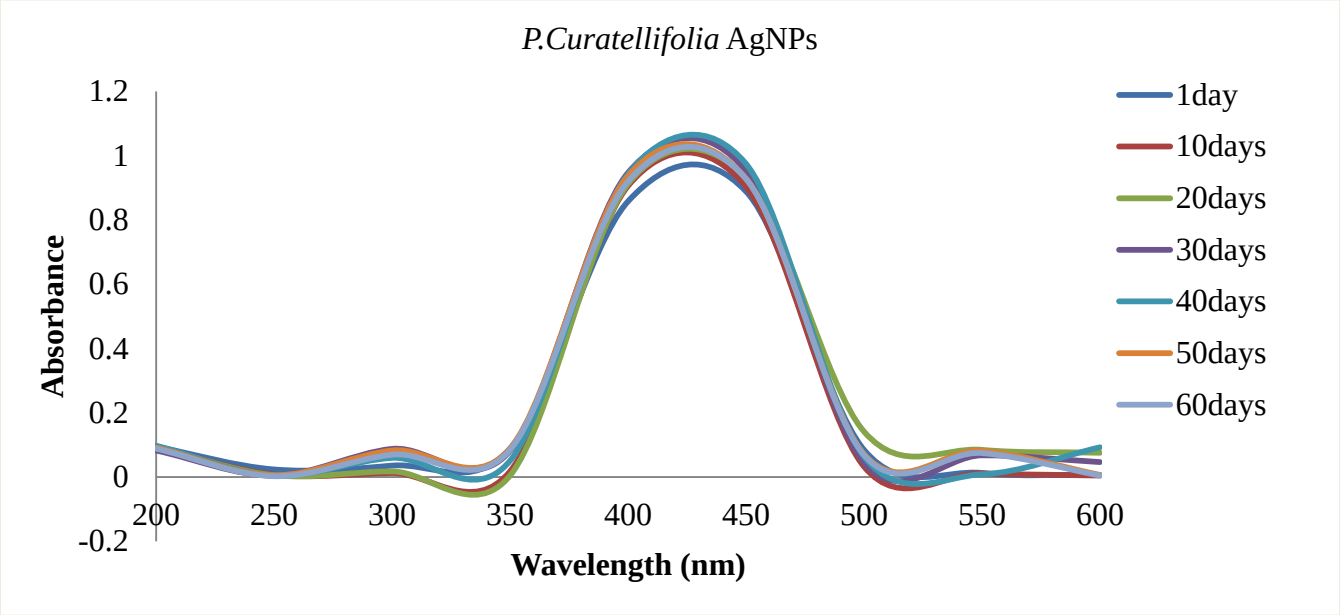


Fig 4.13: Stability studies on silver nanoparticles from a) *V.Infausta* and b) *P.Curatellifolia*

4.4.3 FTIR analysis

FTIR analysis was used to identify the nature of capping ligands that stabilizes the silver nanoparticles formed by the bio reduction process [18]. It is used to probe the chemical composition of the surface of the AgNPs and the local molecular environment of the capping agents on the nanoparticles [9]. The FTIR spectra for the two plant extract showed the peaks at 3337.17 cm^{-1}, 2092.53 cm^{-1}, 1639.56 cm^{-1}, 1014.81 cm^{-1} and 644.08 cm^{-1} *V.Infausta* and at 3652.92 cm^{-1} , 2086.23 cm^{-1} , 1654 cm^{-1} , 1014.03 cm^{-1} and 610.12 cm^{-1} for *P.Curatellifolia* as shown in Tables 4.2 and 4.3 respectively. The FTIR spectra are shown below in Fig 4.13 (a) and (b). The peak at 3317.17 cm^{-1} and 3652.92 cm^{-1} for *V.Infausta* extract and *P.Curatellifolia* extract respectively indicates the presence of O-H stretching vibrations of phenol group [2] which was also confirmed by the phytochemical tests which are shown in table 4.1 above. The band was shifted to higher frequency region in *V.Infausta* and to lower frequency in *P.Curatellifolia* showing the

formation of silver nanoparticles due to the reduction of silver ions by the O-H group. The peak in region 2092.53 cm^{-1} and 2086.23 cm^{-1} indicates the C–H stretching of alkanes. Unsaturated C=C bonds, shown by the peak in the 1639.56 cm^{-1} and 1654 cm^{-1} region, indicates the existence of such groups in the aromatic ring structure [21] . It also shows the presence of the amide I and amide II which arises due to carbonyl (C=O) due to the presence of flavonoids and steroids in the plant extracts [19] and amine (N-H) stretching vibrations in the amide linkages of the proteins, respectively. The proteins may bind to AgNPs through free amine groups or carboxylate ion of amino acid residue in the plant extract [92] . The carbonyl group (C=O) is due to flavonoids and steroids which are present in the leaf extract of the two plants which were responsible for reduction and efficient stabilization of the silver nano particles [19]. The bands 1014.81 cm^{-1} and 1014.03 cm^{-1} could be assigned to the C-N stretching vibrations of aliphatic amines [62]. The FTIR study confirmed that the plant extract has the ability to perform dual functions of reduction and stabilization of AgNPs due to the presence of phytochemicals like proteins over the silver nanoparticle surface [43].

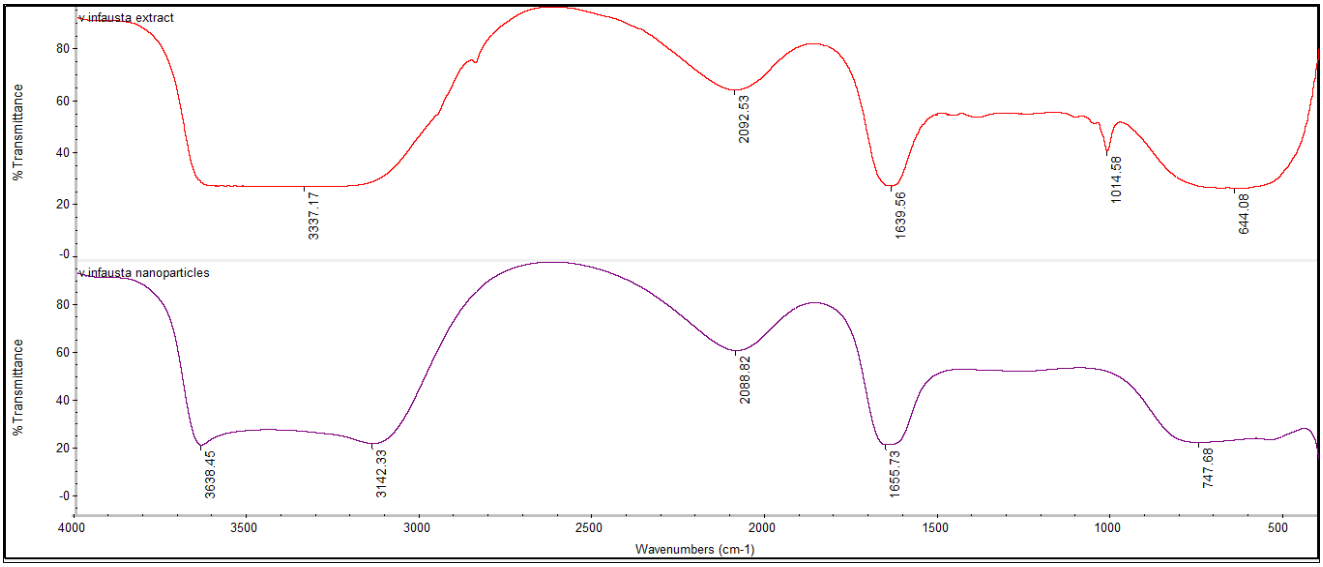
Table 4. 2 : FTIR for *V.Infautsa* extract and AgNPs

Extract	**Silver nanoparticles**
Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
333.17	3638.45
2092.3	3142.33
1639.56	2088.82
1014.8	1655.73
644.08	747.68

Table 4. 3 : FTIR for *P.Curatellifolia* extract and AgNPs

Extract	**Silver nanoparticles**
Wavenumber (cm^{-1})	Wavenumber (cm^{-1})
3652.92	3642.06
2086.23	3136.47
1654.92	2088.73
1014.03	1657.76
610.12	756.34

a)



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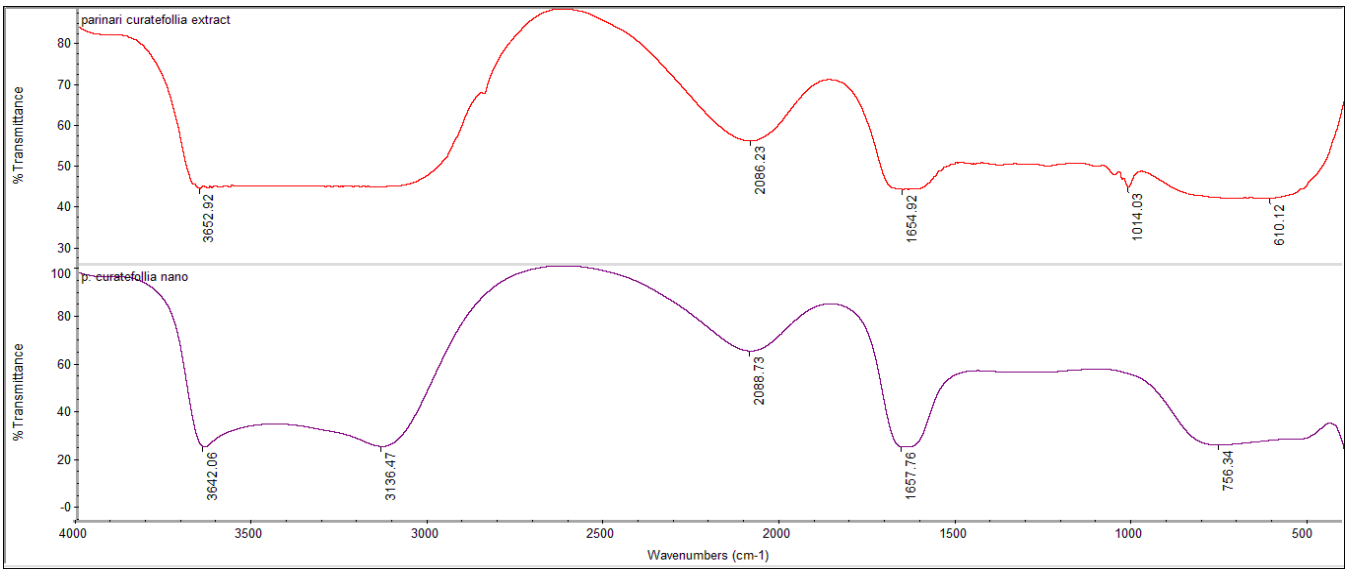


Fig 4.14 : FTIR for *V.Infausta* (a) and *P.Curatellifolia* (b) for both the extract and silver nanoparticles

4.5 Antifungal analysis

The high surface-area-to-volume ratio make the silver nanoparticles more suitable as antimicrobial agents [105]. The antifungal behavior of the synthesised nanoparticles is attributed by the presence of electronic effects that are brought about as a result of changes in local electronic structures of the surfaces due to their smaller sizes [106]. These unique properties of the silver nanoparticles are considered to be contributing towards enhancement of reactivity of their surfaces. The antifungal activities of Itraconazole and Griseofulvin, that are widely used against many fungal infections, were used as positive control for comparison with antifungal activity of the AgNPs [107]. The silver nanoparticles of *Parinari Curatellifolia* compared very well with those of Itraconazole while those from *Vangueria Infausta* had almost the same inhibition zones values as those of Griseofulvin. This means that the synthesised silver nanoparticles from both the plants extract could be used in drug formulations inorder to try and substitute the chemicals that are already being used

to formulate these drugs which are having serious side effects to humans. Most of the phytochemicals have been identified to have antimicrobial activity according to literature review for example flavonoids [23] and these could have been responsible for the antifungal activity shown by these synthesised silver nanoparticles. The antifungal activity of silver nanoparticles derived from *V.Infausta* and *P.Curatellifolia* leaves showed enhancement in activity due to synergistic effect of silver and the components of these leaves. Studies reveal that the antimicrobial activity of the silver particles is due to their positive charge that qualifies them in reacting with the negatively charged proteins on the cell membranes and thus contributing to their antimicrobial activities [6].The silver nanoparticles from both plants showed effective antifungal activity due to their extremely large surface area, which provides better contact with the four fungi used [108],namely *Trichoderma, S.Cerevisiae, Penicillium* and *Fusarium* but the silver nanoparticles of *P.Curatellifolia* showed a remarkable antifungal activity as compared to the silver nanoparticles of *V.Infausta.* This might have been contributed by the size of the silver nanoparticles which are a bit larger than those of *V.infausta* as indicated by the maximum surface plasmon resonance at 430 nm as compared to that of *V.Infausta* at 415 nm. A surface plasmon resonance in the range of 420 nm-430 nm normally increase the antimicrobial activity of the synthesised nanoparticles [67] .It was reported that due to their small size, the silver nanoparticles may attach to the cell surface of the fungi and get into the cells directly even without damaging the cell wall and then cause the death of the cell [96]. The clear zones in the Agar plate after incubation is an indication of the inhibition of fungal growth. The disruption of membranes of bacteria such as *E. coli* by silver nanoparticles significantly increases its permeability, leading to abnormal transport through the plasma membrane and, finally, cell death, is similar to the studies that have also been reported in other fungal species, [109]. It has also been reported that silver nanoparticles might also lead to protein denaturation and

proton pump destruction by binding to the surface proteins of fungi, increasing the permeability of the membrane or protein lipid bilayer, finally resulting in disruption of the cell membrane[96]. Other reports say that the AgNPs attach to the cell membrane and penetrate in the fungi producing a site which has little molecular weight in center of fungi, they then attach to the respiratory sequence releasing silver ion in the fungal cell and finally cell division stop leading to cell death [4].

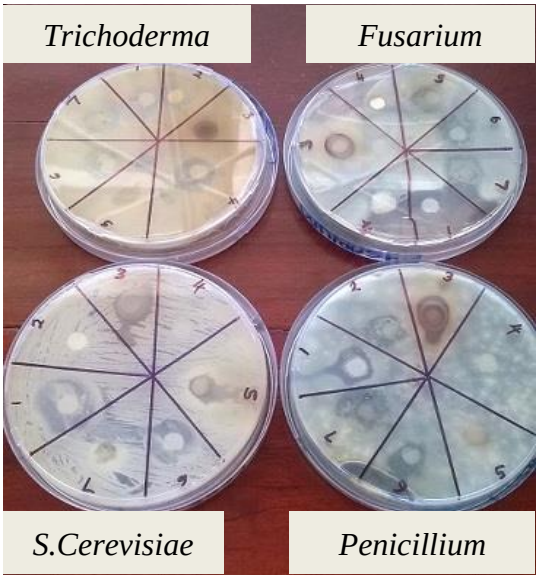
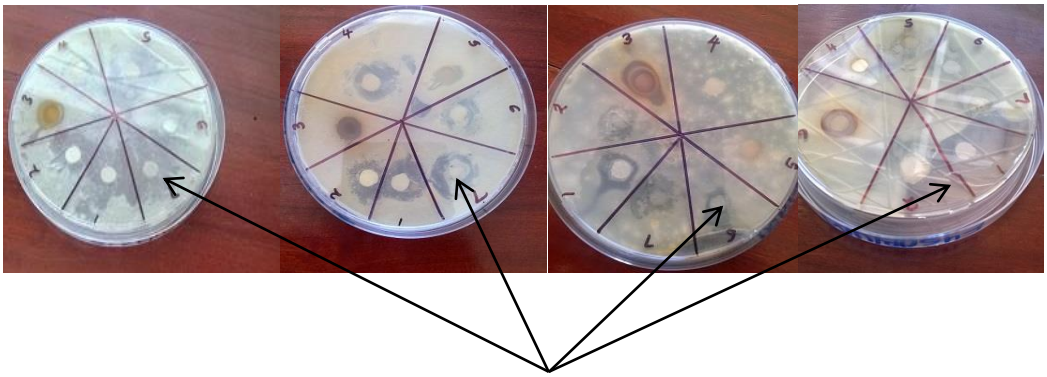


Fig 4.15: Agar Disk Diffusion method used for antifungal evaluation on four different fungi



Clear zones of inhibition

Fig 4.16: Impregnated discs showing clear zones of inhibition

Table 4. 4 : Antifungal results

Fungi	**Average inhibition zone (mm) for**						
	1	2	3	4	5	6	7
	Itraconazole	*P.Curatellifolia* Extract	*P.Curatellifolia* AgNps	*V.Infausta* extract	$AgNO_3$	Griseofulvin	*V.Infausta* AgNps
Trichoderma	19.33	11	16.33	9.33	8	17.33	15.33
S.Cerevisiae	21.33	12	17.33	10.67	9	17.67	16
Penicillium	20	10.33	16.67	10	8.67	18.33	15.67
Fusarium	19	10	16	9	8	17	15

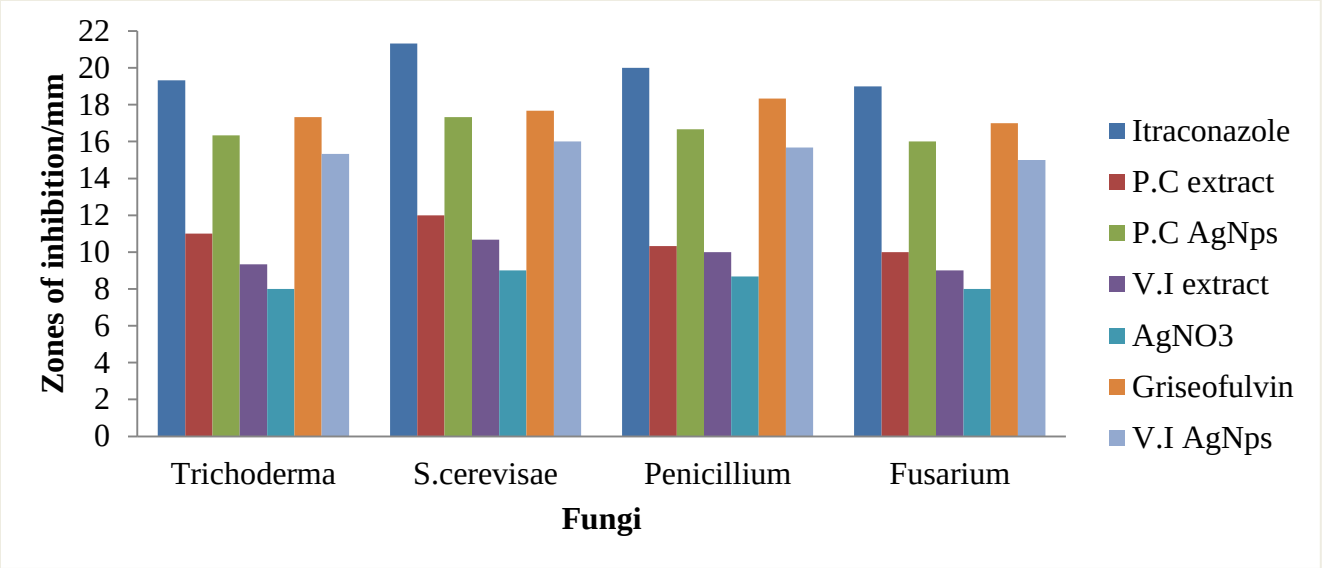


Fig 4.17: Graphical representation of inhibition zones

4.6 Summary

The green approach used in the synthesis of silver nanoparticles in this research showed that this is a simple, one way and eco-friendly technique. The results obtained showed that the silver nanoparticles are stable even at room temperature and this aspect could be exploited in using these nanoparticles in drug delivery systems as they are non-toxic to humans due to the use of harmless chemicals during the synthesis. In addition to their stability the AgNPs showed a high antifungal activity which was comparable to that of antifungal drugs like Itraconazole and Griseofulvin thus they can be used to replace these drugs inorder to eliminate the adverse side effects which are brought about by these antifungals. The AgNPs could also be used in wound dressing and for other medicinal application in future.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The green method used for the synthesis of silver nanoparticles was successfully carried out using plant leaves from *Parinari Curatellifolia* and *Vangueria Infausta*. The presence of phytochemicals such as saponins, flavonoids, phenols, coumarins, steroids glycosides and tannins in the leaves extract made the bio reduction of silver ions to silver nanoparticles successful. The green approach used in this synthesis offered an ecofriendly method due to the use of these phytochemicals as reducing agents which are non-toxic and safe to use without any environmental pollution. The optimum conditions achieved for the synthesis of silver nanoparticles using *Parinari Curatefollia* were a temperature of 70 ^{o}C, 5 mM silver nitrate and 9 ml of the extract. For *Vangueria Infausta* the temperature was at 50 ^{o}C, 5 mM silver nitrate and 10 ml of the plant extract. The silver nanoparticles for both the plant leaves extract were achieved within 6 hours of synthesis. Surface Plasmon Resonance band of the synthesized AgNPs as determined by the UV-Vis Spectroscopy was centered at 430 nm for *Parinari Curatellifolia* and at 415 nm for *Vangueria Infausta*. The presence groups like C= O, N-H, C-O and the -OH due to the presence of phytochemicals like polyphenols and flavonoids played a major role in the synthesis and stabilization of AgNPs. The synthesized silver nanoparticles from both plants were found out to be stable for over one and a half month as evidenced by the UV-Vis Spectroscopy studies. The AgNPs obtained from both the plants leaves extract can be used an alternative source of antifungals as they exhibited strong inhibition against *Trichoderma, Fusarium, Penicillium* and *Saccharomyces Cerevisiae* with the silver nanoparticles from *Parinari Curatellifolia* leaf extract having the highest potential in drug usage.

5.1 Recommendations

The pharmacokinetics and pharmacodynamics (effect of AgNPs on human body and *vice versa*) of the synthesized AgNPs should be studied and eventually pre-clinical and clinical studies on the AgNPs should be done. There is need to characterize the silver nanoparticles using SEM and XRD inorder to understand the size, morphology and the crystallinity of the silver nanoparticles. Further studies should be carried out to isolate, characterize and test the effectiveness of the bio-active compound in the *Parinari Curatellifolia* and *Vangueria Infausta.* The mechanism of the antifungal activity of silver nanoparticles on fungi needs to be studied further.

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APPENDICES

APPENDIX A: MATERIALS

List A: Apparatus used in the synthesis and characterization of the silver nanoparticles

Measuring cylinders , volumetric flasks, Beakers, spatula, pestle , mortar, wash bottles, conical flasks, petri dishes, filter papers, filter funnels, vials, 180μm sieve, droppers and syringes

Table A 1: Reagents and Chemicals

Chemical name	Chemical formula	Manufacturer	Mass/concentration
Silver nitrate	$AgNO_3$	MERCK Chemicals	98%
Acetic anhydride	$(CH_3CO)_2O$	ACE	40%
Ferric chloride	$FeCl_3$	ACE	2 %
Sodium hydroxide	NaOH	Skylabs	2% and 10 %
Sulphuric acid	H_2SO_4	Skylabs	98 %
Mercuric chloride	$HgCl_2$	Skylabs	99.9 %
Potassium iodide	KI	Skylabs	99.9 %
Chloroform	$CHCl_3$	ACE	55 %
Potassium bromide	KBr	ACE	99.9 %
Ethanol	CH_3CH_2OH	Skylabs	75%
Distilled water	H_2O	MSU Lab	-

Table A2: Instrumentation

Name	Model	Manufacturer	Use in research
Analytical balance	GA-110	OHAUS	Weighing
UV-Vis	UV 752	Perkin Elmer	Characterization
FTIR	Nicolet 6700	Thermo scientific	Characterization
Incubator	MISA	LABOTEC	Grow and maintains fungal cultures
Biological Safety Cabinet (BSC)	H14 HEPA	SafeFAST Elite	Provide operator and environmental protection during cell culture
Autoclave	RAU-123	Incotherm	Sterilization
Rotary shaker	HY4	Vision Electrical	Shaking during extraction